The European CLINAM & ETPN Summit, June 23-26, 2013

CLINAM
European Foundation for
Clinical Nanomedicine

CLINAM 6/13
Conference & Exhibition

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

Clinical Nanomedicine & Targeted Medicine

From Antibodies to Nanodrugs, Diagnostic Systems and Targeted Delivery

Previous Events on Sunday, June 23, 2013 • General Assembly of the European Society for Nanomedicine (15.30 h) • Meeting of the International Society for Nanomedicine (16.30 h) • **Welcome Dinner for speakers and invited guests (19.45 h**

Main Sections of the Summit

- **6th European Conference for Clinical Nanomedicine:**
- **4rd ETP Nanomedicine Brokerage Session**
- **3nd ETP Nanomedicine Policy – Industry Table**
- **6th Foyer Exhibition & University Village**

CONFERENCE PROCEEDINGS

Editors: Beat Löffler, MA and Prof. Dr. med. Patrick Hunziker

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CLINAM European Foundation for Clinical Nanomedicine

CLINAM 6/13 with ETPN
European Summit for Clinical Nanomedicine and Targeted Medicine

Basel, Switzerland, Monday, June 24, 09.00 h – Wednesday, June 26, 2013, 17.45 h

Conference Proceedings - Table of Contents

Introduction

CV OF SPEAKERS

Akhter Sohail 7
Alexiou Christoph 7
Allen Theresa Mary 7
Anderson Daniel G. 7
Anzalone Christopher 7
Arber Werner 8
Balogh Lajos 8
Barenholz Ychezkel 8
Bates Katie 9
Behrend Olaf 9
Berger François 9
Binnig Gerd 9
Birngruber Thomas 10
Boccaccini Aldo R 10
Boeck Georg 10
Boisseau Patrick 10
Bouwmeester Hans 11
Brinkmann Ulrich 11
Brun Reto 12
Burckhardt Peter 12
Capurro Rafael 12
Carr Bob 12
Cesselli Daniela 12
Coelho Luiz Felipe 13
Cremer Carsten 13
Cullis Pieter R. 13
Dawson Kenneth 14
De Boer Marco 14
Dell'Ambrogio Mauro 15
Desai Neil P. 15
Dobrovolskaia Marina 15
Dolev Yaniv 15
Dormidontova Elena E. 15
Duschl Albert 16
Dvir Tal 16
Ehmann Falk 17
Ellis-Behnke Rutledge 17
Emanuel Noam 17
Fadeel Bengt 18
Falk Andreas 18
Fernandez-Busquets Xavier 18
Fiandra Luisa 18
Fisch Andreas 19
Frima Heico 19
Gerber Christoph 19
Gouze Nicolas 19
Gramatica Furio 20
Grawunder Ulf 20
Güntherodt Hans-Joachim 20
Hansen Steffi 20
Harrer Stefan 21
Herrmann Inge 21
Horstmann Michael 21
Hunziker Patrick 21
Hwang Larn 22
Iversen Tore-Geir 22
Karagkiozaki Varvara 22
Kavallaris Maria 23
Kemmer Wolfgang 24
Kölker Stefan 24
Koning Gerben A. 25
Kostarelos Kostas 25
Krol Silke 25
Lammers Twan 26
Lee Dong Soo 26
Levy Laurent 26
Limberger Markus 26
Lisziewicz Julianna 27
Löffler Beat 27
Logothetidis Stergios 27
Lojkowski Witold 27
Maiocchi Alessandro 28
Mangge Harald 28
Masserini Massimo 28
Masson Mar 29
McNeil Scott E. 29
Meyre Marie-Edith 29
Miller Robert D. 30
Mitsakis Konstantinos 30
Moghimi Moein 30
Mollenhauer Jan 31
Morin Guy 31
Mous Jan 31
Müller Bert 32
Nidhokubwayo Jean Bosco 32
Needham David 32
Nie Guangjun 33
Nunes Antonio 33
Ofran Ynay 34
Parak Wolfgang J. 34
Patri Anil K. 35
Pikramenou Zoe 35
Prina-Mello Adriele 36
Prud'Homme Robert K. 36
Richter Michael 36
Riebesehl Bernd 36
Robinson Douglas K.R. 36
Romero Eder Lilia 37
Rothen-Rutishauser Barbara 37
Sakai-Kato Kumiko 37
Santos Helder 37
Schipanski Angela 38
Schmid (Baumberger) Ruth 38
Schwartz Simo 39
Scoles Giacinto 39
Seelig Joachim 40
Shahbazian Hripsime 40
Solokov Andrey 40
Sommer Andrei P. 41
Stern Stephan 41
Storm Gert 42
Stroes Erik 42
Strohmeier Rudolf W. 43
Sum Robert 43
Swieszkowski Wojciech 43
Szebeni Janos 44
Teuscher Thomas 44
Timpe Carsten 44
Trohooulos Panagiotis 45
Van der Voorn Hans 45
Van Hoogevest Peter 45
Van Hoorn Danny 45
Varshochian Reyhaneh 46
Vogel Viola 46
Volkov Yuri 47
Wagner Andreas 47
Walt Heinrich 47
Weichold Frank 47
Welten Iris 48
Weltring Klaus-Michael 48
Wenzel Wolfgang 48
Wippermann Marieluise 48
Wolbert Ron 48
Yonath Ada 49
Zinkernagel Rolf M. 49

CV OF POSTER SUBMITTERS

Alewani Walla 53
Anvari Bahman 53
Appeldoorn Chantal 53
Arabi Leila 54
Avgoustakis Konstantinos 54
Bastogne Thierry 54
Bering Olson Sidsel 55
Berin Annabelle 55
Bhatia Pooja 55
Borgos Sven Even 56
Carvalho Flavia Chiva 56
Cella Claudia 56
Chandra Vivek 56
Chan Fu-Hsiung 57
Chen Cheng 57
Chen Peilin 58
De la Fuente Maria 58
De Miguel Laura 58
De Mollerat du Jeu Xavier 58
Defain Maria Victoria 59
Del Ben Fabio 59
Demoulins Thomas 59
Desai Diti 59
Devel Laurent 60
Deyhle Hans 60
Dezsi Laszlo 60
Efthimiadou Eleni 61
El-Sayed Ramy 62
Falk Andreas 62
Fortuna Sara 63
Gaware Vivek Sambhaji 63
Gianfranceschi Giuseppe 63
Grobler Paul 63
Grzeskowiak Bartosz F. 64
Gudkova Olga Ye. 64
Hennig Robert 64
Hieber Simone E. 64
Hilger Ingrid 64
Hobson James 65
Hofmann Margarethe 65
Hossain Lubna 65
Iqbal Samir M. 65
Jordan Olivier 66
Kedzierska Aleksandra E. 67
Kim Hyunjin 67
Kordas George 67
Kristl Julijana 67
Kümmel Anne Maria Rita 68
Kusnieruk Sylwia 68
Kuznetsova Nalia R. 68
Laine Anne-Laure 68
Lehner Roman 69
Li-Blatter Xiaochun 69
Liptrott Neill James 69
Lozano Fernandez Tamara 69
Lund Hansen Pernille 70
Magnusson Ingolfur 70
Mangoni Damiano 70
Mani Aparna 70
Manickam Sivakumar 71
Marianecchi Carlotta 71
Masson Mar 72
Mazzucchelli Serena 72
Meszaros Tamas 73
Milotsevits Gergely 73
Mohamed Mohamed 73
Montes Fonseca Silvia L. 73
Morch Yrr 74
Motamed Kouros 74
Müller Caroline 75
Myagkova Ekaterina 75
Nikitina Liudmila 75
Ozbakir Ulgen Burcin 75
Palma Anita 75
Park Margriet 75
Patko Zsofia Panna 76

Peleteiro Olmedo Mercedes 76
Pietrzykowska Elisabeth 76
Pinkeron Nathalie M. 76
Pinzaru Simona 77
Posocco Paola 77
Pricl Sabrina 78
Rad-Malekshahi Mazda 78
Ramazani Farshad 78
Riedel Angela 78
Robinson Douglas K.R. 78
Russo Anna 79
Sahariah Priyanka 79
Santos Helder 79
Satalkar Priya 80
Schoenenberger Cora-Ann 80
Schulz Georg 80
Seehaas Nicole 80
Sergunova Victoria A. 80
Takacs Laszlo 80
Thalmann Peter 81
Townley Helen E. 81
Ucisik Mehmet Hikmet 81
Vargas Gonzales Mara 81
Varshochian Reyhaneh 82
Vasaikar Suhas V. 82
Vllasaliu Driton 83
Voulgari Estafathia 83
Vroon Anne 83
Wang Chang-Fang 83
Wilhelm Nadine 84
Xu Doudou 84
Yang Kewei 84

INTERVENTION ABSTRACTS

Akhter Sohail 87
Alexiou Christoph 87
Allen Theresa Mary 88
Anderson Daniel G. 88
Anzalone Christopher 88
Balogh Lajos 89
Barenholz Ychezkel 89
Bates Katie 89
Behrend Olaf 90
Berger François 90
Binnig Gerd 90
Birngruber Thomas 90
Boccaccini Aldo R 91
Boeck Georg 91
Boisseau Patrick 91
Bouwmeester Hans 92
Brinkmann Ulrich 93
Brun Reto 93
Burckhardt Peter 94
Capurro Rafael 94
Carr Bob 94
Cesselli Daniela 95
Coelho Luiz Felipe 96
Cremer Carsten 96
Cullis Pieter R. 96
Dawson Kenneth 98
De Boer Marco 98
Desai Neil P. 99
Dobrovolskaia Marina 100
Dolev Yaniv 100
Dormidontova Elena E. 100
Duschl Albert 102
Dvir Tal 102
Ellis-Behnke Rutledge 103
Emanuel Noam 103
Fadeel Bengt 104
Falk Andreas 104
Fernandez-Busquets Xavier 104
Fiandra Luisa 105
Fisch Andreas 106
Frima Heico 107
Gerber Christoph 107
Hansen Steffi 107
Harrer Stefan 108
Herrmann Inge 108
Horstmann Michael 108
Hunziker Patrick 109
Hwang Larn 110
Iversen Tore-Geir 110
Karagkiozaki Varvara 111
Kavallaris Maria 111
Kemmer Wolfgang 111
Kölker Stefan 112
Koning Gerben A. 112
Kostarelos Kostas 113
Krol Silke 113
Lammers Twan 113
Lee Dong Soo 113
Levy Laurent 114
Limberger Markus 114
Lisziewicz Julianna 114
Logothetidis Stergios 115
Lojkowski Witold 115
Maiocchi Alessandro 116
Mangge Harald 117
Masserini Massimo 118
Masson Mar 118
McNeil Scott E. 119
Meyre Marie-Edith 119
Miller Robert D. 120
Mitsakis Konstantinos 120
Moghimi Moein 121
Mollenhauer Jan 121
Mous Jan 121
Müller Bert 122
Needham David 122
Nunes Antonio 124
Ofran Ynay 124
Parak Wolfgang J. 125
Patri Anil K. 125
Pikramenou Zoe 125
Prina-Mello Adriele 126
Prud'Homme Robert K. 126
Richter Michael 126
Robinson Douglas K.R. 127
Romero Eder Lilia 127
Rothen-Rutishauser Barbara 128
Santos Helder 128
Schipanski Angela 130
Schmid (Baumberger) Ruth 130
Schwartz Simo 131
Scoles Giacinto 131
Solokov Andrey 131
Sommer Andrei P. 131
Stern Stephan 132
Storm Gert 132
Stroes Erik 133
Sum Robert 133
Swieszkowski Wojciech 133
Szebeni Janos 134
Teuscher Thomas 136
Timpe Carsten 136
Van der Voorn Hans 136
Van Hoogevest Peter 136
Van Hoorn Danny 136
Varshochian Reyhaneh 137
Vogel Viola 138
Volkov Yuri 138
Wagner Andreas 139
Walt Heinrich 139
Weichold Frank 139
Wenzel Wolfgang 139
Wolbert Ron 140
Yonath Ada 140
Zinkernagel Rolf M. 140

POSTER ABSTRACTS

Alewani Walla 143
Anvari Bahman 144
Appeldoorn Chantal 145
Arabi Leila 146
Avgoustakis Konstantinos 147
Bastogne Thierry 148
Bering Olson Sidsel 149
Berin Annabelle 150
Bhatia Pooja 151
Borgos Sven Even 151
Carvalho Flavia Chiva 152
Cella Claudia 153
Chandra Vivek 154
Chan Fu-Hsiung 154
Chen Cheng 155
Chen Peilin 155
De la Fuente Maria 156
De Miguel Laura 157
De Mollerat du Jeu Xavier 158
Defain Maria Victoria 159
Del Ben Fabio 161
Demoulins Thomas 161
Desai Diti 161
Devel Laurent 162
Deyhle Hans 163
Dezsi Laszlo 164
Efthimiadou Eleni 165
El-Sayed Ramy 166
Falk Andreas 166
Fortuna Sara 167
Gaware Vivek Sambhaji 168
Gianfranceschi Giuseppe 170
Grobler Paul 171
Grzeskowiak Bartosz F. 171
Gudkova Olga Ye. 172
Hennig Robert 173
Hieber Simone E. 174
Hilger Ingrid 175
Hobson James 175
Hofmann Margarethe 176
Hossain Lubna 177
Iqbal Samir M. 178
Jordan Olivier 179
Kedzierska Aleksandra E. 179
Kim Hyunjin 180
Kordas George 180
Kristl Julijana 180
Kümmel Anne Maria Rita 182
Kusnieruk Sylwia 182
Kuznetsova Nalia R. 183
Laine Anne-Laure 184
Lehner Roman 185
Li-Blatter Xiaochun 185
Liptrott Neill James 187
Lozano Fernandez Tamara 188
Lund Hansen Pernille 189
Magnusson Ingolfur 189
Mangoni Damiano 190
Mani Aparna 191
Manickam Sivakumar 191
Marianecchi Carlotta 193
Masson Mar 194
Mazzucchelli Serena 194
Meszaros Tamas 195
Milotsevits Gergely 195
Mohamed Mohamed 196
Montes Fonseca Silvia L. 197
Morch Yrr 198
Motamed Kouros 199
Müller Caroline 201
Myagkova Ekaterina 201
Nikitina Liudmila 203
Ozbakir Ulgen Burcin 204
Palma Anita 204
Park Margriet 205
Patko Zsofia Panna 205
Peleteiro Olmedo Mercedes 206
Pietrzykowska Elisabeth 207
Pinkeron Nathalie M. 207
Pinzaru Simona 208
Posocco Paola 209
Pricl Sabrina 211
Rad-Malekshahi Mazda 212
Ramazani Farshad 213
Riedel Angela 213
Robinson Douglas K.R. 213
Russo Anna 214
Sahariah Priyanka 215
Santos Helder 217
Satalkar Priya 219
Schoenenberger Cora-Ann 220
Schulz Georg 220
Seehaas Nicole 220
Sergunova Victoria A. 221
Takacs Laszlo 222
Thalmann Peter 222
Townley Helen E. 223
Ucisik Mehmet Hikmet 223
Vargas Gonzales Mara 224
Varshochian Reyhaneh 224
Vasaikar Suhas V. 225
Vllasaliu Driton 226
Voulgari Estafathia 226
Vroon Anne 227
Wang Chang-Fang 227
Wilhelm Nadine 229
Xu Doudou 229
Yang Kewei 230

SATELITE SESSIONS

233

EXHIBITORS

Basel Inkubator 237
Endor 238
IBM 239
Izon 240
NanoSight 241
Nanosurf 242
Polymun 243
Scienion 244
SeroScience 245
Tecomedical 246
to-BB 247

INTRODUCTION

Welcome to the European Summit for Clinical Nanomedicine and Targeted Medicine, which the CLINAM-Foundation has organized jointly with the European Technology Platform on Nanomedicine (ETPN).

Our joint annual gathering on Clinical Nanomedicine and Targeted Medicine has become an international landmark and at this sixth conference we are grateful for the many valuable contributions to the programme by many excellent researchers, clinicians and experts in the field of nanomedicine and targeted therapies with all their facets.

This year's focus topic is: "Nanomedicine & Targeted Medicine – the paradigm of precise, highly effective, economic and safe medicine for the benefit of patients and mankind". CLINAM aims at a highly interdisciplinary event to push the boundaries of cutting-edge Nanomedicine and Targeted Medicine.

Nanomedicine is steadily progressing, as evident in the program of this field, which represents an "annual snapshot" of the state of the art. In particular, the number of drugs being translated from research to the clinic, and the number of clinical trials is growing each year. As the awareness of clinicians has increased enormously and a significant number of proof-of-concept, non-clinical and clinical studies are under development, this is attracting the venture capital community, large pharmaceutical companies, health technology companies and SMEs to invest in the field. Increasing investments by global pharmaceutical companies in nanotech-based equipment and in nanostructured agents used for diagnosis and therapy are evident, even if the term "nanomedicine" is not consistently used for such efforts.

We look forward to this meeting since it will identify new challenges and indicate solution strategies for Nanomedicines and Targeted Medicines to provide new tools in support of healthcare systems with cost-effective approaches. Nanomedicine is one of the big building blocks of the coming revolution of personalized medicine as compared to the current "one-size-fits-all".

Debating research and responsible development thoroughly, learning from the leaders in the field, discussing in a balanced manner about the opportunities and risks, investigating pathways in the translation of findings, converging on regulatory aspects and creating new common projects are important activities that are shaping this conference and leading to a highly interdisciplinary melting pot of professionals in the healthcare of tomorrow: This is what the summit is striving to achieve for the benefit of patients and mankind in general.

Beat Löffler
CEO of the CLINAM Foundation

Patrick Hunziker
CSO of the CLINAM-Foundation

Basel, June 2013

CURRICULA VITAE OF SPEAKERS

Sohail Akhter

Dr. Sohail Akhter is currently working as postdoctoral research associate at the Department of Pharmaceutics, Utrecht University, The Netherlands. His work has focused on the pharmaceutical development, pharmacokinetic and pharmacodynamic evaluation of antipsychotics polymeric particles for overcoming non-adherence in psychotic disorders. He did his master and PhD in Pharmaceutical Sciences (Pharmaceutics) on the development of lipid vesicular and polymeric nanoparticulate system for therapeutic targeting and bioavailability enhancement. During his PhD, he was awarded with SRF fellowship of CSIR, DBT and UGC. In addition, he was awarded with travel grants for scientific presentations under young scientist category by DST and ICMR. His research interests involve rational development of nanoparticulates and vesicular systems for effective therapeutic targeting, pharmacokinetics and bioanalysis.

Christoph Alexiou

Prof. Dr. Christoph Alexiou, born 2nd of March 1967, received his Ph.D. in 1995 from the Technical University of Munich, Medical school. After finishing his internship in the Gastroenterology Department at the Universityhospital of the Technical University he started as a physician and researcher at the Department of oto-rhino-laryngology, head and neck surgery and founded a research group working on the field of local chemotherapy with magnetic nanoparticles (Magnetic Drug Targeting). In the year 2000 he received his degree as an ENT-Physician and 2002 he changed to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He is working there as an assistant medical director in the clinic and leads the Section for Experimental Oncology and Nanomedicine (SEON). Since 2009 he owns the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the Universityhospital Erlangen. The aim of his resarch focus on the translation of Magnetic Drug Targeting into human trials and he received for his research several national and international awards.

Theresa Mary Allen

PhD, FRCS
Professor Emeritus of Pharmacology and Oncology, University of Alberta, Edmonton, AB
Strategic Advisor, Drug Delivery, Centre for Drug Research & Development, Vancouver, BC
Adjunct Professor, Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC

EDUCATION

R.T., Medical Technology, 1961 Ottawa General Hospital; B.Sc. Honors, Biochemistry, 1965, University of Ottawa; Ph.D., Oceanography, 1971, Dalhousie University.

RESEARCH

Theresa Allen is a Professor Emeritus of Pharmacology and an adjunct professor of Oncology at the University of Alberta. She has been active in the drug-delivery field for over 30 years, has over 230 peer-reviewed publications, many highly cited, and is an inventor on several patents. The product Doxil,Ò the first anticancer nanomedicines,came out of pioneering research in her laboratory at the University of Alberta. She has also been active in the area of new drugs from natural products, resulting in two drugs proceeding into Phase II clinical trials. Her recent work in developing ligand-targeted therapeutics for small molecule therapeutics and gene medicines are at the leading edge of this new field and the methods she developed are widely used throughout the field. Her former graduate students occupy senior positions in academia and industry throughout the world.
Dr. Allen is a founding member and strategic advisor of the Centre for Drug Research & Development (CDRD, www.cdrd.ca), which is a novel hybrid organization devoted to advancing promising medical discoveries from academia to a commercially attractive stage. CDRD evolved from the recognition by the Founders of the pressing need to improve the translation of medical discoveries made in the universities and teaching hospitals into new drugs and technologies that result in global economic and health benefits. CDRD identifies, advances, and commercializes innovative life science discoveries via a unique structure that connects scientists, health care providers, and industry.

HONORS AND AWARDS

Governor General's award for highest average graduation from high school; Gold Key award for highest average B.Sc. in Biochemistry; Fisheries Research Board of Canada grant for 5 years for Ph.D. in oceanography; Defence Research Board of Canada grant for 1 year for post-doctoral research in Neurochemistry; Killam Professor, U. of Alberta, 1995-96; McCalla Research Professor, U. of Alberta, 1998-99; finalist ASTech Award for Leadership in Alberta Technology, 1999; Cygnus Award (Controlled Release Society) for excellence in guiding graduate student research; Novartis award 2000 (Pharmacological Society of Canada) for significant contributions to the advancement and extension of knowledge in Pharmacology; winner ASTech Award for Leadership in Alberta Technology, 2001; winner Alec Bangham International Award for contributions to liposome research, 2002; winner Leadership Award from the Canadian Society for Pharmaceutical Sciences, 2004; elected a Fellow of the Royal Society of Canada, 2005. Elected to the College of Fellow of the Controlled Release Society, 2012

Daniel G. Anderson

Dr. Daniel G. Anderson is the Goldblith Associate Professor at Massachusetts Institute of Technology, and a member of the Department of Chemical Engineering, the Institute of Medical Engineering and Science, and the David H. Koch Institute for Integrative Cancer. He received his PhD in molecular genetics from the University of California at Davis. At MIT, he pioneered the use of robotic methods for the development of smart biomaterials for drug delivery and medical devices. His work has led to the first methods rapid synthesis, formulation, analysis, and biological evaluation of large libraries of biomaterials for use in medical devices, cell therapy and drug delivery. In particular, the advanced drug delivery systems he has developed provide new methods for nanoparticulate drug delivery, non-viral gene therapy, siRNA delivery, and vaccines. His work has resulted in the publication of over 190 papers, patents and patent applications. These patents have led to a number of licenses to pharmaceutical, chemical and biotechnology companies, and a number of products that have been commercialized or are in clinical development.

Christopher Anzalone

Dr. Christopher Anzalone is President and CEO of Arrowhead Research Corporation, a targeted therapeutics company primarily focused on developing RNAi drugs. Dr. Anzalone has a wealth of experience in nanotechnology, biotechnology, company-building, and venture capital. Prior to joining Arrowhead, Dr. Anzalone founded and built The Benet Group, a private equity firm focused on creating

and building new nanobiotechnology companies from university-generated science. Prior to Benet, Dr. Anzalone was a partner at the Washington DC-based private equity firm Galway Partners, LLC where he was in charge building new business ventures. Dr. Anzalone holds a Ph.D. and M.A. in Biology from UCLA and a B.A. in Government from Lawrence University.

WERNER ARBER

born 1929, Swiss citizen

EDUCATION

Studies of natural sciences at the Swiss Polytechnical School in Zürich (diploma) and at the University of Geneva (PhD). Postdoctoral stay at the University of Southern California in Los Angeles.

EXPERIENCE

- 1960/70 Research assistant, later associate professor in Molecular Biology at the University of Geneva.
- 1970/71 Visiting research Professor, University of California, Berkeley
- 1971/96 Full Professor of Molecular Microbiology, Biozentrum, University of Basel
- 1986/88 Rector of the University of Basel
- 1996/99 President of the International Council for Science (ICSU)
- 1978 Nobel Prize in Medicine/Physiology for the discovery of restriction enzymes and their application to problems of molecular genetics
- 1981 Member and since 2011 Präsident of the Pontifical Academy of Sciences

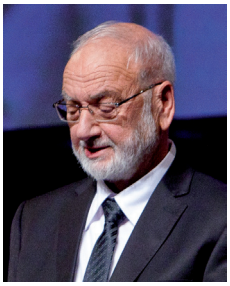
Research topics: Microbial genetics, restriction enzymes, horizontal gene transfer, mobile genetic elements, site-specific recombination, molecular mechanisms of microbial evolution.

Lajos (Lou) P. Balogh

Ph.D., 364 Ocean Ave, #702, Revere, MA 02151 USA Email: baloghl@prodigy.net Phone/Fax: (617) 682-0053, Mobill: (734) 239-3342

Dr. Lajos (Lou) Balogh is Chief Scientific Advisor and Principal of AA Nanomedicine & Nanotechnology, Boston MA, providing expert advice, scientific evaluation, and feasibility assessments for nanomedicine related publications, projects, and small business plans, as well as technology due diligence for government agencies, private companies, and investors in Nanomedicine, Nanobiotechnology, and Nanotechnology since 2000. Dr. Balogh is a former Co-Director of the NanoBiotechnology Center and Director of Nanotechnology Research in the Department of Radiation Medicine at the Roswell Park Cancer Institute. Dr. Balogh received his Ph.D. from the Kossuth L. University in Hungary in Chemical Technology and was invited to the University of Massachusetts Lowell in 1991 as a Visiting Professor. Later he worked at the Michigan Molecular Institute as a senior scientist, and had faculty appointments in the Departments of Internal Medicine and Biomedical Engineering at the University of Michigan Ann Arbor and the University at Buffalo, SUNY. He has authored or coauthored over 150 scientific publications, 1 book and six book chapters in addition to 12 patents in various disciplines.

Dr. Balogh is the Editor-in-Chief of the journal Nanomedicine: *Nanotechnology, Biology and Medicine (Elsevier, Impact Factor=6.692)* and member of numerous USA, European, and International expert committees, including NIH NANO and EPA Nanotechnology study sections and NIH Small Business Study Sections since 1993. He is also a member of the Steering Committee of the American National Standard Institute Nanotechnology Panel, and serves on the US Technical Advisory Committee to the International Standard Organization on Nanotechnology (TC-229). Lou is one of the five Founders of the American Society for Nanomedicine. (http://www.amsocnanomed.org)



Yechezkel (Chezy) Barenholz

Head of Membrane and Liposome Research Lab, Hebrew University-Hadassah Medical School, POB 12272, Jerusalem 91120, Israel,
chezyb@ekmd.huji.ac.il, chezyb@gmail.com

Professor Barenholz (Daniel G. Miller Professor in Cancer Research) received his Ph.D. at the Hebrew University-Hadassah Medical School, Jerusalem in 1971. He has been on the faculty of the Hebrew University since 1968 and was promoted to a Professor in 1981. He was a Visiting Professor it the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville VA, USA from 1973 to 2005. He has been a Visiting Professor at the following universities: University of Utrecht, The Netherlands, 1992; the University of Kyoto, Japan, 1998; La Sapeinza University, Rome, 2006; Jaiotung University, Shanghai, China, 2006; Kings College, University of London, UK, 2006; and, the Danish Technical University DTU, Copenhagen, 2010. The basic research of Professor Barenholz focuses on the biophysics of lipid assemblies such as liposomes and micelles, and on the composition-structure-function relationships of biological membranes (with special focus and contributions related to sphingolipids).

His applied research centers around the development of drug delivery systems (DDS) and drugs based on such DDS including low molecular weight anti-cancer, anti-inflammatory, and local anesthetic drugs, as well as delivery systems for peptides, proteins, nucleic acids, and vaccines. This is exemplified by Doxil®, which was based on his invention and was developed to an FDA- and worldwide-approved anti-cancer drug by Professor Barenholz together with the oncologist Professor Alberto Gabizon, and SEQUUS Pharmaceuticals, Menlo Park CA, USA. Doxil® (Caelyx® in Europe) is the first FDA-approved nano drug and the first FDA-approved liposomal drug (1995). It is distributed today all over the world by Johnson and Johnson. Doxil sales exceeds half a billion dollars a year. Professor Barenholz, with the help of others, based on his inventions, founded the following start-up companies: 1. NasVax Ltd (now a public company on the Israeli stock market), a vaccine developing company, based on, among others VaxiSomeTM , a Barenholz-invented polycationic sphingolipid adjuvant; 2. Moebius Medical, which develops a liposome-based medical device for treatment of osteoarthritis. Moebius finished successfully her first clinical trial and are now preparing for a large pivotal clinical trials; LipoCure Ltd for the development of liposomal nano drugs based on Professor Barenholz' inventions for treatment of cancer and inflammatory diseases [rheumatoid arthritis (RA) and multiple sclerosis (MS)], as well as for special liposomes remote loaded with local anesthetics for prolonging analgesia duration. Two of the liposomal drugs under development in LipoCure are in final preparation for clinical trials.

Professor Barenholz is a coauthor of more than 360 scientific publications having altogether more than 10,000 citations. He is a co-inventor in more than 30 approved patent families. He was an executive editor of Progress in Lipid Research, an editor of 4 Special Issues, and is on the editorial board of 5 scientific journals.

Professor Barenholz was awarded the following prizes and awards: the Donders Chair Professor at the Faculty of Pharmacy, University of Utrecht, The Netherlands {1992); the Kaye award for innovation, twice (1995 & 1997) at the Hebrew University, Jerusalem, Israel; the international Alec D. Bangham (the founder of the Liposome field) award (1998); the Teva Founders Prize (2001), Israel; an Honorary Doctor degree for "outstanding contributions to lipid membrane research and highly innovative achievements in nanomedicine" from the Technical University of Denmark (DTU) in 2012, (Copenhagen, Denmark); and the international Controlled Release Society's (CRS) most prestigious CRS Founders Award for 2012. In 2003 Professor Barenholz founded (from Doxil royalties) the "Barenholz Prizes" for Israeli Ph.D. students to encourage excellence and innovation in applied science.

Professor Barenholz is married to Dr. Hanna Barenholz, together they share 4 daughters and 12 grandchildren

Katie Bates

MPhil/PhD student

Katie obtained her BSc (Hons) Degree (first class) in Biomedical Sciences and Neuropharmacology at King's College London in 2009. She joined the Nanomedicine Laboratory in October 2010 as a PhD student working on a research project focusing on the use of carbon nanotubes as novel gene delivery vectors for the treatment of Parkinson's disease.

Olaf Behrend

- 1970 born in Bonn, Germany

EDUCATION

- 1991 – 1996 University of Karlsruhe, Germany; Process Engineering Student
- 1996 Diploma Degree in Process Engineering (Dipl.-Ing.)
- 1997 – 2002 University of Karlsruhe, Germany; Institute of Food Process Engineering; Research Assistant
- 2002 Doctoral Degree in Process Engineering (Dr.-Ing.) Thesis: "Mechanical Emulsification by Ultrasound" (in German)

EXPERIENCE

- 2002 – 2007 Bayer Technology Services GmbH, Leverkusen, Germany; Process Technology; Scienitst (Development Engineer)
- 2007 – 2009 Bayer Animal Health GmbH, Monheim, Germany; Formulation Development; Principal Scientist (Lab Head)
- since 2009 Boehringer Ingelheim Pharma GmbH & Co KG, Biberach/Riss, Germany; Pharmaceutical Development; Principal Scientist (Lab Head)

François Berger

CLINATEC director
Professor of cell Biology and oncology in Grenoble medical university
Director of the Brain nanomedicine Group, INSERM U 836 CEA-Leti- MINATEC Campus, 17, rue des Martyrs, 38054 Grenoble Cedex 9, France; francois.berger@cea.fr, www.leti.fr
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François Berger, MD,PhD had a dual scientific and clinical education in the field of neurology, oncology and molecular and cell biology. For the last 4 years he coordinated the Brain Nanomedicine Group in INSERM U 836. He continues to have a dual clinical and research activity has professor of cell biology and neuro-oncology. He develops a translational research activity, trying to validate innovative technologies at the preclinical/clinical level in close collaboration with CEA-LETI micro-nanotechnology centre. At the interface between technology and medicine, he is the director of Clinatec. Clinatec is a unique clinical-preclinical research facility devoted to the validation of new implanted micro-nanotechnologies at the human brain interface associating biological and imaging facilities to provide the best environment for the first preclinical and human proof of concept.

Research area: neuro-oncology, neurosciences, biomarkers, nanomedicine

EDUCATION

- 1990 Poitiers Medical school; MD
- 1994 Angers Medical school; neurologist Residency
- 1995 – 1997 San Diego Salk Institute; Postdoctoral fellowship in the F Gage laboratory
- 1995 Grenoble University; PhD
- 1998 Grenoble University; Master of Science

PROFESSIONAL CAREER

- Since 2011: director of CLINATEC INSTITUE, CEA, Grenoble; (Innovative Applications of Micro-Nano-Technologies to Medicine)
- Since 2009: scientific advisor of the French technology Institute associating all the public research agencies in France (CEA, INSERM, CNRS, INRIA)
- Since 2004: Head of the INSERM research laboratory "Brain nanomedicine group" evaluated A plus in 2010 by the national evaluation agency.
- Since 2000: Professor of Cell Biology and Oncology- clinical activity in the field of neuro-oncology
- 1999-2004: Head of the neuro-oncology group in the INSERM research laboratory of AL Benabid.
- 1994- 1995 Assistant in cell biology and neuro-oncology

RECENT PUBLICATIONS

- Nissou MF, El Atifi M, Guttin A, Godfraind C, Salon C, Garcion E, van der Sanden B, Issartel JP, Berger F, Wion D. Hypoxia-induced expression of VE-cadherin and filamin B in glioma cell cultures and pseudopalisade structures. J Neurooncol. 2013 Mar 31.
- Zaccaria A, Bouamrani A, Selek L, El Atifi M, Hesse AM, Juhem A, Ratel D, Mathieu H, Coute Y, Bruley C, Garin J, Benabid AL, Chabardes S, Piallat B, Berger F. A micro-silicon chip for in vivo cerebral imprint in monkey. ACS Chem Neurosci. 2013 Mar 20;4(3):385-92.
- Perles-Barbacaru AT, Berger F, Lahrech H. Quantitative rapid steady state T(1) magnetic resonance imaging for cerebral blood volume mapping in mice: Lengthened measurement time window with intraperitoneal Gd-DOTA injection. Magn Reson Med.
- Lages E, Guttin A, El Atifi M, Ramus C, Ipas H, Dupré I, Rolland D, Salon C,Godfraind C, deFraipont F, Dhobb M, Pelletier L, Wion D, Gay E, Berger F,Issartel JP. MicroRNA and target protein patterns reveal physiopathological features of glioma subtypes. PLoS One. 2011;6(5):e20600.

International patents in the field of nanoproteomic, micro-invasive molecular fingerprints and biomarkers

Gerd Binnig

Gerd Binnig studied physics at the Johann Wolfgang Goethe University in Frankfurt and completed his Ph.D. in 1978. Since 1978, he has been a research staff member of the IBM Zurich Research Laboratory, interrupted by a sabbatical at the IBM Almaden Research Center (1985/86) and a guest professorship at Stanford University (1985-88). From 1987 to 1995, he headed an IBM Physics group at the University of Munich, from which he received an honorary professorship in 1987. For the development of the Scanning Tunneling Microscope (STM), which he invented together with Heinrich Rohrer, he received numerous awards including the Nobel Prize in Physics in 1986. The STM, and the Atomic Force Microscope (AFM), which he invented later and developed together with Calvin Quate and Christoph Gerber, were essential for nanotechnology to emerge. Later he developed a model to describe complex systems and founded in 2000 the company Definiens, where he together with others developed the novel computer language CNL. Today CNL is widely used for complex image analysis.

Thomas Birngruber

MSc
Born: 01.10.1978 in Rainbach, Austria
Citizenship: Austrian
Mail: thomas.birngruber@joanneum.at

EDUCATION

• 1999 – 2006 Master of Science in Biomedical Engineering; Technical University Graz, Austria

Master Thesis: Development of a fiber optical sensor for glucose and oxygen

• 2006 – 2007 Junior Scientist; Medical University of Graz, Department of Radiology

Correction of B1 field inhomogeneity in a 3T MR scanner

• 2007 – 2013 PhD in Biomedical Engineering; Medical University of Graz, Department of Endocrinology (Prof. Thomas Pieber)

Technical University of Graz, Department of Biomedical Engineering (Prof. Scharfetter)

Joanneum Research – HEALTH (Dr. Frank Sinner)

PhD Thesis: Development of a barrier-free sampling system in brain tissue – cerebral Open Flow Microperfusion (cOFM)

CURRENT POSITION

Senior Scientist and Deputy Head of the Research group: Biomedical Technology and Monitoring,
Joanneum Research – HEALTH
Elisabethstrasse 5a, 8010 Graz, Austria

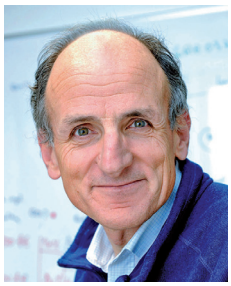
Aldo R. Boccaccini

Prof. Dr.-Ing. habil. Aldo R. Boccaccini is Professor of Biomaterials and Head of the Institute of Biomaterials at the Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Germany. Prior to this appointment, he was Professor of Materials Science and Engineering at Imperial College London, UK (2000-2009). He is currently Visiting Professor of Materials at Imperial College London. He is also visiting professor at Nagoya Institute of Technology (Japan), RWTH Aachen University (Germany) and Universidad Nacional de Cuyo (Argentina). Boccaccini holds a MEng degree from Instituto Balseiro (Argentina), PhD (Dr-Ing.) from RWTH Aachen University (Germany) and Habilitation from Ilmenau University of Technology (Germany). He had post-doctoral appointments at the University of Birmingham (UK), the University of California at San Diego (USA) and the Ilmenau University of Technology, Germany. The research activities of Prof. Boccaccini are in the broad area of biomaterials, focusing on bioactive glasses, ceramics and polymer/glass composites for regenerative medicine, drug delivery, bioactive coatings and medical devices. Prof. Boccaccini has pioneered the development of bioactive glass based scaffolds incorporating polymers with angiogenic properties for bone tissue engineering applications. He also developed electric field assisted methods for production of nanostructured materials and composites with defined surface topography with potential use in the biomedical field. He is the author or co-author of more than 450 scientific papers and 15 book chapters. Boccaccini is Fellow of the Institute of Materials, Minerals and Mining (UK) and of the American Ceramic Society. He is the Editor-in-Chief of the journal "Materials Letters" (Elsevier) and serves in the editorial board of several recognized international journals including Journal of Materials Science, J. Tissue Engineering and Regenerative Medicine, International Materials Review, J. Biomaterials Applications, Advanced Engineering Materials, Advances in Applied Ceramics, Materials, Scientific Reports, Key Engineering Materials and J. Mater. Processing Technology.

He has been a visiting scholar at different universities around the world, including Japan, Italy, Singapore, Germany, Argentina, Netherlands, Slovenia, Spain and Poland. Boccaccini has been member of the organizing committee of numerous international conferences and he is the founder of the International Conferences Series on Electrophoretic Deposition (sponsored by Engineering Conferences International) having served as the Chairman of the 4 conferences of the series held so far in Canada (2002), Italy (2005), Japan (2008) and Mexico (2011). He was a member of the first European Continental Council of the Tissue Engineering and Regenerative Medicine International Society (TERMIS), 2005-2010. Selected achievements in academia and for the scientific community, awards and indicators of esteem include: Adolf-Martens Prize, Adolf-Martens-Fonds, Berlin, Germany (2000), Verulam Medal and Prize in recognition of distinguished contributions to ceramics, Institute of Materials, Minerals and Mining, London (UK) (2003), Federation of European Materials Societies (FEMS) "Materials Science and Technology Prize 2003", Ivor Jenkins Medal, Institute of Materials, Minerals and Mining, UK (2010), McMahon Lecture Award, Alfred University, USA, 2011. Boccaccini is the Representative of Germany to the Management Committee of COST action MP1005, since 2011. He was elected Member of the Reviewer Panel of the German Science Foundation (DFG) in 2011.

Georg Boeck

Georg Boeck, Ph.D., studied pharmaceutical sciences in Regensburg, Germany, and finished his thesis in Pharmaceutical Technology at the University of Heidelberg, Germany. He has been working with Boehringer Ingelheim since 1996 and he started his industrial career as laboratory head in the Respiratory Drug Delivery Department in Ingelheim and was responsible for GMP bulk manufacturing of clinical trial supplies of solid oral dosage forms and capsules for powder inhalation. In the year 1999 he became head of "Device Development and was responsible for the development of the Respimat® Soft MistTM Inhaler. In 2004 Georg Boeck moved to the Pharmaceutical Development Department in Biberach, Germany, and established the group "New Technologies & External Cooperations". The main areas of research include solid oral dosage forms with a special focus on poorly water soluble and poorly bioavailable compounds.



Patrick Boisseau

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EXPERIENCE

M. Patrick BOISSEAU joined the French Atomic Energy and Alternative Energies Commission (CEA) in 1987 to work for 7 years as academic research fellow in plant biology. He then spent 4 years at the Foresight & Strategy Division at the CEA headquarters as expert on strategy in life sciences and environment.

From 2001 to 2004, he was committed to the design, organisation and funding of the NanoBio innovation cluster in Grenoble. The NanoBio center is an integral part of the Minatec Innovation Center, the model for France's competitive clusters and #1 European centre for micro- and nanotechnologies.

From 2004 till 2008, he was coordinator of the European network of excellence in nanobiotechnology, Nano2Life (www.nano2life.org). This network of excellence integrates 23 full academic partners and 41 associate companies (>400 scientists) in a comprehensive joint programme of activity. Since then, he has been part of more than 12 European projects and main coordinator of 5 Framework Projects.

Since 2012, he has been elected Chairman of the European Technology Platforms on Nanomedicine that he joined in 2005.

Since 2008, he has been Programme Manager on nanomedicine, at CEA-Leti."; 15 scientists are devoted there to the preclinical and clinical development of Lipidots® lipid nanocarriers.

Since 2012 he is head of the Strategic Planning on Healthcare at CEATech.
Patrick Boisseau is graduate of the Institut National Agronomique (1983) and of the Ecole Nationale du Génie Rural, des Eaux et des Forêts(1985). He holds a Master's Degree in Human Nutrition (2005).

MANDATES

• Foundation in 2004 of the nanobiotech section at the European Federation of Biotech.
• Member In 2004-2005 of the steering committee of the European Science Foundation Forward Look on Nanomedicine, responsible for the working group on "nanodiagnostics."
• Chairman since late 2012 of the European Technology Platform on Nanomedicine.
• Board Member in charge of Development of the European Platform on PhotoDynamic Medicine since 2011
• Experts for several international organizations such as the European Science Foundation, and the European Commission. Reviewers of numerous articles.

Hans Bouwmeester

Dr. ir. Hans Bouwmeester is senior scientist at RIKILT- Wageningen University and Research center. There he coordinates research on human health risks of nanoparticles, leads national projects and supervises Post-Doc and PhD-students. He studied biology at Wageningen University and obtained his Ph-D at the Rudolf Magnus Institute of Neuroscience at the Medical Faculty of the Utrecht University. He has considerable experience in experimental nanotoxicology. He participated in the EFSA WGs on Nanotechnologies and the WHO/FAO expert meeting on nanotechnologies and is member of the Dutch national Nano platform and the ILSI nanorelease project. His research is funded by the Dutch NanoNextNL program, ZonMW (Netherlands Organization for Health Research and Development), the Dutch department of Economic Affairs and the Netherlands Food and Consumer Product Safety Authority. Internationally he participates in the FP 7 projects Qnano, Marina and NANoREG. Research in his group is focused on the oral route of administration. Oral feeding studies with nanoparticles have been performed, that are directly of use for the risk assessment of nanoparticles. In currently ongoing studies in vitro models for the human gastrointestinal tract are being developed. To simulate the harsh conditions in the stomach and gut an in vitro digestion model is used and triple culture in vitro models that reflect the physiological complexity of the gut epithelium are used to study the uptake of nanoparticles through the gut.

At RIKILT nanotoxicology studies are combined with newly developed analytical chemical separation and characterization techniques, mainly FFF-Single Particle ICP-MS based for metal containing nanoparticles and FFF-MALDI based for organic based nanoparticles. Lastly, at RIKILT decision support systems are developed to facilitate risk prioritization.

MOST RELEVANT PAPERS

•Walczak, AP; Fokkink, R; Peters, R; Tromp, P; Herrera Rivera, Z E.; Rietjens, I; Hendriksen, P J.M.; Bouwmeester, H. Behavior of Silver Nanoparticles and Silver Ions in an In Vitro Human Gastrointestinal Digestion Model. Nanotoxicology 2012. DOI: 10.3109/17435390.2012.726382

• Van der Zande M, Vandebriel RJ, Van Doren E, Kramer E, Herrera Rivera Z, Serrano-Rojero CS, Gremmer ER, Mast J, Peters RJ, Hollman PC, Hendriksen PJ, Marvin HJ, Peijnenburg AA, Bouwmeester H. Distribution, Elimination, and Toxicity of Silver Nanoparticles and Silver Ions in Rats after 28-Day Oral Exposure. ACS Nano. 2012 Aug 28;6(8):7427-42.

• Peters, R.; Kramer, E.; Oomen, A. G.; Herrera Rivera, Z. E.; Oegema, G.; Tromp, P. C.; Fokkink, R.; Rietveld, A.; Marvin, H. J.; Weigel, S.; et al. Presence of Nano-Sized Silica During in Vitro Digestion of Foods Containing Silica as a Food Additive. ACS Nano 2012 Mar 27;6(3):2441-51

• Dekkers S, Bouwmeester H, Bos P, Peters R, Rietveld A, Oomen A.Knowledge gaps in risk assessment of nanosilica in food: evaluation of the dissolution and toxicity of different forms of silica. Nanotoxicology. 2012 DOI: 10.3109/17435390.2012.662250

• Bouwmeester, H.; Poortman, J.; Peters, R. J.; Wijma, E.; Kramer, E.; Makama, S.; Puspitaninganindita, K.; Marvin, H. J.; Peijnenburg, A. A.; Hendriksen, P. J. Characterization of Translocation of Silver Nanoparticles and Effects on Whole-Genome Gene Expression Using an in Vitro Intestinal Epithelium Co-Culture Model. ACS Nano 2011, 5, 4091-4103.
• Dekkers, S.; Krystek, P.; Peters, R. J.; Lankveld, D. X.; Bokkers, B. G.; van Hoeven-Arentzen, P. H.; Bouwmeester, H.; Oomen, A. G., Presence and risks of nanosilica in food products. Nanotoxicology 2011, 5 (3), 393-405.

• Bouwmeester, H.; Lynch, I.; Marvin, H.; Dawson, K.; Berges, M.; Braguer, D.; Byrne, H.; Casey, A.; Chambers, G.; Clift, M.; Elia, G.; Fernandes, T.; Fjellsbø, L.; Hatto, P.; Juillerat, L.; Klein, C.; Kreyling, W.; Nickel, C.; Riediker, M.; Stone, V., Minimal analytical characterisation of engineered nanomaterials needed for hazard assessment in biological matrices Nanotoxicology 2011,5(1), 11.

Ulrich Brinkmann

Ph.D.
Senior Principle Scientist, Scientific Director
Roche Pharma Research and Early Development- Large Molecule Research
Penzberg, FRG

Dr. Ulrich Brinkmann heads a New Technology / Protein Engineering unit within Roche Pharma Research in Penzberg, FRG. His Ph.D thesis covered development of expression systems to produce recombinant reteplase. Subsequently, he held positions as Postoc and Associate Scientist at the NIH/NCI (Ira Pastan Lab) focusing on antibody stabilization/engineering and recombinant immunotoxins for cancer therapy. Prior to joining Roche, he served as CSO in Functional Genetics and Pharmacogenetics companies, Xantos and Epidauros (now Beckmann Coulter) respectively. Ulrich Brinkmann is author and inventor of numerous publications and patents in the field of antibody engineering.

RELATED PUBLICATIONS

• Schneider B, Grote M, John M, Haas A, Bramlage B, Ickenstein LM, Jahn-Hofmann K, Bauss F, Cheng W, Croasdale R, Daub K, Dill S, Hoffmann E, Lau W, Burtscher H, Ludtke JL, Metz S, Mundigl O, Neal ZC, Scheuer W, Stracke J, Herweijer H, Brinkmann U Targeted siRNA Delivery and mRNA Knockdown Mediated by Bispecific Digoxigenin-binding Antibodies. Molecular therapy. Nucleic acids; 2012 Sep 18;1:e46 PMID:23344238

• Metz S, Panke C, Haas AK, Schanzer J, Lau W, Croasdale R, Hoffmann E, Schneider B, Auer J, Gassner C, Bossenmaier B, Umana P, Sustmann C, Brinkmann U Bispecific antibody derivatives with restricted binding functionalities that are activated by proteolytic processing. Protein engineering, design & selection : PEDS; Sep 13; 2012

• Haas AK, Maisel D, Adelmann J, von Schwerin C, Kahnt I, Brinkmann U Human-protein-derived peptides for intracellular delivery of biomolecules. The Biochemical journal; Mar 15;442(3):583-93 2012

• Metz S, Haas AK, Daub K, Croasdale R, Stracke J, Lau W, Georges G, Josel HP, Dziadek S, Hopfner KP, Lammens A, Scheuer W, Hoffmann E, Mundigl O, Brinkmann U Bispecific digoxigenin-binding antibodies for targeted payload delivery. Proceedings of the National Academy of Sciences of the United States of America; 2011 May 17;108(20):8194-9

Reto Brun

Reto Brun is a well-known parasitologist who mainly worked on malaria and African sleeping sickness. His main interest is drug discovery and development for those diseases. At the Swiss Tropical and Public Health Institute he established a Screening Centre for protozoan parasites which was involved in the discovery of most of the clinical candidates for malaria and sleeping sickness. He is also a founder of the Eastern Africa Network for Trypanosomiasis which is doing research and control of this neglected disease. As a Professor at the University of Basel he supervised over 50 MSc and PhD students and as an author he published >480 research articles and book chapters.

Peter Burckhardt

Peter Burckhardt is CEO of „EVA – the Basel life sciences start-up agency“ since 2006. In addition he is CEO of the BASEL INKUBATOR. He has an education as a chemist and achieved his PhD at the University of Basel. In 1984 he joined Ciba-Geigy where he started his career as a research chemist in Pharma Research. Later he held various management positions in research, development, manufacturing, and communications in Ciba-Geigy / Novartis. From 1996 – 1999 he was CEO of Novartis Animal Health Germany. From 2001 to 2006 he worked as a consultant in human resources. As CEO of EVA he is member of the board in several portfolio companies. Since 2009, Peter Burckhardt is president of the BioValley Business Angels Club BioBAC.

Rafael Capurro

Born 1945 in Montevideo, Uruguay.

ACADEMIC DEGREES / EXPERIENCE

• Licentiate in Philosophy from Colegio Máximo, Universidad del Salvador, Buenos Aires, Argentina (1970).
• Dr.phil. in Philosophy from Düsseldorf University (1978).
• Information management at Fachinformationszentrum Karlsruhe, Germany (1980-85).
• Postdoctoral teaching qualification (Habilitation) in Practical Philosophy (Ethics) from Stuttgart University (1989).
• Lecturer at the Institute of Philosophy, Stuttgart University (1987-2004).
• Professor (em.) of information management and information ethics at Stuttgart Media University Germany (1986-2009).
• Steinbeis-Transfer-Institute Information Ethics (STI-IE), Director (2008-2013)

(CO-) FOUNDER

• Capurro-Fiek-Foundation for Information Ethics (CFF): http://www.capurro-fiek-foundation.org/ (since 2011)
• International Center for Information Ethics (ICIE) (Since 1999) http://icie.zkm.de/

• RELEI
Red Latinoamericana de ética de la información
http://redeticainformacion.ning.com/
• ANIE
Africa Network for Information Ethics
http://www.africainfoethics.org/index.html

FELLOWSHIPS AND AWARDS

• Research Fellow of the Graduate School of Library and Information Science (GSLIS), University of Illinois at Urbana-Champaign, USA (2003-2005).
• Senior Fellow in Information Ethics, Center for Information Policy Research, School of Information Studies, University of Wisconsin-Milwaukee, USA (2007-2008; 2009-2010).
• Distinguished Researcher in Information Ethics, School of Information Studies, University of Wisconsin-Milwaukee, USA (2010-2012).
• Former European Group on Ethics in Science and New Technologies to the European Commission (EGE) (2000-2005; 2005-2010)
• Distinguished Researcher at the ACEIE, Department of Information Science, University of Pretoria, South Africa (since 2012).

SELECTED PUBLICATIONS

• Leben im Informationszeitalter. Berlin 1995.
• Ethik im Netz. Stuttgart 2003.
• Rafael Capurro, Michael Eldred and Daniel Nagel: Digital Whoness: Identity, Privacy and Freedom in the Cyberworld. Frankfurt 2013.
• Medicine in the Information and Knowledge Society. Basel 2013. Online: http://www.capurro.de/Medicine2_0.html

See complete list of publications: http://www.capurro.de/publi.html

More at: http://www.capurro.de/home-eng.html

Bob Carr

Dr Bob Carr is the inventor of the NanoSight technology and is currently the Chief Technical Officer of NanoSight and was one of the two Founders in 2002. NanoSight has had a compound annual growth rate (CAGR) of around 70% for the last 5 years and now has over 600 systems installed World-Wide.
Originally a microbial biochemist, his 20 year career at the leading UK Government Research Establishment at Porton Down (CAMR) led him into laser/fibre optics instrumentation, biophysics and nanotechnology and specifically the application of biophysical techniques in the detection and analysis of nanoparticles. In 1996 he left CAMR and formed his own R&D and Consultancy Company, Macranal Ltd., which developed the basic ideas and principles on which NanoSight is currently based. With John Knowles, he formed NanoSight (through acquiring Macranal) in 2003/4.
Bob has significant multidisciplinary experience in the areas of microbiology, laser-based nanoparticle detection and biosensor technology and has had published over 200 scientific papers, technical notes and Conference proceedings and 10+ patents. He has initiated, as Principle Investigator and/or Coordinator, a wide range of UK- and EU-funded international R&D projects and continues to lead the development of NanoSight technology in an increasingly wide range of applications and Industry sectors.

Daniela Cesselli

EDUCATION AND TRAINING

• 1998: medical degree, University of Udine, Faculty of Medicine.
• 2002: specialization in Oncology, University of Udine, Faculty of Medicine.
• 2006: PhD in “Quantitative and molecular diagnostic pathology”, University of Siena, Faculty of Medicine.

RESEARCH ACTIVITY

• June 2012- : Associate Researcher within the –European Grant, 7FP, Ideas, ERC Advanced Grant ”Molecular Nanotechnology For Life Science Application: QUantitative Interactomics for Diagnostics, Proteomics and QUantitative Oncology,(Quidroquo) proposal

n. 269025. P.I. Prof. G. Scoles, University of Udine. Studies focused on the application of nanotechnologies at specific biological and clinical issues.

• 2003-2013: In collaboration with Dr. A.P. Beltrami, coordination of a research team, lead by Prof. C.A. Beltrami, at the Interdipartimental Center for Regenerative Medicine, University of Udine, involved in:

- Isolation and characterization of multipotent adult stem cells from several adult human tissues.
- Use of stem cells in cardiac, hepatic and neural regenerative medicine.
- Optimization of patient-based in vitro model of diseases.
- Study of cardiac stem cell senescence in human heart failure
- Tumor initiating cells and tumor supporting cells in human ovarian, liver and brain cancer.

• 2009-2013: Principal investigator of the project: "Employment of Adipose tissue derived Multipotent Adult Stem Cells for cardiovascular regeneration", University of Udine.

• 2010: Visiting scientist at the Brigham and Women's Hospital, Boston, USA, for a project focused on cardiac stem cell senescence.

• 2003: Research Fellow at the Cardiovascular Research Institute, New York Medical College, Valhalla NY (USA). Studies on:

- the effects of hyperglycemia and oxidative stress on myocyte death and heart failure
- Identification and characterization of cardiac stem cell niches in a normal murine model.
- evaluation, by long term BrdU labeling assay, of the cardiac myocyte turnover in young healthy mouse.

PUBLICATIONS IN SCIENTIFIC JOURNALS

• Cesselli D, et al. (2013) Cardiac stem cell senescence. Methods Mol Biol 976: 81-97.

• Zeppieri M, et al. (2013) Human adipose-derived stem cells for the treatment of chemically burned rat cornea: preliminary results. Curr Eye Res 38: 451-463.

• Garrovo C, et al. (2013) In vivo tracking of murine adipose tissue-derived multipotent adult stem cells and ex vivo cross-validation. Int J Mol Imaging 2013: 426961.

• Bergamin N, et al. (2013) A human neuronal model of Niemann Pick C disease developed from stem cells isolated from patient's skin. Orphanet J Rare Dis 8: 34.

• Katare R, et al. Boosting the pentose phosphate pathway restores cardiac progenitor cell availability in diabetes. Cardiovasc Res 2012.

• Ferro F, et al. Dental pulp stem cells differentiation reveals new insights in Oct4A dynamics. PLoS One 2012; 7: e41774.

• Ferro F, et al. Acellular bone colonization and aggregate culture conditions diversely influence murine periosteum mesenchymal stem cell differentiation potential in long-term in vitro osteoinductive conditions. Tissue Eng Part A 2012; 18: 1509-19.

• Beltrami AP, Cesselli D, Beltrami CA. Stem cell senescence and regenerative paradigms. Clin Pharmacol Ther 2012; 91: 21-9.

• Amadesi S, et al. Role for substance p-based nociceptive signaling in progenitor cell activation and angiogenesis during ischemia in mice and in human subjects. Circulation 2012; 125: 1774-86, S1-19.

• Katare R, et al. Transplantation of Human Pericyte Progenitor Cells Improves the Repair of Infarcted Heart Through Activation of an Angiogenic Program Involving Micro-RNA-132. Circ Res 2011.

• Katare R, et al. Intravenous gene therapy with PIM-1 via a cardiotropic viral vector halts the progression of diabetic cardiomyopathy through promotion of prosurvival signaling. Circ Res 2011; 108: 1238-51.

• Fortini C, et al. Circulating stem cell vary with NYHA stage in heart failure patients. J Cell Mol Med 2011; 15: 1726-36.

• Ferro F, et al. Adipose tissue-derived stem cell in vitro differentiation in a three-dimensional dental bud structure. Am J Pathol 2011; 178: 2299-310.

• Cesselli D, et al. Role of tumor associated fibroblasts in human liver regeneration, cirrhosis, and cancer. Int J Hepatol 2011; 2011: 120925.

• Cesselli D, et al. Effects of age and heart failure on human cardiac stem cell function. Am J Pathol 2011; 179: 349-66.

• Beltrami AP, Cesselli D, Beltrami CA. At the stem of youth and health. Pharmacol Ther 2011; 129: 3-20.

• Kajstura J, et al. Myocyte Turnover in the Aging Human Heart. Circ Res 2010.

• Ferro F, et al. Biochemical and biophysical analysis of tissue engineered bone obtained from 3D culture of a subset of bone marrow mesenchymal stem cells. Tissue Eng Part A 2010.

• Corallini F, et al. TNF-alpha modulates the migratory response of mesenchymal stem cells to TRAIL. Cell Mol Life Sci 2010.

• Campagnolo P, et al. Human Adult Vena Saphena Contains Perivascular Progenitor Cells Endowed With Clonogenic and Proangiogenic Potential. Circulation 2010.

• Kostic I, et al. Influence of iodide excess and interferon-gamma on human primary thyroid cell proliferation, thyroglobulin secretion, and intracellular adhesion molecule-1 and human leukocyte antigen-DR expression. Thyroid 2009; 19: 283-91.

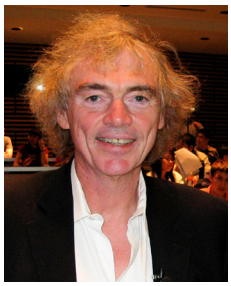
• Cesselli D, et al. Multipotent progenitor cells are present in human peripheral blood. Circ Res 2009; 104: 1225-34.

Luiz Felipe Coelho

Luiz Felipe Coelho is graduated in Biological Science at the Catholic University of Minas Gerais (2000) and has a PhD in Microbiology at the Federal University of Minas Gerais (2006). Currently he is a Professor at the Department of Microbiology and Immunology at Federal University of Alfenas, Brazil. He works in the field of microbiology, with particular emphasis on Molecular Biology and Immunology of Dengue virus applied to development of new vaccines and diagnostic tests for this Neglected disease.

Karsten Cremer

Karsten Cremer, Ph.D., is Founder and Principal of Pharma Concepts GmbH, a pharmaceutical IP consultancy located in Basel (Switzerland). He is a European patent attorney and pharmaceutical scientist with more than 20 years of professional experience in the field of drug delivery and pharmaceutics. Karsten also founded the Drug Delivery Focus Group of the International Association for Pharmaceutical Technology, for which he serves as Chairman. In previous appointments, he was CEO of Capsulution NanoScience AG, Director of Oral Drug Delivery at LTS Lohmann Therapy Systems, and Lecturer of Pharmaceutics at the University of Marburg. As an IP consultant, Karsten Cremer works with large pharmaceutical companies as well as small and medium-size drug delivery companies. His specialties include IP issues relating to pharmaceutical formulations and drug delivery technologies. In addition to drafting, filing and defending patent applications, he is experienced in developing and implementing tailor-made patent strategies, managing patent portfolios, and performing freedom-to-operate analyses, technical and IP due diligences.



Pieter R. Cullis

Professor, Department of Biochemistry and Molecular Biology, university of B.C.
Director, Nanomedicines Research Group

EDUCATION AND TRAINING

• 1964-1967: BSc, Physics, UBC
• 1964-1972: PhD, Physics, UBC
• 1973-1976: Postdoc, Biochemistry, University of Oxford, UK
• 1977: Postdoc, Biochemistry, University of Utrecht, Holland

RESEARCH AND PROFESSIONAL EXPERIENCE

- 1978 Assistant Professor, Biochemistry and Molecular Biology, UBC, Vancouver
- 1982 Assistant Professor, Biochemistry and Molecular Biology, UBC, Vancouver
- 1985 - pres. Professor, Biochemistry and Molecular Biology, UBC, Vancouver
- 1985 - 2000 Co-Founder and Chairman, Lipex Biomembranes, Vancouver
- 1986 - 1991 Co-Founder, Director and President, Canadian Liposome Company, Vancouver
- 1992 - 2008 Co-Founder and Director,Inex Pharmaceuticals Corp (now Tekmira Pharmaceuticals), Burnaby
- 1996 - 2000 Director, Synapse Technologies, Vancouver
- 2001 - 2005 Co-founder, Director and Chairman SAB, Protiva Biotherapeutics , Burnaby
- 2004 Co-founder, Scientific Director 2005-2010, Centre for Drug Research and Development
- 2009 - pres. Co-Founder and Chairman, AlCana Technologies, Vancouver
- 2010 - pres. Co-Founder and Chairman, Precision NanoSystems, Vancouver
- 2011 - pres. Co-Chair, BC Personalized Medicine Initiative, Vancouver

ACADEMIC AND PROFESSIONAL AWARDS

- 1973 - 1976 MRC Postdoctoral Fellowship, Medical Research Council
- 1977 - 1977 EMBO Postdoctoral Fellowship, European Molecular Biology Organization
- 1978 - 1982 MRC Scholar, European, Medical Research Council
- 1983 - 1989 MRC Scientist, European, Medical Research Council
- 1986 Ayerst Award, Canadian Biochemical Society
- 1987 Faculty of Medicine Distinguished Lecturer, University of British Columbia
- 1988 - 1989 UBC Killam Research Prize, University of British Columbia
- 1988 Jacob Bierly Research Prize, University of British Columbia
- 1991 Gold Medal for Health Sciences, BC Science Council
- 2000 Alec Bangham award, International Liposome Society
- 2002 Award for Innovation and Achievement, BC Biotechnology Alliance
- 2004 Fellow of the Royal Society of Canada, Royal Society of Canada
- 2005 Barre Award, University of Montreal
- 2005 UBC Alumni Award, Science & Medicine, University of British Columbia
- 2010 CSPS Leadership Award , Canadian Society for Pharmaceutical Sciences
- 2011 Webber Lifetime Achievement Award, University of British Columbia, Faculty of Medicine
- 2011 Prix Galien Canada Research Award, Prix Galien Canada
- 2012 Senior Faculty Award-Outstanding Contributions, University of British Columbia Faculty of Medicine

PROFESSIONAL INTERESTS

My research interests are focused on the roles of lipids in biological membranes and the use of lipid nanoparticle (LNP) systems to deliver drugs. I have also been extensively involved in founding companies to commercialize technologies developed, at least in part, in my UBC laboratory. In all, these efforts have led to 10 drugs that have been approved or are in clinical testing (see Table below). My interests in the functional roles of lipids concern the widespread ability of membrane lipids to adopt non-bilayer structures and the roles of such structures in processes such as membrane fusion, transport processes across bilayer lipid systems induced by ion gradients and the development of increasingly sophisticated model membrane systems. My interests in LNP drug delivery systems concern first the in vivo delivery of small molecules, particularly drugs used in cancer chemotherapy, with the aim of increasing efficacy and reducing toxicity. In this work the aim is to take advantage of the proclivity of long-circulating LNP systems to accumulate at sites of disease such as tumor sites and sites of inflammation to maximize the benefits inherent in providing local sustained release of drugs at sites of disease. A second area concerns the design of LNP systems that are able to effectively deliver their contents into the cytoplasm of target cells following i.v. administration, thus enabling the therapeutic use of genetic drugs such as siRNA, antisense oligonucleotides, mRNA and plasmids for gene therapy. Finally, a large part of my professional life has been aimed at developing potential therapeutics to the point where they are of interest to the commercial world. This led me to co-found the Centre for Drug Research and Development, which is aimed at developing, within academia, the extensive proof-of-concept data packages necessary to attract investment to preclinical drug candidates.

Kenneth Dawson

Kenneth Dawson is Director of the Centre for BioNano Interactions (CBNI). His scientific focus is to understand interaction of nanoparticles with living systems (www.cbni.eu). The Centre seeks to clarify the controlling factors for those interactions, to support applications in nanotheraputics and nanosafety. He has received several international prizes, including the 2007 Cozzarelli prize from the US National Academy of Sciences USA, as well as IBM, Packard, Canon, Sloan and Dreyfus prizes.

Professor Dawson is Director of CBNI, Chair of Physical Chemistry at UCD and co-ordinator of the European Infrastructure in the arena. He has experience in the management of large scale EU projects, including multi-sectoral cross-disciplinary research projects, and other international programs.

Prof. Dawson's professional roles include representing Ireland on the OECD and ISO working groups on standards for Nanotechnology, in DG SANCO, and in European Medicines Agency working group.. He chaired the launch of the International Alliance on NanoEHS Harmonisation (http://nanoehsalliance.org/), a new global partnership of scientists from EU, US, and Japan. He is currently Editor of Current Opinion in Colloid Science, Senior Editor of Physica, Associate Editor of Journal of Nanoparticle Research, and former President of the European Colloid and Interface Society.

Marco de Boer

Marco holds an MSc degree in chemistry/molecular biology from the University of Amsterdam, and a Ph.D. degree in molecular biology from the Vrije Universiteit Amsterdam.

Before joining to-BBB in 2010, he headed the Department Bioprocessing at the R&D center of Kerry Ingredients & Flavours in Almere. From 2000 to 2005, Marco was Director Biomolecules at Isogen Life Science, and responsible for R&D and the Biomolecules Production Facility. Before that, he has worked as research manager for Unilever Research.

SELECTED PUBLICATIONS

Marco de Boer, Corine C Visser & Pieter J Gaillard (2013). Upcycling drugs for brain-related diseases: a sustainable future for targeted drug delivery. Therapeutic Delivery, Vol 4 (4), pages 435 – 438.

Mauro Dell'Ambrogio

Mauro Dell'Ambrogio, the holder of a Doctorate in Law from the University of Zurich, held a number of public offi ces in canton Ticino from 1979 to 1999 after passing his bar exam: Judge, Chief of the Cantonal Police, Secretary-General for Education and Culture, project manager for the creation of the University of Lugano (USI), and Secretary-General of the USI. After four years heading up a group of private clinics, he was made Director of the University of Applied Sciences of Southern Switzerland (SUPSI) in 2003. He has been mayor of Giubiasco, a member of the Ticino cantonal parliament and chairman of the Ticino electricity works. He took up the post of State Secretary for Education and Research in January 2008.

Neil P. Desai

PhD

Vice President, Strategic Platforms
Abraxis Bioscience, LLC
A wholly owned subsidiary of Celgene Corp
Los Angeles, CA

Neil Desai is currently Vice President of Strategic Platforms at Abraxis Bioscience / Celgene. Prior to its acquisition by Celgene in Oct 2010, he was Sr. Vice President of Global Research and Development at Abraxis Bioscience, in Los Angeles, California, USA, where he was responsible for the company's growing product pipeline and the development of the company's intellectual property portfolio. Dr. Desai is an inventor of ABI's nanotechnology and nanoparticle-albumin bound (nabTM) drug delivery platform, was primarily responsible for the development of its nanotechnology drug, Abraxane® and the discovery of the novel targeted biological pathway utilized by nab®-drugs. This platform has been clinically proven to enhance the efficacy and safety of cytotoxic drugs through a novel targeted biological pathway and is the first protein-based nanotechnology product to be approved globally for the treatment of cancer. Prior to his positions at Abraxis, Dr. Desai was Senior Director of Biopolymer Research at VivoRx, Inc and VivoRx Pharmaceuticals, Inc. (predecessor companies of Abraxis), where he worked on the early discovery and development of Abraxane, developed novel encapsulation systems for living cells and was part of the team that performed the world's first successful encapsulated islet cell transplant in a diabetic patient.
Dr. Desai has more than 20 years of experience in the research and development of novel drug delivery systems and biocompatible polymers. He holds over 100 issued patents and peer-reviewed publications and has made over 150 presentations at scientific meetings. He has served as reviewer for several scientific journals in the area of cancer therapeutics and drug delivery. He also is an active participant in FDA and EU Nanotechnology initiatives. Dr. Desai holds an M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin, USA, and a B.S. in Chemical Engineering from the University Institute of Chemical Technology in Mumbai, India.

Marina Dobrovolskaia

Ph.D., Senior Scientist

At the NCL, Dr. Dobrovolskaia directs characterization related to a nanomaterials' interaction with components of the immune system. She monitors acute/adverse effects of nanoparticles as they relate to the immune system, both in vitro and in animal models. Dr. Dobrovolskaia is also responsible for the development, validation and performance qualification of in vitro and ex vivo assays to support preclinical characterization of nanoparticles, and for monitoring nanoparticle purity from biological contaminants such as bacteria, yeast, mold and endotoxin. Additionally, she leads structure activity relationship studies aimed at identifying the relationship between nanoparticle physicochemical properties and their interaction with macrophages, components of the blood coagulation cascade, and complement systems.
Prior to joining the NCL, Dr. Dobrovolskaia worked as a Research Scientist in a GLP laboratory at PPD Development, Inc. in Richmond, VA. She was responsible for the design, development and validation of bioanalytical ligand-binding assays to support pharmacokinetic and toxicity studies in a variety of drug development projects. She received her M.S. degree from the Kazan State University in Russia, her Ph.D. from the N.N. Blokhin Cancer Research Center of the Russian Academy of Medical Sciences in Moscow, Russia, and completed two postdoctoral trainings in immunology at the National Cancer Institute in Frederick, MD and the University of Maryland in Baltimore, MD. Her areas of expertise include cell signaling, innate immunity, immunogenicity and analytical methodology.

Yaniv Dolev

DVM, MBA
Moebius Medical Ltd..
Founder & CEO

Born and raised in Israel, Dr. Dolev earned his title as Vetrinary Surgeon at the Faculty of Veterinary Science, National University of La Plata, Argentina. He earned an M.B.A. as well from the School of Macro-Economic Studies, Buenos Aires, Argentina.
Dr. Dolev served as a manager of the European accounts for Arava Export Growers Ltd. for organic products. He continued his commercial carrier serving as Director in charge of Latin America for Maktheshim Chemical Works, a global leader in the field of plant protection Dr. Dolev designed and executed the marketing plans, managing the distributors and directed negotiations with key clients.
Dr. Dolev started his entrepreneurial endeavors founding and managing LovaDerm Ltd., a start-up in the field of dermatology. Later he founded Moebius Medical Ltd. where he still serves as CEO. The company is developing a new injectable intra-articular treatment. Dr. Dolev brought the company from it's very early steps through a fund raising rounds to a successful termination of a large clinical trial.
Dr. Dolev also assists the European Commission as an independent expert, evaluating projects in the FP7-Health program.

Elena E. Dormidontova

The Institute for Materials Science and Physics Department, University of Connecticut, 97 North Eagleville Road, Storrs, CT 06269-3136, Phone: (860)-486-3542, Email: eed@ims.uconn.edu

EDUCATION AND SCIENTIFIC DEGREES

- 1994 Ph. D in Physics and Mathematics, Physics Department, Moscow State University
- 1991 B.A., M.S. in Physics, Physics Department Moscow State University

ACADEMIC POSITIONS

- 2013- Associate Professor, Institute for Materials Science and Physics Department, University of Connecticut
- 2002- 2012 Assistant/Associate Professor, Department of Macromolecular Science and Engineering, Case Western Reserve University

• 1999-2002 Postdoctoral Associate, Dept. Chem. Eng. and Mat. Science, University of Minnesota
• 1996-1999 Postdoctoral Researcher, Dept. of Polymer Chemistry, University of Groningen
• 1994-1996 Researcher, Physics Dept., Moscow State University, Chair Polym. Crystal Physics

RESEARCH INTERESTS

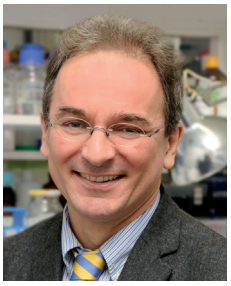
Computer simulations and analytical theory of nanoparticle targeting for drug delivery and imaging, receptor clustering, bio-recognition, self-assembly and reversible interactions in biopolymers, thermodynamics and kinetics of self-assembly and drug encapsulation.

PUBLICATION AND PRESENTATION HIGHLIGHTS

Hirsch Index - 18, 41 published peer-reviewed articles cited 913 times (citation rate 22.3)
The highest number of citations by a singly-authored paper 156

RECENT SELECTED PEER-REVIEWED PUBLICATIONS

• S. Wang; E. E. Dormidontova, "Selectivity of Ligand-Receptor Interactions between Nanoparticle and Cell Surfaces" Physics Rev. Letters, 109, 238102-4, 2012
• S. Wang; E. E. Dormidontova, "Nanoparticle Targeting using Multivalent Ligands: Computer Modeling", Soft Matter, 2011, 7, 4435-4445, 2011
• S. Wang; E. E. Dormidontova, "Nanoparticle Design Optimization for Enhanced Targeting: Monte Carlo Simulations", Biomacromolecules 11, 1785–1795, 2010
• H. Djohari, E. E. Dormidontova, "Kinetics of Nanoparticle Targeting by Dissipative Particle Dynamics Simulations", Biomacromolecules, 10, 3089–3097, 2009
• M. Hagy, S. Wang, E. E. Dormidontova, "Optimization of Functionalized Polymer Layers for Specific Targeting of Mobile Receptors on Cell Surfaces", Langmuir, 24, 13037-13047, 2008
• D. Sutton, S. Wang, N. Nasongkla, J. Gao, E.E. Dormidontova, "Doxorubicin and b-lapachone Release and Interaction with Micelles Core Materials: Experiment and Modeling", Experimental Biology and Medicine, 232, 1090-1099, 2007
• C.-C .Chen, E.E. Dormidontova, "Architectural and Structural Optimization of Protective Polymer Layer for Enhanced Targeting", Langmuir, 21, 5605-5615, 2005



Albert Duschl

University of Salzburg, Department of Molecular Biology
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+43 (662) 8044 5731
albert.duschl@sbg.ac.at
http://www.uni-salzburg.at/tapir

Born: 17. November 1957, Deggendorf/D

MAIN RESEARCH AREA

Immunology, allergy, signal transduction and gene regulation in immune cells, biochemistry, nanomaterials and the immune system, nano/bio interactions

EDUCATION AND SCIENTIFIC CAREER

Training

• 1977 - 1984 Study of Biology, Justus-Liebig-University, Gießen, D
• 14. 2. 1984 Diploma in Biology
• 1984 - 1986 Scholarship from Studienstiftung des Deutschen Volkes. Doctoral thesis.
• 11. 12. 1986 Doctorate awarded, Dr. rer. nat.
• 02. 6. 1997 Habilitation at the Medical Faculty of the University of Würzburg degree Dr. med. habil. awarded
• 26. 8. 1997 Permission for independent teaching (venia legendi), Privatdozent.

Career History

• 4/1987 - 12/1989 Research fellow, Department of Physiology and Biophysics, University of California, Irvine, USA. Scholarship from DFG.
• 1/1990 - 5/1990 Scientist at the MPI for Biochemistry, Martinsried, Germany, Scholarship from the Max-Planck-Society.
• 6/1990 - 1/2001 Assistant/group leader at the Institute for Physiological Chemistry, University of Würzburg, Germany.
• Since 1 / 2001 Full professor (chair) of Biochemistry at the Institute for Chemistry and Biochemistry, now Department of Molecular Biology, University of Salzburg, Austria.
• 2003 - 2011 Vice Rector (vice president) for Research at the University of Salzburg.

ACADEMIC ACHIEVEMENTS (Selected)

Review Activities; Memberships, Advisory Functions

• Reviewer for ACS Nano, Biointerphases, Cellular and Molecular Life Sciences, ChemBioChem, Environmental Toxicology, European Journal of Immunology, International Archives of Allergy and Immunology, Journal of Allergy and Clinical Immunology, Journal of Immunological Methods, Journal of Infectious Diseases, Life Sciences, Molecular Biosystems, Particle and Fibre Toxicology
• Reviewer for National Institutes of Health, National Institute of Allergy and Infectious Disease (NIH/NIAID), USA
• Reviewer for Deutsche Forschungsgemeinschaft (DFG)
• EURO-Nanotox-LETTER: Advisory board member
• Member of the "Collegium Internationale Allergologicum (CIA)
• Advisory expert for the European Food Safety Authority (EFSA) 2009-2012
• Advisory expert for Immunosafety at F. Hoffmann-La Roche Ltd.
• Secretary General of the Austrian Society for Allergology and Immunology (since 2012)

PUBLICATIONS

Total number of scientific publications: 87, Overall number of citations: 2381; Hirsch-index: 28, all according to Google Scholar®

GRANTS (ongoing only)

• FP7 EID HUMUNITY: Cell-Based Models of Human Mucosal Immunity with Multiple Commercial Applications, Associated partner.
• 2012-2015: FP7 CSA NanoEIS: "Nanotechnology education for industry and society", Coordinator
• 2011 - 2014: FWF Doctoral Program W1213: Immunity in Cancer and Allergy (ICA), Principal investigator
• 2011 - 2016: FP7 NanoValid, "Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials", WP leader
• 2010 - 2014: FP7 ITN NanoTOES, "Nanotechnology: Training of Experts in Safety", Coordinator

Tal Dvir

Dr. Dvir obtained a B.Sc. (2003) and a Ph.D (2008) degrees in Biotechnology Engineering from Ben-Gurion University of the Negev in Israel. His Ph.D research was supervised by Prof. Smadar Cohen and focused on cardiac tissue engineering and regeneration. Dr. Dvir continued his postdoctoral studies in the laboratory of Prof. Robert Langer in the department of Chemical Engineering at MIT. His postdoc research focused on nanotechnological strategies for engineering complex tissues. He received numerous awards for his graduate and postgraduate studies including a prestigious fellowship from the American Heart Association. On October 2011 Dr. Dvir was recruited by the Department of Biotechnology and the center for Nanotechnology at Tel Aviv University to establish the Laboratory for Tissue Engineering and Regenerative Medicine. His current research interests include nanoscale technologies for cardiac and brain tissue engineering and regeneration.

Falk Ehmann

MD, PhD, MSc

Falk Ehmann is currently working in the Scientific Support and Project Section of the European Medicines Agency (EMA) were his main responsibilities include holding the Scientific Secretariat of the Pharmacogenomics Working Party and the Innovation Task Force promoting Innovation and new methodologies in drug development and being involved in the development of policies, guidelines and the annual working program in these areas. He held various positions and responsibilities at the EMA since 2004, including Scientific Secretariat of the Vaccine Working Party and Biosimilar Working Party (BMWP).
Prior to joining the EMA Dr. Ehmann was a Public Health Researcher at the Robert Koch Institute in Berlin and Medical Intern at different University Hospitals including Bordeaux, Munich, Berlin, Geneva and Tanzania.
Falk Ehmann wrote his PhD thesis in the department for Cellular Signal Transduction at the University Hospital Hamburg-Eppendorf in the Centre of Experimental Medicine of the Institute of Biochemistry and Molecular Biology.

Rutledge Ellis-Behnke

Ph.D.

Director, Nanomedicine Translational Think Tank
Medical Faculty Mannheim of the University of Heidelberg

Augenklinik, Haus 1
Theodor-Kutzer-Ufer 1-3
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Germany
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Affiliate Professor, Dept of Neurosurgery & Brain Repair
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rutledg@mit.edu

Professor Rutledge Ellis-Behnke is the Director of the Nanomedicine Translational Think Tank at the Medical Faculty Mannheim of the University of Heidelberg in Germany. In addition, he is Affiliate Professor in the Department of Neurosurgery & Brain Repair at the University of Florida Morsani School of Medicine, and Research Affiliate in the Brain and Cognitive Sciences department at the Massachusetts Institute of Technology. His primary research interest is using nanotechnology to reconnect the disconnected parts of the brain in order to restore function. Through additional discoveries, his work in Nanomedicine has broadened to include hemostasis without clotting; preserving stem cells; preservation and restoration of vision; and immobilizing cancer stem cells.
Ellis-Behnke received his PhD from MIT in Neuroscience, BSci from Rutgers University and graduated from Harvard Business School's International Senior Manager's Program (AMP/ISMP). He is Associate Editor for the journal Nanomedicine: Nanotechnology, Biology and Medicine; an Editorial Board member for Nanomedicine & Biotherapeutic Discovery; a member of both the Executive and Scientific Advisory Boards for the Glaucoma Foundation; and a member of the Executive Board of the Asia Foundation for Cancer Research. He has more than 100 patent applications and his "Nano Neuro Knitting" and "Immediate Hemostasis" technologies have each been licensed for translation to humans.

SELECTED PAPERS

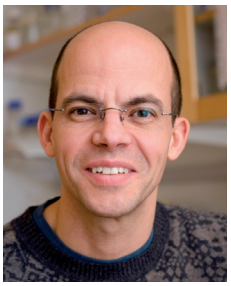
• **Ellis-Behnke RG**. "Molecular Medical Devices for Nanoneurosurgery." In: Textbook of Nanoneurosurgery, Babak Kateb and John Heiss (eds.), New York, Taylor & Francis (In press).
• Sang, YH, Liang, YX, Liu, LG, **Ellis-Behnke RG**, Wu, WT, So, KF, and Cheung, RTF. (2013) A Rat Model of Intracerebral Hemorrhage Permitting Hematoma Aspiration plus Intralesional Injection. Experimental Animals 62: 63-69.
• Jonas RA, Yuan TF, Cheung SWH, Liang YX, Jonas JB, Tay DKC and **Ellis-Behnke RG**. (2012) The spider effect: Morphological and orienting classification of microglia in response to stimuli in vivo. PLoS One 7: e30763.
• Wu KS, Tang B, Li SY, Lo ACY, Ngan AHW, Wong DSH, So KF and Ellis-Behnke RG. (2011) Micro-scale stiffness change of cornea tissues suffered from elevated intraocular pressure investigated by nanoindentation. Soft Materials DOI:10.1080/1539445X.2011.622030.
• **Ellis-Behnke RG** and Schneider GE. "Peptide Amphiphiles and Porous Biodegradable Scaffolds for Tissue Regeneration in the Brain and Spinal Cord." In: Biomedical Nanotechnology, Methods in Molecular Biology series, Sarah Hurst (vol. ed.), vol. 726, pp 259-281. New York, Springer, 2011.
• **Ellis-Behnke RG** and Jonas JB. (2011) Redefining tissue engineering for nanomedicine in ophthalmology. Acta Ophthalmol. 89: e108-14.
Liang YX, Chan KCW, Cheung SWH, Tay DKC, Wu EX and Ellis-Behnke RG. (2010) CNS regeneration after chronic injury using a self-assembled nano material and MEMRI for real-time in vivo monitoring. Nanomedicine 7: 351-359.
• **Ellis-Behnke RG**, Liang YX, Guo J, Tay DKC, Schneider GE, Teather LA, Wu W and So KF. (2009) Forever young: how to control the elongation, differentiation and proliferation of cells using nanotechnology. Cell Transplantation 18: 1047-1058.
• **Ellis-Behnke RG**, Teather LA, Schneider GE and So KF. (2007) Using nanotechnology to design potential therapies for CNS regeneration. Current Pharmaceutical Design 13: 2519-2528.
• **Ellis-Behnke RG**. (2007) Nano Neurology and the 4 Ps of CNS Regeneration: Preserve, Permit, Promote and Plasticity. Med Clin North Am. 91: 937-62.
• Guo J, Su H, Zeng Y, Liang YX, Ellis-Behnke RG, So KF and Wu W. (2007) Reknitting the injured spinal cord using self-assembling peptide nanofiber scaffold. Nanomedicine 3: 311-21.
• **Ellis-Behnke RG**, Liang YX, You SW, Tay DKC, Zhang S, So KF and Schneider GE. (2006) Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision, Proc Nat Acad Sci USA 103: 5054-5059.
• **Ellis-Behnke RG**, Liang YX, Tay DKC, Kau PWF, Schneider GE, Zhang S, Wu W and So KF. (2006) Nano hemostat solution: immediate hemostasis at the nanoscale. Nanomedicine 2: 207-15.
• Schneider GE, **Ellis-Behnke RG**, Liang YX, Kau PWF, Tay DKC and So KF. (2006) Behavioral testing and preliminary analysis of the hamster visual system. Nat Protoc.1: 1898-1905.

Noam Emanuel

Ph.D.

Dr. Emanuel has vast experience in biotechnology projects, including development of drug delivery systems and immunology. His extensive expertise includes immunotherapy, vaccines, immunodiagnostics, systemic and local drug-delivery,

and medical devices. Dr. Emanuel has a number of approved patents in the field of drug delivery and diagnostics. Dr. Emanuel is a co-founder of PolyPid and served as its CEO during the company's first three years. He received his Ph.D. degree from the Faculty of Medicine at the Hebrew University of Jerusalem.
Falk Ehmann wrote his PhD thesis in the department for Cellular Signal Transduction at the University Hospital Hamburg-Eppendorf in the Centre of Experimental Medicine of the Institute of Biochemistry and Molecular Biology.



Bengt Fadeel

Bengt Fadeel is Professor of Medical Inflammation Research at the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, and Adjunct Professor of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA. He received his M.D. and Ph.D. degrees from Karolinska Institutet. He is Head of the Division of Molecular Toxicology and Vice Chairman of the Institute of Environmental Medicine, and a Fellow of the Academy of Toxicological Sciences (ATS). Dr. Fadeel is engaged in several EU projects on nanosafety including FP7-MARINA, FP7-NANOREG, FP7-NANOSOLUTIONS, and the COST action MODENA, as well as the Future Emerging Technology (FET) Flagship Project GRAPHENE. He served as Editor of "Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health" (Elsevier, 2012). Dr. Fadeel has organized several conferences and workshops on nanosafety or nanomedicine including the 1st (2006) and 2nd (2010) Mini-Symposium on Nanotoxicology, Nobel Forum, Karolinska Institutet, and he is co-chair of the International Nanotoxicology Congress (nanotox2014.org) in Antalya, Turkey (2014). Dr. Fadeel was awarded the Environmental Medicine Prize (2011) by the Cancer and Allergy Foundation for his research on the opportunities and risks of the emerging nanotechnologies.

Andreas Falk

Andreas Falk is CEO of BioNanoNet Forschungsgesellschaft mbH and works on several projects in the thematic field of nanotechnology, nanotoxicology, nanohealth and sensor solutions (nano-health, NanoProdEx, NANOFORCE, NANoREG, SPIDIMAN). He is setting up an international hub system focusing on fostering the communication between experts in the field of nano-safety, using already existing national structures as e.g. EURO-NanoTox (European Center for Nanotoxicology) which is coordinated by him. He represents Austria, BioNanoNet and its members within several national and international working groups (NanoSafetyCluster, European Technology Platform Nanomedicine, NANOfutures, COST-Action TD1204, OECD-WPMN,etc.), always having future project ideas in his mind, and promoting collaborations amongst the scientific clientele. He holds a degree in biomedical science (1999) and business administration (2011), combining two major scientific and economic fields into one central contact point for the whole Austrian Nano-Community in the KET's biotechnology and nanotechnology.

Xavier Fernàndez Busquets

Associate Researcher, Biomolecular Interactions Team, Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona Science Park, Baldiri Reixac 10-12, E-08028 Barcelona, Spain. www.ibecbarcelona.eu.

Assistant Research Professor, Nanomalaria Group, Barcelona Centre for International Health Research (CRESIB), Rosselló 132, E-08036 Barcelona, Spain. www.cresib.cat.

Coordinator, Biomolecular Interactions Group, Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Martí i Franquès 1, E-08028 Barcelona, Spain.

Work phone: +34 93 227 5400 (ext. 4581)
e-mail: xfernandez_busquets@ub.edu

Current research: Nanobiomedicine
1. Single-molecule studies of proteoglycan and glycosaminoglycan interactions.
2. Application of nanotechnology to the study of functional amyloids.
3. Development of nanovectors for the targeted delivery of antimalarial drugs.

ACADEMIC BACKGROUND

• 1986: Graduate in Biological Sciences, area of Biochemistry. Universitat Autònoma de Barcelona, Spain.
• 1988: Dissertation for University degree, area of Enzymology/Organic Chemistry. CIBA-GEIGY AG, Basel, Switzerland / Universitat Autònoma de Barcelona.
• 1988: Master in Biochemistry and Molecular Biology. Universitat Autònoma de Barcelona.
• 1992: PhD Thesis in Biological Sciences. Universitat Autònoma de Barcelona.
• 2004: Diploma in University Teaching, Institut de Ciències de l'Educació, Universitat de Barcelona.

POSITIONS HELD

• November 2001 - November 2006: 5-year tenure track Ramón y Cajal Position. Research Center for Bioelectronics and Nanobioscience. Barcelona Science Park, Universitat de Barcelona, Spain.
• May 1999 - November 2001: Postdoctoral position. Plant Biotechnology Group. Department of Biochemistry and Molecular Biology, School of Pharmacy, Universitat de Barcelona, Spain.
• April 1993 - April 1999: Postdoctoral position. Novartis AG-Friedrich Miescher Institut, Basel, Switzerland, and Marine Biological Laboratory, Woods Hole, USA.
• October 1992 - March 1993: Postdoctoral position. Institute of Agroalimentary Research and Technology (IRTA), Cabrils, Spain.
• February 1987 - September 1992: PhD Thesis. Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Spain.
• July - October 1985 and July - December 1986: Trainee student. Zentrale Forschungslaboratorien, CIBA-GEIGY AG, Basel, Switzerland.

Peer-Reviewed Publications: 57

Conference Contributions: 112

Luisa Fiandra

PhD

PROFESSIONAL PREPARATION AND APPOINTMENTS

• 1992-1998 Graduate studies (Natural Sciences-University of Milan, Italy); specialization in Transport Physiology
• 1999-2002 PhD in Animal Biology (University of Milan, Italy)
• 2003-2010 Postdoctoral fellowship (University of Milan, Italy) on Cell Biology and Transport Physiology
• 2010-present Fixed-term researcher in "L. Sacco" University Hospital of Milan, Italy.
Manager and scientific coordinator of the project "Development of novel nanostructured materials for the diagnosis of breast cancer, inflammatory bowel disease (IBD) and modulation of antiretroviral therapy (HAART) in HIV" supported by "Assessorato alla Sanità", Regione Lombardia, and Sacco Hospital (NanoMeDia Project)

INTERNATIONAL WORKING EXPERIENCES

• 2004-2005 Research activity on separation of the different cell types from crustacean hepatopancreas and transport experiments in the isolated cells (Univ. of North Florida, USA)

SIGNIFICANT TRAINING

• 2000 Training on electrophysiological techniques applied to epithelia (Univ. of Lecce, Italy)
• 2003 School of Physiology organized by the Italian Physiology Society on "Transmembrane transport in cells and epithelia" (Univ. of Lecce, Italy)
• 2010 Theoretical and practical course on "Nanoparticles: characterization and biological interactions" (Univ. of Milano Bicocca, Italy)

SCIENTIFIC ACTIVITY

After a long experience in the field of transport physiology with a specific competence in permeability processes through intact epithelia and cellular membranes, my present field of interest is the study of nanocomplexes delivery in animal models for future applications in oncology and Intestinal Bowel Desease (IBD) management. In particular, I coordinate all in vivo and ex vivo studies of the Surgical Research Nanotechnology Laboratory (SNRL) in "L. Sacco" Hospital of Milan, aimed to define targeting, accumulation, intracellular fate and therapeutic effects of different nanoconstructs in murine breast cancer models and to determine the delivery and the anti-inflammatory activity of nanostructured tools in IBD mouse model.
Recently I also focused on the study of conjugation of nanoparticles with antiretroviral drugs, aimed to enhance their passage trhough the blood-brain barrier and maximise the antiviral action in the central nervous system in HIV treatment.

The results of my past and present research are reported in 17 peer-reviewed publications and have been presented in 40 congresses (24 national and 16 international).

Andreas Fisch

Andreas Fisch holds a position as Senior Fellow in the Pharmaceutical Development of parenteral dosage forms at Novartis Pharma AG in Basel, Switzerland. He is leading several projects in the Technology Platform Parenteral Nanomedicine.
He received his PhD in Pharmacy from Mainz University, Germany, in 1992 after completing this graduate research at the Institute of Immunology in Mainz on MCH class II restricted antigen presentation. He continued his academic career in Clinical Pharmacology at the University Hospital of Mainz on blood cell and tissue interactions.
After two years research fellowship in the Biomedical Research Center of Baxter Bioscience in Vienna, Austria, he headed the Pharmaceutical Development Center for parenteral colloids of B.Braun in Crissier, Switzerland from 1997-2007 before joining Novartis.

Heico Frima

Heico Frima obtained his Masters Degree in Applied Physics from the Technical University of Delft in 1980 and then worked in various R&D and product management functions in the semiconductor equipment industry. Since 1990 he is Programme Officer in the Directorate-General for Research & Innovation of the European Commission in Brussels in the field of micro-technology and then from 2002 as Programme Officer for nanoscience and nanotechnology. As Programme Officer he contributes to programme policy development, the organisation of research proposal evaluations, contract negotiations and follow-up of research projects that are funded by the European Commission. Presently he is responsible for research policy in the field of nanomedicine, working in the Unit 'Nano Sciences and Nano Technologies'. Heico has the Dutch nationality, is married and has two children.

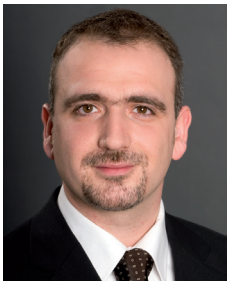
Christoph Gerber

Christoph Gerber is a titular professor and the Director for Scientific Communication of the NCCR (National Center of Competence in Research Nanoscale Science) at the Department of Physics, University of Basel, Switzerland, and a founding member of the NCCR. He was formerly a Research Staff Member in Nanoscale Science at the IBM Research Laboratory in Rueschlikon, Switzerland, and has served as a project leader in various programs of the Swiss National Science Foundation and in the European Framework 6

For the past 35 years, his research has been focused on Nanoscale Science. He is a pioneer in Scanning Probe Microscopy, and he made major contributions to the invention of the Scanning Tunneling Microscope and the Atomic Force Microscope (AFM), he is also a co-inventor of Biochemical sensors based on AFM Technology.
He is the author and co-author of more than 160 scientific papers that have appeared in peer-reviewed journals and has been cited more than 24'000 times in cross-disciplinary fields. He belongs to the one hundred worldwide most cited researchers in Physical Sciences. He has given numerous plenary and invited talks at international conferences.
His work has been recognized with multiple honorary degrees and various awards and appeared in numerous articles in daily press and TV coverage. He is a Fellow of the American Physical Society, a Fellow of the World Technology Network and a Fellow of the IOP Institute of physics UK. He serves in the advisory board of several nano institutes and has chaired and co-chaired various international conferences. His IP portfolio contains 37 patents and patent publications. His private interest range from literature (scientific and a good novel) to art and sports (he is a passionate skier and plays an acceptable round of golf).

His current interests include
• Biochemical sensors based on AFM Technology
• Chemical surface identification on the nanometer scale with AFM
• Nanomechanics, nanorobotics, and molecular devices at the ultimate limits of measurement and fabrication
• Atomic Force microscopy research on insulators
• Single Spin Magnetic Resonance Force Microscopy (MRFM)
• Self-organization and self-assembly at the nanometer scale

http://www.nccr-nano.org/nccr/contact/
http://en.wikipedia.org/wiki/Christoph_Gerber



Nicolas Gouze

Nicolas Gouze has an engineer's degree in optronics from the University Paris XI and studied Innovation Management at the University of Valenciennes (France). Since 2004 he is working with the Department Future Technologies and Europe of VDI/VDE-IT. From 2004-2010 he was involved in technology transfer and innovation issues within the Innovation Relay Centre (IRC) and Enterprise Europe Network (EEN). Since 2010 Nicolas is also involved in the management of the ETP Nanomedicine, by offering support to the members and the Executive Board and being responsible for the ETPN Secretariat activities.
The ETP Nanomedicine was established in 2005 as a joint venture of the European Commission and CEOs of large industrial companies, SMEs and academic research institutions to investigate and

advance joint activities in the area of nanotechnology in medicine. Since 2005 the ETPN published a number of strategic documents outlining the needs and roadmaps for nanomedicine research in Europe. The ETPN contributed to set up numerous European funded projects providing a first impression of the conditions for a suitable social and economic environment and the structural requirements for an efficient translation of R&D results into innovative Nanomedicines. The ETPN supports its members in coordinating their joint research efforts and improving communication amongst the members as well as towards the EC and the European Member States.

Furio Gramatica

Chair, Centre for Innovation and Technology Transfer (CITT)
and
Head, Lab. Of Nanomedicine and Clinical Biophotonics (LABION)
Fondazione Don Carlo Gnocchi ONLUS, Milano

Furio Gramatica, physicist, was born in Milano in 1964. He is the Chair of the Centre for Innovation and Technology Transfer (CITT) at Don Gnocchi Foundation – an Italian chain of 30 healthcare and research centres specialized in rehabilitation – where he also leads the Nanomedicine and Clinical Biophotonics Laboratory (LABION). For five years he served as Director of the Biomedical Technology Department "Polo Tecnologico" in the same Foundation. Formerly, he spent several years at CERN (Geneva), at Italian Institute of Nuclear Physics and in high-technology companies, with R&D management roles. Dr. Gramatica is fellow professor of physics at Milan University Medical School, member of the Executive Board of the European Technology Platform of Nanomedicine; member of the board of experts, evaluators and reviewers of the European Commission and of Wellcome Trust.

Ulf Grawunder

PhD (founder and CEO)

Ulf Grawunder is an experienced Swiss Life-Science entrepreneur with over 10 years experience in the therapeutic antibody development industry. With NBE-Therapeutics, he has recently founded his second Swiss Biotech company and is leading NBE-Therapeutics as its CEO since June 2012. Before that, Ulf Grawunder co-founded the Swiss Biotech company 4-Antibody, where since 2004 he served as founding CEO and after 2006 assumed the role of CSO. During his tenure at 4-Antibody, Ulf Grawunder raised about CHF 50 million capital for the company, secured two pharma/biotech collaborations with Boehringer Ingelheim, Germany, and Human Genome Sciences, U.S.A. and grew the company to 50 employees. Ulf Grawunder is serving on various Boards of non-profit and for-profit life-science organizations and is vice-president and Board member of the Swiss Biotech Association. In 2003 Ulf Grawunder was awarded the New Entrepreneurs in Technology and Science Award from the Gebert-Rüf-Foundation, Switzerland. Ulf Grawunder holds a PhD in Cell Biology from the University of Basel, for work on early B cell development performed at the Basel Institute for Immunology. In addition, Ulf Grawunder holds a Diploma in Technology Entrepreneurship from the Entrepreneur and Business School in St. Gallen, Switzerland (HSG=Hochschule St. Gallen).

Hans-Joachim Güntherodt

- 1963 Diploma in Physics at the Swiss Federal Institute of Technology (Eidgenössische Technische Hochschule – ETH) in Zurich
- 1967 PhD degree from ETH Zurich
- 1968 Several Visits at research centers in Jülich and Karlsruhe for neutron scattering experiments
- 1971 Visiting Professor at FU Berlin
- 1973 Habilitation at ETH Zurich
- 1974 Full Professor in physics at the Institute of Physics in Basel and department head
- 1974/1981 On sabbatical leave at the IBM Thomas J. Watson Research Center, Yorktown Hights, USA
- 1986-87 Dean of the Faculty of Natural Science of the University of Basel
- 1992 Rector designatus of the University of Basel
- 1993 Sabbatical leave at Tokyo Institute of Technology, Japan
- 1994-96 Rector of the University of Basel
- 1996 Member of the Board and Module Coordinator of the Swiss Priority Program Micro and Nanosystems Technology (MINAST)
- 1998 Director of the Swiss Priority Program Micro and Nanosystems Technology (MINAST) of the ETH Board
- 1999 Scientific Director of the Technology Oriented Program 'The Nanometer in Science and Technology of the 21st Century' 'TOP NANO 21'.
- 2001 Director of the National Center of Competence in Research (NCCR) 'Nanoscale Science – Impact on Life Sciences, Sustainability, Information and Communication Technologies'
- 2004 Member of the Executive Board of IUVSTA (International Union of Vacuum Science, Technology and Applications). Head of the division 'Nanometer Structures'.
- 2005 Honorary Doctor (Dr. h.c.) of the University of Neuchâtel
- 2006 Organization of ICN+T2006 – International Conference on Nanoscience and Technology 2006 in Basel; Alliance of Nano09 and STM06

Steffi Hansen

Dr. Steffi Hansen (HIPS/DDEL) graduated from Ernst-Moritz-Arndt University Greifswald with a diploma in pharmacy and obtained her approbation as a pharmacist (2004). After her PhD (2009) with Prof. Claus-Michael Lehr at Saarland University at the chair of Biopharmaceutics and Pharmaceutical Technology, she did a postdoctorate at the James L. Winkle College of Pharmacy, University of Cincinnati (OH). This stay was financially supported by the DAAD (German Academic Exchange Service). Since her return Dr. Hansen is a scientist at HIPS/DDEL where she runs several projects concerned with the absorption of drugs and nanoparticles across the skin.
Dr. Hansen is the author of 15 manuscripts in international peer-reviewed research journals, 3 book-chapters, co-editor of 1 Special Issue of the journal "Advanced Drug Delivery Reviews", and works as a reviewer for several pharmaceutical and dermatological journals. She is a member of several German and international pharmaceutical research societies (e.g. Controlled Release Society, American Assoc. of Pharmaceutical Scientists, Int. Assoc. for Pharmaceutical Technology, and German Pharmaceutical Society).
Dr. Hansen's research focusses on transdermal drug delivery. She develops modern drug delivery systems especially in the context of developing needle free vaccination methods to combat infectious diseases. For this purpose she uses excipients which are pharmaceutically well accepted or widely used as food additives in order to improve the transdermal bioavailability of poorly permeable drugs and biomolecules with stability issues. An important aspect in her

work is the standardization of skin explant models for the in vitro testing of such drug delivery systems and vaccination methods. A further focus of her research is the development of diffusion based in silico models of the skin which she applies to improve the understanding of transdermal absorption.

Stefan Harrer

Stefan Harrer received the B.Sc., Diploma, and Ph.D. degrees in electrical engineering and computer science with special focus on medical engineering, nanotechnology and nanoelectronics from the Technical University of Munich, Munich, Germany, in 2003, 2004, and 2008, respectively, and a Honors Masters Certificate in technology management from the Technical University of Munich and the Ludwig-Maximilians Universitaet Muenchen, Munich, in 2003. He studied technology management at the Center for Digital Technology and Management, Ludwig-Maximilians Universitaet Muenchen, the Technical University of Munich, and the University of California, Berkeley from 2001-2003. During 2005-2006 he was a Visiting Graduate Student in the Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, where he was involved in implementing new nanoimprint lithography and nanotransfer lithography techniques. In 2008 he joined the International Business Machines Corporation as a process development engineer in the Advanced Lithography Group, IBM Research Division, Albany, NY, working on nanofabrication technologies with special focus on nanoimprint lithography, immersion lithography, and direct self-assembly technology. In 2009 he became a research scientist in the Systems Biology and Functional Genomics Group at the IBM T. J. Watson Research Center, Yorktown Heights, NY, where he worked on implementing and developing novel DNA-sequencing technologies as an investigator of the National Human Genome Research Institute (NHGRI) Grant "Nanopore-based Electrical Device for DNA Sequencing". Since 2011 he is the Lead of BioNanotechnology Research and a senior Research Staff Member in the IBM Australia Research Laboratory, Melbourne, where he leads the Bio-Nanosensors Research Group. He is also a member of the Senior Lab Leadership Team and the Head of the Lab Innovation Development Team. Since February 2012 he also holds an Honorary Associate Professor position in the Department of Electrical and Electronic Engineering, Center for Neural Engineering, at the University of Melbourne, Australia. He has authored or coauthored numerous technical publications, and has over 20 patents issued or pending. Dr. Harrer was awarded the IEEE Best Student Paper Award at the 6th IEEE International Conference on Nanotechnology 2006, Cincinnati, OH, the IBM PreT0 Best Poster Presentation Award 2009 at IBMs PreT0 Conference 2009, Albany, NY, the IEEE Most Innovative Paper Award 2009 at the Annual IBM Semiconductor Technology Symposium 2009, Yorktown Heights, NY, the IBM Patent Application Invention Achievement Award 2010, the IBM First Plateau Invention Achievement Award 2010, the IBM Second Plateau Invention Achievement Award 2011, the IBM Third Plateau and Fourth Plateau Invention Achievement Awards 2012, and the IBM Fifth Plateau Invention Achievement Award 2013. He is a member of several scientific societies, including the IEEE and the New York Academy of Sciences.

Inge Herrmann

Inge Herrmann was born in 1985. She studied Chemical and Bioengineering at the ETH Zurich with a stay at the TU Delft in 2007. During her PhD studies in the group of Professor Wendelin Stark at the ETH Zurich, she developed a nanomagnet-based blood purification technology in collaboration with the University Hospital Zurich. After her PhD in biomedical engineering and postgraduate studies in clinical trials management, she worked as at the Center of Clinical Research at the University Hospital in Zurich and the University of Illinois (visiting scientist). Since spring 2013, Inge Herrmann works as a research fellow in the Stevens group at the Imperial College in London. Her research interests include therapeutic applications of magnetic nanoparticles, organ-protective small molecule with applications in sepsis and ischemia reperfusion injuries and the development of point-of-care biosensors.

Michael Horstmann

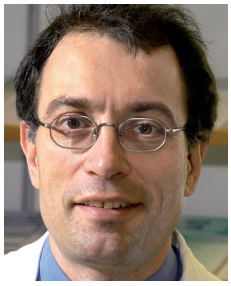
(Ph.D.)
born 9.5.55 in Essen, Germany.

- since 4/2013 Acino AG, Aesch
  Head Technology Excellence
- 2011-2013 tesa SE, Hamburg (Beiersdorf AG): Head pharma Hamburg, then CTO of the two sites
- 1987-2011 LTS Lohmann Therapie-Systeme AG: Laboratory head and finally ressort head of R&D
- 1985-1987 Beiersdorf AG, Pharma: Laboratory head, transdermal systems
- 1983-1985 University of Münster: Development of nicotine patches Pharmacology of metabolism

EDUCATION

- 1979-83 University of Münster: dissertation: nicotine implantable system
- 1974-1979 University of Marburg: pharmaceutical sciences

About 60 patents and 30 publications and 50 speaker lectures



Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. As a fellow the Massachusetts General Hospital, Harvard Medical School, worked on cardiac imaging in a joint project with the Massachusetts Institute of Technology, Cambridge.
His professional activities in Europe, the U.S., Africa and China gave him a broad insight into the needs for the medicine of the future in a variety of settings. Hunziker became involved in medical applications of Nanoscience in the late nineties and has been the pioneer physician in Nanomedicine in Switzerland since then. With improved prevention, diagnosis and cure of cardiovascular disease as his main research topic, he worked in the nanoscience fields of atomic force microscopy, nanoptics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting.
He is the co-founder and president of the European Society of Nanomedicine, co-founder of the European Foundation for Clinical Nanomedicine and co-initiator of the European Conference for Clinical Nanomedicine and is clinically active as deputy head of the Clinic for Intensive Care Medicine at the University Hospital Basel, Switzerland. In November 2008 Patrick Hunziker became professor for Cardiology and Intensive Care Medicine at the University of Basel.

Larn Hwang

Ph.D.
Chief Operating Officer
IGDRASOL
e-mail: lhwang@igdrasol.com

EDUCATION

Ph.D., Molecular Microbiology
University of Texas Southwestern Medical Center at Dallas, Texas - 1994

AWARD

Leukemia Society of North Central Texas Postdoctoral Fellowship 1994 – 1998

EMPLOYMENT

• 2011-present Chief Operating Officer, IGDRASOL, Fountain Valley, CA
IGDRASOL is the leader in next generation nanoparticle paclitaxel therapeutics.
• 2005-2011 Head of Cell Biology, Abraxis Bioscience Inc, Marina Del Rey, CA
• 2005 QC Microbiologist, Carlsbad Technology, Carlsbad, CA
• 2003-2004 Postdoctoral Scientist, Salk Institute, La Jolla, CA
• 1999 – 2002 Postdoctoral Research Scientist, Johnson & Johnson Pharmaceutical Research & Development, La Jolla, CA
• 1994 – 1999 Postdoctoral Fellow, University of Texas Southwestern Medical Center, Dallas, TX
• 1987 – 1994 Teaching and Research Assistant
University of Texas Southwestern Medical Center, Dallas, TX

PUBLICATIONS

1. Sachdeva M, Wu H, Ru P, **Hwang L**, et al. (2011) Oncogene 30:822-31
2. Desai NP, Trieu V, **Hwang LY,** et al. (2008) Anticancer Drugs. 19:899-909.
3. **Hwang, L.-Y.**, et al. (2001). Immuno. Res. 24, 245-272
4. Simmons, A. D., M.M. Musy, C. S. Lopes, **L.-Y. Hwang**, et al. (1999) Hum. Mol. Genet., 8, 2155-2164.
5. Ayi, T.-C., J. T. Tsan, **L.-Y. Hwang**, et al. (1998) Oncogene 16:2143-2148
6. Baer, R., **L.-Y. Hwang**, et al. (1997) Chromosomal Translocations and Oncogenic Transcription Factors, F.J. Rauscher III and P.K. Vogt, Editors. Springer P.55-65
7. Tsan, J. T., Z. Wang, Y. Jin, **L.-Y. Hwang**, et al. (1997) In The Yeast Two-hybrid System, P. L. Bartel, & S. Fields, Editors. Oxford University Press: Oxford. P. 217-232
8. Wu, L.C., Z.W. Wang, J. T. Tsan, M .A. Spillman, A. Phung, X. L. Xu, M.-C. W. Yang, **L.-Y. Hwang**, et al. (1996) Nature Genetics 14:430-440
9. **Hwang, L.-Y.**, et al. (1995) Curr Opin in Immuno 7:659-664
10. Xia, Y., **L.-Y. Hwang**, et al. (1994) Oncogene 9:1437-46.
11. **Hwang, L.-Y.**, et al. (1993) Oncogene 8: 3043-6
12. Cheng, J.-T., H.-L. Hsu, **L.-Y. Hwang**, et al. (1993) Oncogene 8: 677-83
13. Sanz, I., **L.-Y. Hwang**, et al. (1988) Ann. N. Y. Aca. Sci. 546:133-42

PATENTS

1. US20090220589 A1 TREATMENT, DIAGNOSTIC, AND METHOD FOR DISCOVERING ANTAGONIST USING SPARC SPECIFIC MIRNAS. V Trieu, L Hwang, N Desai
2. WO2009137660 A3 ENHANCEMENT OF DRUG THERAPY BY miRNA. V Trieu, L Hwang, N Desai
3. WO2009111375 A2 TREATMENT, DIAGNOSTIC, AND METHOD FOR DISCOVERING ANTAGONIST USING SPARC SPECIFIC MIRNAS. V Trieu, L Hwang, N Desai
4. US20090280167 A1 ENHANCEMENT OF DRUG THERAPY BY MIRNA. V Trieu, L Hwang, N Desai
5. US20120183538 A1 SPARC ANTISENSE COMPOSITIONS AND USES THEREOF. V Trieu, L Hwang, N Desai
6. WO2011060040 A1 DIAGNOSTIC AND THERAPEUTIC APPLICATIONS OF THE U6 snRNA. V Trieu, L Hwang, N Desai
7. WO2011068869 17-AAG POTENTIATING MICRORNAS. V Trieu, L Hwang, N Desai
8. WO2011130464 A1 TRANSLATIONAL REGULATION OF SPARC BY THE MICRORNAS MIR29A, B AND C. V Trieu, L Hwang, N Desai
9. WO2011068869 A1 MICRORNAS THAT CHEMOSENSITIZE THE APOPTOTIC EFFECT OF 17-AAG. V Trieu, L Hwang, N Desai
10. EP2257626 A2 TREATMENT, DIAGNOSTIC, AND METHOD FOR DISCOVERING ANTAGONIST USING SPARC SPECIFIC MIRNAS. V Trieu, L Hwang, N Desai
11. EP2288704 A2 ENHANCEMENT OF DRUG THERAPY BY MIRNA. V Trieu, L Hwang, N Desai

Tore-Geir Iversen

Tore-Geir Iversen is a senior researcher and Project Group Leader at the Centre for Cancer Biomedicine, The Norwegian Radium Hospital in Oslo, Norway. He received his PhD at the Norwegian University of Science and Technology (NTNU), Trondheim in 1995, at that time studying microbial genetics. He joined the group of professor Kisrten Sandvig in 1997, a group that for many years has contributed significantly to our present knowledge about endocytosis and intracellular transport focusing on different protein toxins as tools. In 2006 Iversen turned his focus into studying how nanoparticles are endocytosed and transported in cells. His group was the first to demonstrate that accumulation of nanoparticles within endosomes could induce changes in the normal intracellular transport of the cell. Current research interests also include more applied biological studies about nanoparticles and the criteria required for their clinical use in therapy and imaging.

MAIN CONTRIBUTIONS TO THE FIELD OF NANOPARTICLE RESEARCH

• Tekle, C., B.van Deurs, K.Sandvig, and **T.G.Iversen**. 2008. Cellular Trafficking of Quantum Dot-Ligand Bioconjugates and Their Induction of Changes in Normal Routing of Unconjugated Ligands. *Nano Letters* 8:1858-1865.
• **Iversen, T.G.**, T.Skotland, and K.Sandvig. 2011. Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. *Nano Today* 6:176-185.
• Skotland, T., **T.G.Iversen**, and K.Sandvig. 2010. New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. *Nanomedicine* 6:730-737.

Varvara Karagkiozaki

Nanomedicine Group, LTFN Lab, Aristotle University of Thessaloniki, Greece
E-mail: vakaragk@physics.auth.gr

Dr. Karagkiozaki Varvara is a Specialist Cardiologist that holds a BSc in medicine from the Medical School, Aristotle University of Thessaloniki, Greece (AUTh), an MSc in Nanosciences & Nanotechnologies (N&N), and a PhD in Nanomedicine. Her career is mostly devoted to nanomedicine research in cardiovascular field and especially in atherosclerosis and thrombosis diagnosis and treatment.
She is the Head of Nanomedicine Group of LTFN (Lab of Thin Films - Nanosystems & Nanometrology, AUTh, http://www.ltfn.gr) and a member of European, American, and International Nanomedicine societies. She is also a member of the Scientific Board of North Greek Society of Atherosclerosis. She received a scholarship from the North Greek Society of Atherosclerosis and research awards from Greek Cardiology and Atherosclerosis Societies. She is the Coordinator and Principal Investigator in a R&D Project funded by

European Commission (EC) & Greek Ministry of Education, Lifelong Learning and Religious Affairs entitled 'NanoCardio: Nanomedicine for Advanced, Bio-Active/-Mimetic Materials for Cardiovascular Implants'. She is the co-editor of a book entitled 'Horizons in Clinical Nanomedicine' that will be published from Pan Stanford Publishing in June 2013.

She published several academic research papers in peer reviewed journals and chapters on Nano-cardiology and Nanomedicine issues. She gave lectures at Nanomedicine Summer Schools (ISSON9, ISSON10 and ISSON11) and at N&N Postgraduate Program of AUTH, during the period of 2010-2013. Finally, she is the Chair of the Organizing Committee of the Nanomedicine Workshop and a member of the Organizing Committee of the Bioelectronics Workshop during the International Conferences on Nanosciences & Nanotechnologies (N&N) (http://www.nanotexnology.com).

Maria Kavallaris

Tel: +61 2 9385 2151
Mob: +61 4084780855
Email: mkavallaris@ccia.unsw.edu.au
Qualifications: BAppSc (UTS), PhD (UNSW)

CURRENT POSITION(S)

(1) Professor and Head, Tumour Biology & Targeting Program, Children's Cancer Institute Australia, Lowy Cancer Research Centre, University of New South Wales (UNSW); (2) NHMRC Senior Research Fellow; (3) Co-Director, Australian Centre for Nanomedicine. UNSW.

PREVIOUS POSITIONS

• 2006 – 2010 Associate Professor (Conjoint), Faculty of Medicine, UNSW
• 2007 President, Australian Society for Medical Research (ASMR) (Honorary position)
• 2007 Invited visiting Professor, University of Marseille, F
2005 – 2006 Presiden-Elect and Honorary Treasurer, ASMR (Honorary position)
• 2004 – 2005 Executive Director & Honorary Secretary, ASMR (Honorary position)
• 2003 – 2007 Group Leader, Pharmacoproteomics Group, CCIA
• 2001 – 2006 Senior Lecturer (Conjoint), Faculty of Medicine, UNSW 1998 – 2000 Project Leader, Experimental Therapeutics Program, CCIA
• 1998 – 2000 Lecturer (Conjoint), Faculty of Medicine, UNSW
• 1996 – 1997 WHO International Agency for Research on Cancer (IARC) Research Fellow, Albert Einstein College of Medicine, NY, USA
• 1994 – 1995 Senior Research Officer, CCIA

PRIZES, AWARDS & HONOURS (selected)

• 2013 Invited speaker, The Cytoskeleton in Tissue Repair and Diseases, Switzerland (Sept)
• 2013 Invited speaker, Workshop on Chemistry and Biology of Microtubules and Microtubule Interacting Agents, Beijing China (Sept)
• 2013 Keynote Speaker, International Science School, University of Sydney (July)
• 2013 Invited speaker, CLINAM (Clinical Nanomedicine), Switzerland (June)
•2013 Colloquium speaker, University of California Santa Barbara USA (April)
• 2012 Plenary Speaker, Nanotechnology, Medicine & Dentistry, Mexico
• 2012 Plenary Speaker, International Nanomedicine Conference, Sydney
• 2011 Invited Speaker, Australasian Gene Therapy Society, Melbourne
• 2011 Invited Speaker, COMBIO, Cairns
• 2009 Invited Member, Bristol Myers Squibb Advisory Board, Boston
• 2009 Awarded a MHMRC Senior Research Fellowship (5 years)

SERVICE TO DISCIPLINE (selected)

• 2010-13 Member, National Health and Medical Research Council Assigners Academy
• 2010-13 co-Chair, International Nanomedicine Conference, Sydney
• 2010- Senior Editor, American Association for Cancer Research
• 2010- Editorial Advisory Board Member, Open Proteomics Journal
• 2009 Member, NHMRC Grant Review Panel
• 2008- Member of the Board, Australian Institute for Policy and Science
• 2008 Fellowship–Panel–Member, National Health and Medical Research Council Training Awards
• 2008 Committee Member, Medical Research Support Program, NSW Government
• 2007 President, Australian Society for Medical Research (1 year appointment)
• 2005-6 Member, NHMRC Grant Review Panel
• 2004 Co-Chair, Program Committee, Small-Molecules Section, Annual Meeting of the American Association for Cancer Research
• 2004 Convenor, 2nd Australian Health & Medical Research Congress

PATENTS

Kavallaris M, Gan PP, McCarroll JA (2007). Modulation of β-tubulin expression in tumour cells. Patent family: PCT/AU2008/000298 – WO08106730 (national patents/patent applications in AU, US, JP, IL, CA, EP, SG). Commercial Licence Agreement held with Target Discovery Inc (executed on 21 December 2010) and Benitec Biopharma Pty Ltd.

PUBLICATIONS (selected)

1. Boyer C , Teo J , Phillips P , Erlich RB , Sagnella SM , Sharbeen G , Dwarte T, Duong HTT , Goldstein D , Davis TP* , **Kavallaris M*** , McCarroll JA*. Effective Delivery of siRNA into Cancer Cells and Tumors Using Well-Defined Biodegradable Cationic Star Polymers. *Molecular Pharmaceutics* (In press April 23, 2013) *equal senior authors
2. Byrne FL, Yang L, Phillips PA, Hansford LM, Fletcher JI, Ormandy CJ, McCarroll JA*, **Kavallaris–M***. RNAi-mediated stathmin suppression reduces lung metastasis in an orthotopic neuroblastoma mouse model. *Oncogene* Epub 11 Feb, 2013 *equal senior authors
3. Kow SC, McCarroll J, Valade D, Boyer C, Dwarte T, Davis TP, **Kavallaris M**, Bulmus V. Dicer-labile PEG conjugates for siRNA delivery. *Biomacromolecules*. 12:4301-10, 2011.
4. McCarroll JA, Gan PP, Liu M, **Kavallaris M**. βIII-Tubulin is a multifunctional protein involved in drug sensitivity and tumorigenesis in non-small cell lung cancer. *Cancer-Research*. 70(12):4995-5003, 2010
5. Po'uha ST, Shum MSY, Goebel A, Bernard O, **Kavallaris M**. LIM-kinase 2, a regulator of actin dynamics, is involved in mitotic spindle integrity and sensitivity to microtubule-destabilising drug. *Oncogene*. 29:597-607, 2010
6. **Kavallaris M**; Microtubules and resistance to tubulin-binding agents; Nature-Reviews-Cancer, 10: 194-204, 2010
7. Gan PP, Pasquier E, **Kavallaris M.** Class III β-tubulin mediates sensitivity to chemotherapeutic drugs in non-small cell lung cancer. Cancer-Research. 67:9356–9363, 2007
8. Chan Y, Bulmus V, Hadi Zareie M, Byrne FL, Barner L, **Kavallaris M**. Acid-Cleavable Polymeric Core- Shell Particles for Delivery of Hydrophobic Drugs. *Journal of Controlled Release*. 115:197–207, 2006
9. Verrills NM, Po'uha ST, Liu MLM, Liaw TYE, Larsen MR, Ivery MT, Marshall GM, Gunning PW, **Kavallaris M**. Alterations in γ-Actin and Tubulin-Targeted Drug Resistance in Childhood Leukemia. *Journal of the National Cancer Institut*e. 98:1363-74, 2006
10. Verrills NM, Flemming C, Liu M, Ivery MT, Cobon GS, Norris MD, Haber M, **Kavallaris M**. Microtubule Alterations and Mutations induced by DesoxyepothiloneB: Implications for Drug-Target Interactions. *Chemistry & Biology*. 10:597-607, 2003
11. **Kavallaris M**, Tait AS, Walsh BJ, He L, Horwitz SB, Norris MD, Haber M. Multiple microtubule alterations are associated with Vinca alkaloid resistance in human leukemia cells. *Cancer Research 61*: 5803-5809, 2001
12. Kavallaris–M, Kuo DYS, Burkhart CA, Regl DL, Norris MD, Haber M. Horwitz SB. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific {beta}-tubulin isotypes. *Journal of Clinical Investigation*. 100:1282–1293, 1997

Wolfgang Kemmner

PhD

Since 1995, Dr. Kemmner is heading the Research Group for Molecular Oncology at the Experimental and Clinical Research Center (ECRC), Berlin, Germany. Besides, he is head of the Tumorbank of Charité Comprehensive Cancer Center. Before, he was a working group leader at the Institute of Biochemistry, University Heidelberg, where he also received his venia legendi for Biochemistry in 1995. Dr. Kemmner is a member of the editorial board of the journal "Nano Life", member of the SmartHEALTH steering committee for Integrated Biodiagnostic Systems for Healthcare (FP6) and a member of the ProGERD (Progression in Gastroesophageal Reflux Disease) steering committee, reviewer for CANCER Research UK and the Association for International Cancer Research. He has published about 60 papers in international peer reviewed journals and is the holder of two international patents. The focus of Dr. Kemmner's work is translational research on human carcinomas. Current projects encompass the search for genes for the early detection of breast and colorectal carcinomas and the development of a molecular imaging procedure using Protoporphyrin-IX. Dr. Kemmners' work on the use of Protoporphyrin-IX is funded by a national funding agency and fundings of the European Community. The mission of the Experimental and Clinical Research Center (ECRC) is to expand and strengthen interdisciplinary activities between basic and clinical researchers and to shorten the pathway from discovery to clinical applications. As a common structure established by the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine the ECRC offers excellent opportunities for translational research and unique conditions for patient-oriented research and clinical studies in a research-driven environment.

Stefan Kölker

Consultant in Pediatric Metabolic Medicine

Prof. Dr. Stefan KÖLKER, MD, is Head of the Division of Inherited Metabolic Diseases at the University Children's Hospital in Heidelberg, Germany. He graduated in Medicine (1998) at the University of Marburg, Germany, and further qualified as Paediatrician in 2006. In 2007, he was certified for Pediatric Metabolic Medicine by the European Union of Medicial Specialists (UEMS) and since then has worked as a Consultant for Paediatric Metabolic Medicine at the University Children's Hospital Heidelberg. He is coordinator of multi-centre studies on intoxication type metabolic diseases (focusing on organic acidurias and urea cycle disorders) chairman of an international guideline group, organizer and teacher at the European courses for trainees in Pediatric Metabolic Medicine, and organizer of previous international workshops on IEM. In addition to his clinical and diagnostic expertise, he has a long-standing experience in basic research focusing on organic acidurias, energy metabolism, and the blood-brain barrier. He receives grants from the European Union, German Research Community (DFG), Ministry of Education and Science (BMBF), and private sponsors. He has received prestigious awards such as the Horst Bickel Award for translational studies on glutaric aciduria type I in 2007. He has published more than 90 original scientific articles. He is member of various national and international societies for IEM. Since 2011, he is coordinator of the "European registry and network for intoxication type diseases (E-IMD)" focusing on patients with organic acidurias and urea cycle defects.

POSITIONS AND HONORS

Professional Career

- 1998-2005 Advanced training in Pediatrics, University Children's Hospitals Marburg (1998-1999) and Heidelberg (2002-2005), D
- 2006 Board certification by the German Society of Pediatrics
- 2006-2007 Advanced training in Pediatric Metabolic Medicine according to the SSIEM syllabus
- 2007 European Certificate of Pediatric Metabolic Medicine (European Academy of Pediatrics)
- Since 2007 Consultant for Pediatric Metabolic Medicine
- Since 2010 Head, Division of Inherited Metabolic Diseases, University Children's Hospital Heidelberg, Germany

Scientific Career

- 1998 MD thesis
- 1999-2001 Research fellow (DFG), Universities of Marburg and Heidelberg
- 2002-2006 Head, Study group on "Cerebral organic acidurias and energy metabolism"
- Since 2003 Coordinator, Observational clinical studies on glutaric aciduria type I and other organic acidurias
- Since 2003 Coordinator, International guideline group for glutaric aciduria type I
- Dec. 2006 Postdoctoral lecturer qualification (Habilitation).
- Since 2007 Scientific Coordinator, Division of Inherited Metabolic Diseases, Heidelberg
- Since 2009 Leader, Study group of the Interdisciplinary Centre for Neurosciences, Heidelberg

Since 2011 Coordinator, European consortium "E-IMD" (www.e-imd.org)

- June 17, 2011 Appointment as Assistant Professor ("außerplanmäßiger Professor")

AWARDS

- May 24, 1999 SSIEM traveler scholarship
- April 4, 2002 Young scientists' award of the Gesellschaft für Neuropädiatrie e.V. (Society for Neuropediatrics)
- Nov.15, 2007 Horst Bickel Award

SELECTED PEER-REVIEWED PUBLICATIONS

1. Cullup T, Kho AL, Dionisi-Vici C, Brandmeier B, Smith F, Urry Z, Simpson M, Yau M, Bertini E, McClelland VM, Al-Owain M, **Kölker S**, Koerner C, Hoffmann G, Wijburg F, ten Hodt AE, Rogers C, Manchester D, Miyata R, Hayashi S, Said E, Soler D, Kroisel PM, Windpassinger C, Filloux FM, Al-Kaabi S, Hertecant J, Del Campo M, Buk S, Bodi I, Goebel H, Sewry CA, Abs S, Mohammed S, Josifova D, Gautel M, Jungbluth H (2013) Recessive mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy. Nat Genet; 45: 83-87.
2. Danhauser K, Sauer SW, Haack TB, Wieland T, Staufner C, Graf E, Zschocke J, Strom TM, Traub T, Okun JG, Meitinger T, Hoffmann GF, Prokisch H, **Kölker S** (2012) DHTKD1 mutations cause 2-amino-/2-oxoadipic aciduria. Am J Hum Genet; 91. 1082-1087.
3. Sauer SW, Opp S, Hoffmann GF, Koeller DM, Okun JG, **Kölker S.** Therapeutic modulation of cerebral L-lysine transport and metabolism in glutaric aciduria type I. Brain; 134: 157-170.
4. Heringer J, Boy NPS, Ensenauer R, Assmann B, Zschocke J, Harting I, Lücke T, Maier EM, Mühlhausen C, Haege G, Hoffmann GF, Burgard P, **Kölker S**. Use of guidelines improves the neurological outcome in glutaric aciduria type I. Ann Neurol; 68: 743-752.
5. Galy B, Ferring-Appel D, Sauer SW, Kaden S, Lyoumi S, Puy H, **Kölker S**, Gröne HJ, Hentze M (2010) Iron regulatory proteins secure mitochondrial iron sufficiency and function. Cell Metabol; 4: 194-201
6. Sauer SW, Opp S, Mahringer A, Kaminski MM, Thiel C, Okun JG, Fricker G, Morath MA, **Kölker S** (2010) Glutaric aciduria type I and methylmalonic aciduria: simulation of cerebral import and export of accumulating neurotoxic dicarboxylic acids in in vitro models of the blood-brain barrier and the choroid plexus. BBA Molecular Basis of Disease; 1802:552-560.
7. Rauschenberger K, Schöler K, Sass JO, Sauer SW, Djuric Z, Rumig C, Wolf NI, Okun JG, **Kölker S**, Schwarz H, Fischer C, Grziwa B,

Runz H, Nümann A, Shafqat N, Kavanagh KL, Hämmerling G, Wanders RJA, Shield JHP, Wendel U, Stern D, Nawroth PP, Hoffmann GF, Bartram CR, Arnold B, Bierhaus A, Oppermann U, Steinbeisser H, Zschocke J (2010) A non-enzymatic function of 17β-hydroxysteroid dehydrogenase type 10 is required for mitochondrial integrity and cell survival. Embo Molecular Medicine; 2: 51-62.

8. Harting I, Neumaier-Probst E, Seitz A, Maier EM, Assmann B, Baric I, Troncoso M, Mühlhausen C, Zschocke J, Boy NPS, Hoffmann GF, Garbade SF, **Kölker S** (2009) Dynamic changes of striatal and extrastriatal abnormalities in glutaric aciduria type I. Brain; 132: 1764-1782

Gerben A. Koning

http://www.linkedin.com/in/gerbenkoning
http://www.surgical-oncology.nl/

Gerben A. Koning is Assistant Professor at the Erasmus Medical Center, Rotterdam, The Netherlands, Section Surgical Oncology, Department of Surgery and leader of the Innovative Targeting group. Through various grants he has established an international research team working on targeted nanomedicine with the aim to improve tumor therapy using combination therapies (e.g. hyperthermia) and nanomedicine to enhance delivery of drugs to solid tumors. He is co-founder and head of the Nanomedicine Innovation Center Erasmus MC (NICE), a platform for innovation, development and application of nanotools for basic, translational and clinical research within Erasmus MC. Based on one of his inventions Ceronco Biosciences was established in 2011, focusing on tumor cell membrane modulation to enhance drug uptake. He has a broad national and international network in targeted nanomedicine and molecular imaging in which he collaborates with multiple universities, research institutes and companies. He has over 80 original publications, book chapters and reviews, and is inventor in a patent. He has been invited speaker at many international meetings. At present he is board member of the European Society for Hyperthermic Oncology (ESHO) and management committee member of European COST Action TD1004; "Theranostics imaging and therapy" and therein leader of Workgroup 4 on stimuli-responsive nanocarriers. Next to that, he acts as referee for several peer-reviewed journals on targeted nanomedicine and oncology, and various granting agencies and is member of various national and international societies on drug delivery, imaging and cancer.

SELECTED PUBLICATIONS

• Cordeiro Pedrosa LR, van Hell A, Süss R, van Blitterswijk W, Seynhaeve ALB, van Cappellen WA, Eggermont AMM, ten Hagen TLM, Verheij M and **Koning GA**. Improving intracellular doxorubicin delivery through nanoliposomes equipped with selective tumor cell membrane permeabilizing short-chain sphingolipids. Pharm Res. In press 2013.

• Li L, ten Hagen TLM, Hossann M, Suss R, van Rhoon GC, Eggermont AMM, Haemmerich D, **Koning GA**. Mild hyperthermia triggered doxorubicin release from optimized stealth thermosensitive liposomes improves intratumoral drug delivery and efficacy. J. Controlled Release. 2013 Mar 21;168(2):142-150.

• Li L, ten Hagen TLM, Bolkestein M, Gasselhuber A, Yatvin J, van Rhoon GC, Eggermont AMM, Haemmerich D, **Koning GA**. Improved intratumoral nanoparticle extravasation and penetration by mild hyperthermia. J. Controlled Release. 2013, 167(2):130-137

• Dicheva BM, Hagen TL, Li L, Schipper D, Seynhaeve AL, Rhoon GC, Eggermont AM, Lindner LH, **Koning GA**. Cationic Thermosensitive Liposomes: A Novel Dual Targeted Heat-Triggered Drug Delivery Approach for Endothelial and Tumor Cells. Nano Lett. 2012, in press.

• Manzoor AA, Lindner LH, Landon CD, Park JY, Simnick AJ, Dreher MR, Das S, Hanna G, Park W, Chilkoti A, **Koning GA**, Ten Hagen TL, Needham D, Dewhirst MW. Overcoming limitations in nanoparticle drug delivery: triggered, intravascular release to improve drug penetration into tumors. Cancer Res. 2012 Nov 1;72(21):5566-5575

• Guenoun J, **Koning GA**, Doeswijk G, Bosman L, Wielopolski PA, Krestin GP, Bernsen MR. Cationic Gd-DTPA liposomes for highly efficient labeling of mesenchymal stem cells and cell tracking with MRI. Cell Transplant. Cell Transplant. 21(1):191-205, 2012

van Lummel M, van Blitterswijk WJ, Vink SR, Veldman RJ, van der Valk MA, Schipper D, Dicheva BM, Eggermont AMM, ten Hagen TLM, Verheij M, **Koning GA**. Short-chain glucosylceramide strongly improves liposomal doxorubicin uptake and anti-tumor efficacy. FASEB J. 25(1): 280-289, 2011

• **Koning GA**, Li L, ten Hagen TLM, Thermosensitive liposomes for delivery of cancer therapeutics. Therapeutic Delivery 1(5): 707-711, 2010.

Kaijzel EL, van Heijningen PM, Wielopolski PA, Vermeij M,**Koning GA**, van Cappellen WA, Que I, Chan A, Dijkstra J, Ramnath NWM, Hawinkels LJAC, Bernsen MR, Löwik CWGM and Essers J. Multimodality Imaging reveals a gradual increase in Matrix Metalloproteinase Activity at Aneurysmal Lesions in Live Fibulin-4 Mice. Circulation, 3(5):567-77, 2010.

• Djanashvili K, ten Hagen TLM, Blangé R, Schipper D, Peters JA, **Koning GA**. Development of a liposomal delivery system for temperature-triggered release of a tumor targeting agent, Ln(III)-DOTA-phenylboronate. Bioorganic & Medicinal Chemistry, in press, 2010.

• **Koning GA**, Eggermont AMM, Lindner LH, ten Hagen TLM. Hyperthermia and thermosensitive liposomes for improved delivery of chemotherapeutic drugs to solid tumors. Pharm Res, Aug;27:1750-4, 2010.

• Li L, Ten Hagen TL, Schipper D, Wijnberg TM, van Rhoon GC, Eggermont AM, Lindner LH, **Koning GA**. Triggered content release from optimized stealth thermosensitive liposomes using mild hyperthermia. J Control Release., 143:274, 2010.

• **Koning GA** and Krijger GC. Targeted lipid-based nanocarriers for image-guided drug delivery. Anti-cancer agents in medicinal chemistry 7:425, 2007

Kostas Kostarelos

Chair of Nanomedicine

School of Medicine and National Graphene Institute, University of Manchester,
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website: http://www.nanomedicinelab.org

Kostas has recently been appointed Professor of Nanomedicine at the School of Medicine and to lead biomedical research and applications of the National Graphene Institute at the University of Manchester. He has been invited Fellow of the Royal Society of Medicine (FRSM), Fellow of the Institute of Nanotechnology (FIoN) and Fellow of the Royal Society of Arts (FRSA) all in the United Kingdom. In 2010 he was awarded the Japanese Society for the Promotion of Science (JSPS) Professorial Fellowship with the National Institute of Advanced Industrial Science and Technology (AIST) in Tsukuba, Japan. Kostas obtained his PhD from the Department of Chemical Engineering, Imperial College London. Previously he was at the faculty at Cornell University Weill Medical College, NY, USA and Imperial College London, UK. He held the Chair of Nanomedicine and was Head of the Centre for Drug Delivery Research at UCL School of Pharmacy, University College London for the last ten years.

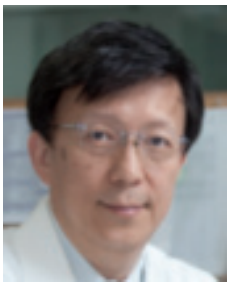
Silke Krol

Silke Krol was born in Gronau (Germany) and received her degree in Chemistry and Biochemistry at the University of Muenster, Germany. Her aim of study for the Ph.D. was the "Biophysical characterization of the surfactant proteins B and C in phospholipid monolayers". Then she studied at the University of Genoa (Italy) as fellow in a EU project in the 5th framework "Nanocapsules with functionalized surfaces". Here she studied the encapsulation of liv-

ing cells (yeast, fungi etc.) and their possible use as biosensor. In a second EU project "Development of a Bioartificial Pancreas for Type I Diabetes Therapy" she focused her interest on encapsulation of pancreatic islets in order to improve long-term survival and stability. Then she worked for three years in CBM (Cluster in Biomedicine), working as head of the NanoBioMed lab @ LANA3DA (laboratory for Nanodiagnostics, drug delivery and Analysis).
Actually she is with Fondazione IRCCS Neurologic Institute "Carlo Besta" in Milan as Principal Investigator and assistant to the director. Her major research interests is the design, development and characterization of nanoparticles as multifunctional drug delivery or as selective drugs for different diseases such as diabetes, cancer, cardiovascular disease, or antiviral drug. Additionally she studies the nanoparticle biodistribution and delivery to the brain and their passage of the blood brain barrier. She continues also her works about multilayer nanocoated pancreatic islets as immune protected transplants for diabetes therapy.
She is lecturing infrequently as contract professor for "Nanomedicine" at the University of Udine and as guest lecturer for "Nantoxicology" in Turin. She is adjunct faculty member of the Pakistan Institute for engineering and applied sciences (PIEAS) in Islamabad. In 2009 she worked as a expert consultant for the United Nations. She is member of the advisory board of "Euro-Nanotox-Letters" and of the CLINAM-Foundation. She serves as external expert reviewer for National projects in France, Italy, and Greece. She was announced as project technical advisor for 3 EU projects.

Twan Lammers

Twan Lammers studied Pharmacy at the University of Utrecht. He obtained a DSc degree in Radiation Oncology from Heidelberg University in 2008, and a PhD degree in Pharmaceutics from Utrecht University in 2009. From 2007 until 2010, he worked as a Postdoctoral Fellow at the Department of Pharmaceutics at Utrecht University. Since 2009, he has been appointed as a Group Leader at the Department of Experimental Molecular Imaging at the University Clinic and the Helmholtz Center for Biomedical Engineering at RWTH Aachen University. In 2012, he was awarded an ERC starting grant (NeoNaNo), and took up a part-time position as an Assistant Professor at the Department of Targeted Therapeutics at the University of Twente. He has published approximately 80 research articles and reviews, and is an Editorial Board Member of the American Journal of Nuclear Medicine and Molecular Imaging, Clinical and Translational Imaging, the Journal of Controlled Release, and Theranostics. His primary research interests include drug targeting to tumors, image-guided drug delivery and tumor-targeted combination therapies.



Dong Soo Lee

M.D.Ph.D.

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CURRENT APPOINTMENT

- President of Korean Society for Nanomedicine (2012)
- President of PET Association of Korea (since 2008 April)
- Professor and Chairman, Department of Nuclear Medicine, Seoul National University College of Medicine; Interdisciplinary Programs for Cognitive and Brain Science, Seoul National University; Interdisciplinary Program of Radiation-Applied Life Sciences
- Professor, WCU Department of Molecular Medicine and Biopharmaceutical Sciences, Seoul National University
- Past-President of Korean Society of Nuclear Medicine
- Past-President of Korean Society of Human Brain Mapping
- Past-Vice-President of Korean Society of Cognitive Science
- Director, Innovation Cluster of Advanced Medical Imaging, (2005-11)

ACADEMIC DEGREE

M.D. 1982 Seoul National University
Ph.D. 1990 Seoul National University

Field of specialization:
Nuclear Medicine (Neurology and Cardiology) and Molecular Imaging.

SHORT SCIENTIFIC BIOGRAPHY

- Published 279 articles in SCI journal (1999-2012).
- Received Daiichi, Abbott, KOFST, SNUH, IBA, Pfizer, SNU Alumni Academic Awards in 1996, 1999, 2001, 2001, 2002, 2003, 2006.
- Editorial Board of Journal of Nuclear Medicine, European Journal of Nuclear Medicine and Molecular Imaging, Journal of Nuclear Cardiology, The Open Journal of Nuclear Medicine, Journal of European Journal of Nuclear Medicine Research, Nanomedicine: Nanotechnology, Biology, and Medicine, American Journal of Nuclear Medicine, Current Molecular Imaging
- Fellow of American College of Cardiology.

Laurent Levy

PhD
CEO and President of the Executive Board
NANOBIOTIX

www.nanobiotix.com

Dr. Laurent Levy has over 15 years' experience in nanomedicine, supported by a solid understanding of physics, chemistry and biology. He obtained his doctorate in nanotechnology from the Pierre & Marie Curie University and the CEA, France, before pursuing a post-doctoral fellowship at the Institute for Lasers, Photonics and Biophotonics at the State University of New York, USA. Here Dr. Levy collaborated with the Director of the Institute, Dr. Paras Prasad, a key scientist working in the nanophotonic and nanobiotech field, to develop a unique vision for adopting nanotechnology to treat cancer.

Dr. Levy's cutting-edge biotechnology and nanotechnology research culminated in the development of a nanomedicine platform called NanoXray to address the biggest drawbacks of radiation therapy, namely, the destruction of healthy tissue, amongst other adverse effects, caused by the high radiation doses necessary to destroy tumor targets. In 2003, he co-founded Nanobiotix, a Paris based nanomedicine company pioneering novel approaches for the local treatment of cancer. Prior to Nanobiotix, Dr. Levy was a consultant in the development of nanotechnology applications for pharmaceutical company Sanofi, medical imaging company Guerbet and chemical company Rhodia, in addition to biotechnology start-ups.

Currently, Dr. Levy is involved in nanomedicine-focused international groups and think-tanks, aside from being an expert for EU funding programs. He is the Vice-President of the European Technology Platform of Nanomedicine (ETPN) and the author of 35 international scientific publications and several patent applications.

Markus Limberger

Dr. Markus Limberger studied Chemistry at the University of Saarland. He finalized his PhD thesis in pharmaceutical and medicinal chemistry in 1999 (Herrmann-Schlosser-studentship, Phoenix Pharmazie science award 2000). After a study visit at the University of Surrey, Guildford and job activities in the field of drug development, characterization of drug substances and analytical development, he started his career at PHAST GmbH in 2002. Actually he

holds the position Technical Director. He looks back on more than ten years of GxP experience. His main focus is concentrated on the areas quality control and quality assurance of drug products and biopharmaceuticals as well as the lean management and optimization of lab processes in the regulated GMP area. The GMP compliant implementations of new innovative analytical techniques into quality control is also a point of interest. Dr. Limberger is member of the APV and GdCh.

Julianna Lisziewicz

PhD,
President,
Chief Executive Officer, Genetic Immunity

Dr. Julianna Lisziewicz co-founded Genetic Immunity in 1998 and has served as the President and Chief Executive Officer of Genetic Immunity since its founding. In 1994, Dr. Lisziewicz co-founded the non-profit Research Institute for Genetic and Human Therapy (RIGHT) and directed its research and business affairs in the USA. RIGHT was focusing on the treatment of HIV/AIDS from multiple perspectives: virology, molecular biology, immunology and medicine. From 1990 to 1995, she was Head of the Antiviral Unit in the Laboratory of Tumor Cell Biology at the National Cancer Institute of the NIH in Bethesda, Maryland. While at NIH, she discovered and developed antisense oligonucleotide therapy and gene therapy for HIV/AIDS treatment. In 2005, she was appointed as the Marie Curie Chair at the Semmelweis University Budapest. She received her Ph.D in molecular biology from the Max-Planck Institute (Goettingen, Germany) and two Masters of Science in Chemistry and Biochemistry from the Technical University (Budapest, Hungary). She has co-authored over 100 peer reviewed scientific publications.

Beat Löffler

Beat Löffler studied Communication Sciences, Philosophy and Political Science. He received an MA at Freie Universität Berlin and improved himself in Life Sciences / Biology for further 2 years. In 1983 he started his first company for concepts and new media. Six years later he became Director of the International Hightech-Forum of the Swiss Industries Fair. After working for further 6 years in the new technology sector as developer and conference organiser, creating concepts for emerging technology events, he started in1994 his present company "L&A Concept Engineering" and specialised in the fields of the development of innovation concepts and of science and knowledge promotion initiatives as well as in leadership-training and interdisciplinary bridging events. Fields of work are · Computational Fluid Dynamics · Materials Science · Energy Technology and · Life Sciences. Beat Löffler held numerous mandates for projects developed by his company, e.g.: He wrote and developed the BioValley Upper Rhine Network-Initiative and coached it as Secretary General for 6 years, signed responsible for the Trade Fair for Simulation and Visualisation SIMPAT and developed the Leadership Training EUROPRENEUR together with INSEAD, Fontainebleau and HSG, St. Gallen. He had a mandate during 4 years of the Centre Européen de Management in Colmar and spent 4 years as life science business development-consultant with lead EMEA for the Japanese company NEC High Performance Computing in Düsseldorf, Germany. In 2005 he conceived and realised the European Summit for New Materials in Energy and Mobility in Essen, Germany for "Initiativkreis Ruhrgebiet". He coconceived and realised the 1st World Summit for New Materials in Energy Technology in Lisbon, 2006. In the same year he started the development of a concept for a conference for applied Nanomedicine. In 2007, he founded together with Patrick Hunziker, MD, the European Foundation for Clinical Nanomedicine, started up the European Society for Nanomedicine and co-founded the International Society for Nanomedicine. Since 2007, he is CEO of the CLINAM Foundation and Secretary General of the European Society for Nanomedicine and the International Society for Nanomedicine. His own company has since then developed towards new fields and includes Nanotechnology in Health. Beat Löffler signs responsible for the European Annual Summit for Clinical Nanomedicine and Targeted Medicine which is in Europe the largest platform for this discipline. He is leading the Dissemination Package within 3 FP7 Projects.

Stergios Logothetidis

PhD

Director of Nanotechnology Lab LTFN,
Department of Physics, Aristotle University of Thessaloniki, Greece
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Prof. S. Logothetidis is the Director and Founder of the Lab of Thin Films - Nanosystems & Nanometrology (LTFN) (http://www.ltfn.gr) at AUTh. His research activity is complemented by over 850 papers, review articles and monographs in peer reviewed International Journals (~290) and International Conference Proceedings (~390), and Proceedings of National Conferences. He has given more than 160 Invited talks on nanotechnologies and nanomedicine and he has over 5500 citations. He is a referee in more than 25 International Scientific Journals and in the board of several Journals. He is the editor of Nanotechnology, Nanomedicine and Nanobiotechnology Books by Springer-Verlag, Berlin Heidelberg (2012) and the co-editor of a new book entitled 'Horizons in Clinical Nanomedicine' that will be published from Pan Stanford Publishing in June 2013.
He has been the coordinator and principal investigator in more than 70 R&D projects funded by European Commission in the fields of Nanotechnologies, Materials Science, Organic Electronics and Nanomedicine.

He is the director and founder of the Interdisciplinary Post-Graduate Program "Nanosciences & Nanotechnologies" (N&N) (http://nn.physics.auth.gr), of AUTH with participation of Depts. of Physics, Chemistry, Biology and Polytechnic School in 2002, and other Depts. and Institutes from Greece and abroad. He is the founder and Coordinator of the Thematic Research Network on Nanotechnologies and Nanobiotechnologies "NANONET" (http://www.nanonet.gr/) with more than 300 members (of Laboratories, Research Centers and companies) from all over the world. Finally, he annually organizes a platform of multiple events since 2004 with two International Conferences, three International Summer Schools and one Exhibition) on Nanotechnologies, Nanomedicine & Organic Electronics under the umbrella of NANOTEXNOLOGY (http://www.nanotexnology.com).

Witold Łojkowski

Prof., Institute of High Pressure Physics, Head of Laboratory of Nanostructures, Faculty of Management, Białystok University of Technology. MsC in physics at the Warsaw University, Warsaw, Poland, PhD in Materials Science
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R&D INTERESTS

- Mechanisms of plastic deformation of metals, role of nanostructure
- Structure of grain boundaries and interfaces
- Solvothermal synthesis of nanoparticles and their applications
- Development of microwave reactors for nanoparticle synthesis

- Foresight and roadmapping in the field of nanotechnology
- Project management with application of the Theory of Constraints

MAJOR COORDINATED PROJECTS

- 2000 - 2003 Center of Excellence Project at the Institute of High Pressure Physics
- 2006 - 2008 Research Network WITNANO: Virtual Institute for Nanoparticles Technology.
- 2004 - 2009 Working Group "High Pressure Microwave Synthesis of Nanoparticles" in COST actions.
- 2006 - 2008 Foresight Project FOREMAT: Scenarios for development of technology of advance ceramic, metallic and composite materials.
- 2008 - 2011 DONANO – Doped Nanoparticles for Innovative Industry
- 2009 – 2012 OXYNANOSEN: Oxygen sensor based on luminescence of nano zirconia. t
- 2011 -2014 SONOSCA: Sonochemical technology for bone regrowth.

MEMBERSHIPS AND FUNCTIONS

- Polish speaker in the European NMP – Nanotechnology, Materials and Production
Technology Programme Committee of the European Commission.
- Polish Speaker in the COST domain committee "Materials, Physics and Nanosciences". Selection of projects to be funded.
- Polish speaker at the OECDWorking Party on Nanotechnology.
- Member of the Advisory Board of CLINAM – Clinical Nanomedicine Association
- Member of the Executive Committee of the European Materials Society and Chair of the E-MRS Fall Meeting in 2013
- Polish Ambasador of the Mummert Scholarship program of the Robert Bosch Stiftung

Alessandro Maiocchi

Alessandro Maiocchi graduated in Industrial Chemistry in 1989 at the Science Faculty of the University of Milan. He is working in the Bracco Group companies since 20 years as a senior scientist covering several roles in the R&D organization. Currently he is the Research Projects Manager at the Bracco Research Centre in Italy. From 2004-2010 he served as contract professor at the Dept. of Biotechnology and Molecular Sciences at the University of Varese in Italy. His current research activity is focused on the design and development of small and nanosized probes for molecular imaging applications in Magnetic Resonance, Ultrasound and Optical Imaging. He is member of several societies and author of more than 150 scientific publication on international journals and conference proceedings in the field of drug design, contrast agents characterization, pharmaceutical product development and imaging methods.

Harald Mangge

Univ.Prof. Dr.med.
Head of the Research Unit on Lifestyle and Inflammation-associated Risk Biomarkers
Vicespeaker of the Cardiovascular Research Field
MUG Coordinator BioTechMed Initiative
Clinical Institute for Medical and Chemical Laboratory Diagnosis
Medical University of Graz
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http://www.medunigraz.at/styjobs

Harald Mangge is a clinical scientist in the fields of obesity related pathologic sequels, inflammation, nanomedicine, and biomarker research. His main field of expertise is laboratory medicine. Current research activities focuse on obesity and lifestyle-related diseases, adipokines, cardiovascular diseases and molecular imaging of atherosclerotic (AS) lesions. An improved diagnosis of vulnerable AS plaques is aimed by means of Nanotechnology since several years. Small (mice) and larger (rabbit) animal models for AS were established, and biomarkers coupled to nanoparticles investigated for molecular vascular imaging of AS. A EU FP7 project in which an improved diagnosis and treatment of atherosclerosis is investigated by means of nanotechnology has started in February 2013 (Project NanoAthero). Harald Mangge is member of the steering committee and WP leader (Preclinical Validation). He conducts further the STYJOBS/EDECTA project, a large prospective, observational study to improve the understanding of metabolic and cardiovascular risk in obesity. Details for STYJOBS/EDECTA please refer: http://clinicaltrials.gov/ct2/show/NCT00482924

- 26.12.1961 born in Villach, Austria

EDUCATION

- 1980-1986 Medical studies at the Medical University of Graz, A
- 1986-1988 Research assistant at the Institute of General and Experimental Pathology, Medical University of Graz (Immunology, Flowcytometry, Tissue culture)
- 1988 Advanced Course in Immunology, St.Louis, USA
- 1988-1990 Clinical rotationship (Departments of Internal Medicine, Pediatrics
- 1988-1991 Obstretics, Gynecology, Surgery, Medical University of Graz)

AWARDS

- 1995 Austrian License as Specialist in Pediatrics
- 1999 Austrian License as Specialist in Laboratory Medicine
- 1999 Associate Professor for Laboratory Medicine (Habilitation)
- 2003 Management education for medical leadership
- 2008 Universitäts-Professor by the Federal President

RESEARCH AND EXPERIENCE

Biomarkers, Atherosclerosis, Obesity (Development of the STYJOBS/EDECTA, Cohort http://clinicaltrials.gov/ct2/show/NCT00482924), Nutrition, Nanomedicine, Immunology, Inflammation (Cytokines, Adipokines)

PUBLICATIONS

>100 original articles, book chapters, letters to the editor, 175 abstracts

MEMBERSHIPS

- Austrian Society for Clinical Chemistry and Laboratory Medicine
- Scientific Board Austrian Society for Obesity
- Austrian Society for Allergology und Immunology
- Wissenschaftliche Gesellschaft für Ärzte, Steiermark
- European Childhood Obesity Group (ECOG)

Massimo Masserini

Massimo Masserini is full Professor of Biochemistry and Molecular Biology at the Medical School, University of Milano-Bicocca , Italy, where he is Head of the Nanomedicine Center, an interdepartmental Consortium devoted to the bio-medical applications of nanotechnology . His research has always been focused on neurochemistry and molecular mechanisms of neurodegeneration, biochemistry of cell membranes and on physicochemical studies on membrane models, in particular liposomes. He has authored or co-authored over 120 papers in national and international journals. Currently, he is Coordinator of "Nanoparticles for therapy and diagnosis of Alzheimer Disease (NAD)", a large FP7 Project involving 18 Partners of 13 European Countries, which aims to take advantage of nanotechnologies to produce multifunctional particles envisioned for the treatment of this disease. Until now, the activities of the Project led to file 4 patents and to the publication of 40 papers on the top journals of nanomedicine.

Már Másson

Professor in Medicinal Chemistry
Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Hagi, Hofsvallagata 53 107 Reykjavik, Iceland. Contact Number: +354-5254483
E-mail: mmaosson@hi.is

My current research is directed towards synthesis of bioactive chitosan derivatives and investigation of the structure activity relationship. As a part of this work developed protection group strategies to allow reproducible synthesis of chitosan based nanomaterials. I am involved in numerical mathematical modeling of drug delivery systems and processes. The investigated application, which we do in collaboration with other groups, include disinfection and antimicrobial treatment, pulmonary drug delivery, transdermal delivery, delivery of photosensitizers, delivery anticancer agent and bone regeneration.

ACADEMIC PROFILE

- Ph.D (Engineering, Biotechnology) Tokyo Institute of Technology, Tokyo, Japan (1995)
- M.S (Organic Chemistry) University of Copenhagen, Denmark (1990)
- B.S (Chemistry) University of Iceland, Reykjavik (1987)

PROFESSINAL PROFILE

- Professor, University Iceland, Faculty of Pharmaceutical Sciences, (2002)
- Assoc. Professor, University Iceland, Faculty of Pharmaceutical Sciences, (2002)
- Researcher, University of Iceland, Faculty of Medicine (1995)

SOME RECENT PUBLICATIONS

- Vivek S. Gaware, Monika Håkerud , Kristján Leósson, Sigríður Jónsdóttir, Anders Høgset, Kristian Berg, **Már Másson**. Tetraphenylporphyrin Tethered Chitosan Based Carriers for Photochemical Transfection. *Journal of Medicinal Chemistry* 2013 56 807–819
- Berglind Eva Benediktsdóttir, Thórarinn Gudjónsson, Ólafur Baldursson (2013)**Már Másson**. Relationship between the structure of N-alkyl-N,N-dimethyl chitosan derivatives and their permeation enhancing effect in bronchial epithelia in vitro 2013. *European Journal of Pharmaceutica and Biopharmaceutics* in Press
- Berglind E. Benediktsdóttir, Ari J. Arason, Skarphédin Halldórsson. Thorarinn Gudjónsson, **Már Másson**, Ólafur Baldursson Drug delivery characteristics of the progenitor bronchial epithelial cell line VA10. *Pharmaceutical Research* 2013 30 (3) 781-791
- Ramona Lieder, Vivek S. Gaware, Finnbogi Thormodsson, Jon M. Einarsson, Chuen-How Ng, Johannes Gislason, **Már Másson**, Petur H. Petersen, Olafur E. Sigurjonsson. Endotoxins affect bioactivity of chitosan derivatives in cultures of bone marrow-derived human mesenchymal stem cells 2013- *Acta Biomaterialia*, 9 4771-4778
- Berglind E. Benediktsdóttir, Kasper K. Sørensen, Mikkel B. Thygesen, Knud J. Jensen, Thórarinn Gudjónsson, Ólafur Baldursson, **Már Másson** (2012) Regioselective fluorescent labeling of N,N,N-trimethyl chitosan via oxime formation. *Carbohydrate Polymers* 90 1273– 1280

Scott E. McNeil

Ph.D., Director

Dr. McNeil serves as the Director of the Nanotechnology Characterization Laboratory (NCL) for SAIC-Frederick and Frederick National Laboratory for Cancer Research, where he coordinates preclinical characterization of nanotech cancer therapeutics and diagnostics. At the NCL, Dr. McNeil leads a team of scientists responsible for testing candidate nanotech drugs and diagnostics, evaluating safety and efficacy, and assisting with product development -- from discovery-level, through scale-up and into clinical trials. NCL has assisted in characterization and evaluation of more than 300 nanotechnology products, several of which are now in human clinical trials. Dr. McNeil is a member of several working groups on nanomedicine, environmental health and safety, and other nanotechnology issues. He is an invited speaker to numerous nanotechnology-related conferences and has several patents pending related to nanotechnology and biotechnology. He is also a Vice President of SAIC-Frederick.
Prior to establishing the NCL, he served as a Senior Scientist in the Nanotech Initiatives Division at SAIC where he transitioned basic nanotechnology research to government and commercial markets. He advises industry and State and US Governments on the development of nanotechnology and is a member of several governmental and industrial working groups related to nanotechnology policy, standardization and commercialization. Dr. McNeil's professional career includes tenure as an Army Officer, with tours as Chief of Biochemistry at Tripler Army Medical Center, and as a Combat Arms officer during the Gulf War. He received his bachelor's degree in chemistry from Portland State University and his doctorate in cell biology from Oregon Health Sciences University.

Marie-Edith Meyre

PhD

Born July, 4th, 1981
65, boulevard Soult – 75012 Paris
Phone number: +33(0)140262684
marie-edith.meyre@nanobiotix.com

EDUCATION

- 2004-2007 PhD, Paul Pascal Research Center, Bordeaux 1 University, France
- 2004 Master 2 in Colloids, Bordeaux 1 University, France

Interface - Formulation - Inorganic synthesis - Physico-chemical characterization of colloids

- 2002-2003 Licence (3 years university degree) and Master 1 in Chemistry, Bordeaux 1 University, France

Organic synthesis - Analytical chemistry - Polymers

WORK EXPERIENCE

- 2013 Chemistry Researcher (Nanobiotix, France): NanoXray platform; Synthesis of "next generation" inorganic nanoparticles for radiotherapy
- 2008-2012 Chemistry Researcher (Nanobiotix, France): EU project Sonodrugs; Image-controlled ultrasound-induced drug delivery; Thermosensitive magnetoliposomes; superparamagnetic nanoparticles; monitoring of drug release thanks to temperature-sensitive Magnetic Resonance Imaging (MRI) contrast agents
- 2004-2007 PhD (Paul Pascal Research Center, France): Incorporation of inorganic nanoparticles in « onion-type » multilamellar lipidic vesicles; Intravesicular synthesis of gold (radiolysis, photoreduction, oxidoreduction) and iron oxide (precipitation) nanoparticles; encapsulation of superparamagnetic nanoparticles inside "onion-type" vesicles and characterization; magnetic properties as MRI contrast agents; magnetic hyperthermia
- 2003-2004 Research training (2 months and 5 months - Paul Pascal Research Center, France): Synthesis of inorganic-organic hybrid nanoparticles (gold/onions); Formulation; nanoparticles synthesis; characterization of synthesis mechanism by Fourier-transform Infrared (FTIR)

Robert D. Miller

Robert D. Miller received his PhD in Organic Chemistry from Cornell University and spent a postdoctoral year at Union Carbide Research Institute in Tarrytown NY working on flash vacuum pyrolysis and matrix isolation spectroscopy of reactive intermediates. He joined IBM at the T.J. Watson Research Laboratories in Yorktown Heights NY after his postdoctoral year and moved to the San Jose Research laboratory. He currently manages the Advanced Organic Materials Department at the IBM Almaden Research Laboratory. His research activities have included: basic photochemical processes and mechanisms, radiation sensitive polymers and microlithography, synthetic methods utilizing multifunctional synthons, synthetic applications of strained ring materials, spectroscopy and chemistry of reactive intermediates, organic reaction mechanisms and processes including catalysis, new polymeric materials for nonlinear optics, polymeric light emitting diodes, novel polymeric architectures, silicon and germanium containing polymers, controlled polymerization techniques, functionalized organic and inorganic nanoparticles, materials for molecular and organic electronics, organic materials for magnetic storage, polymeric electronic materials for semiconductor applications, nanoporous thin films for Bioscience, Optics and Photonics, photovoltaic materials and structures, sublithographic self assembly using block copolymers, air bridge dielectrics, radiation definable dielectrics, solution and CVD precursors for low-k applications, and others. He is a member of the American Chemical Society and the Materials Research Society and currently serves on the editorial advisory boards of Chemical Reviews and Advanced Functional Materials. During his career, he has received five IBM awards for outstanding technical achievements, 32 invention plateau awards and is a member of the IBM Academy of Technology. Dr Miller was elected a Fellow of the Division of Polymeric Materials Science and Engineering (PMSE) in 2006 and the Materials Research Society (MRS) in 2007. In 2009 he was elected to the National Academy of Engineering and is the recipient of the 2010 American Chemical Society award for Chemistry of Materials Dr. Miller is a co-inventor on more than 90 patents and patent publications and has published more than 350 articles in refereed technical journals.

Konstantinos Mitsakis

Dr. Konstantinos Mitsakakis studied Physics at the University of Crete, Greece and did his MSc on Nanoscience & Nanotechnology at Aristotle University of Thessaloniki, Greece. He acquired his doctoral degree (2009) from the Department of Materials Science & Technology, University of Crete, Greece, working at Biosensors Technology Lab, Institute of Molecular Biology & Biotechnology, Foundation for Research & Technology Hellas (IMBB-FORTH), Greece. His PhD thesis was on developing a multi-analyte acoustic biosensor platform for cardiac marker detection. He joined the Lab-on-a-Chip Group at IMTEK, University of Freiburg with a Humboldt Foundation fellowship and is currently the Coordinator of the EU FP7-ICT project "DiscoGnosis". His research interests lie in the broad field of micro/nanotechnology for life sciences and diagnostics, biosensor technologies, lab-on-a-chip and microanalytical systems.

Moein Moghimi

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http://nano.ku.dk/english/research/cpnn/

Moein Moghimi (British Citizen) is Professor of Nanomedicine and Head of the Nanomedicine Group at the Department of Pharmacy (Faculty of Health and Medical Science), University of Copenhagen, Denmark. He further serves as the Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxiocology (CPNN) and Group Leader in Pharmaceutical Nanotechnology at the NanoScience Center (Faculty of Science) of the Copenhagen University. He is also the Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University (China) and the elected Fellow of the Institute of Nanotechnology (FIoN) in UK. Before joining the University of Copenhagen, he was Senior Lecturer in Biopharmacy and Molecular Pharmaceutics at the School of Pharmacy, University of Brighton (UK) and The University Research Fellow in Advanced Drug Delivery at the Department of Pharmaceutical Sciences, University of Nottingham (UK). His research activities are focused on experimental nanomedicine, nanotoxicology and pharmaceutical nanosciences with continuous external funding support. Since 2009 Prof. Moghimi has secured over €8 million in competitive research funds in nanomedicine research as the principal investigator and currently partnering two large-scale competitive European Commission FP-7 programmes in experimental and clinical nanomedicines, addressing Alzheimer's disease and atherosclerosis with allocated budgets of €11 million and €8.5 million, respectively. He has pioneered research in design and surface engineering of nanoparticles and functional nanosystems for parenteral site-specific targeting and imaging modalities (e.g., splenotropic entities, lymphotropic agents, 'phagocyte-resistant' nanoparticles and cancer nanomedicines) as well as the molecular basis of nanomaterial immune toxicity and cytotoxicity.

Professor Moghimi has over 170 peer-reviewed publications/patents to his credit with over 5500 citations and h-index of 36 (as of Jan 2013). He has served as invited Theme Editor for four Theme Issues of the prestigious Advanced Drug Delivery Reviews (Elsevier, The Netherlands) and currently acts as Associate Editor for both Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) and Journal of Biomedical Nanotechnology (American Scientific Publishers, USA). Furthermore, he is an active member of editorial/advisory board of 15 peer-reviewed international journals to include Advanced Drug Delivery Reviews, Nanomedicine-UK (Future Medicine), Journal of Liposome Research (Informa Healthcare) and Drug Delivery (Informa Healthcare).

He further practices in the capacity of consultant to numerous pharmaceutical, biotechnology, health, and food industries as well as investment banks, management consultancy firms and other entrepreneurial enterprises world-wide, and serves on advisory board of two companies. Other responsibilities include being a regular invited assessor and expert evaluator in nanomedicine for different government bodies, various research councils and private organizations world-wide (over 50 establishments in 20 countries). He is a frequent keynote and guest speaker at many international conferences and organizations (with over 250 invited engagements) as well as being regular conference chair and organizer.

Following completion of secondary education at Oxford (UK), he graduated with Honors in Biochemistry from The University of Manchester (UK) in 1985 and in 1989 completed a PhD in Biochemistry (liposome immunobiology) at the Charing Cross Hospital Medical School (Imperial College, University of London, UK).

Professor Moghimi is listed in Marquis Who's Who in the World, USA, Marquis Who's Who in Science and Engineering, USA, and Marquis Who's Who in Medicine and Healthcare, USA (by invitation).

Jan Mollenhauer

Prof. Dr.

Jan Mollenhauer was born in Kiel, Germany, in 1968, studied biology from 1989-1994 at the University of Cologne, Germany, and received his PhD in 1998 from the University of Heidelberg, Germany. In 2003 he received his habilitation in Molecular Medicine from the University Heidelberg, which was mentored by the Nobel laureate in Medicine or Physiology 2008, Prof. Harald zur Hausen. Until 2008 he worked as group leader in the Division of Molecular Genome Analyses (Head: Prof. Annemarie Poustka) at the German Cancer Research Center, Heidelberg. In 2008 he joined the University of Southern Denmark, Odense, as Professor for Molecular Oncology. Since 2010, he is director of the Lundbeckfonden Center of Excellence NanoCAN (Nanomedicine Research Center for Cancer Stem Cell Targeting Therapeutics). Jan Mollenhauer received the Future Award in Health Sciences 2005 and was listed in the 2007 edition of the Who Is Who of Emerging Leaders. In 2009 he was awarded with the Leo og Ingeborg Dannins Fondens Legat, and received the Fyens Stiftstidende Researcher Award in 2010. Research focuses on the role of epithelial protection factors in cancer, on the development and application of novel functional genomics techniques for cancer drug target discovery, and the design of tailored nanodrugs for personalized cancer therapy.

Guy Morin

President of the Executive Council
Head of the Department of Presidential Affairs of the Canton of Basel-Stadt

Born 4 December 1956

Married to Christa Züger Morin, 2 children
Member of the Green Party of the Canton of Basel-Stadt

PROFESSIONAL CAREER

- 1978-1984 Medical studies at Basel University
- 1986-1993 Further training as a general practitioner [Arzt Allg. Medizin, FMH], in a number of hospitals within the Canton of Basel-Stadt
- 1993-2004 Practice as general practitioner in St. Johann, Basel
- 1996-2004 President of the HMO Family Doctor Association [HMO Hausärztevereinigung (VIPA)]

POLITICAL ACTIVITIES

- 1985-1990 Secretary of the Swiss Section of "Doctors for Social Responsibility" ["Ärzte für soziale Verantwortung" (PSPR/IPPNW)]
- since 1988 Member of Doctors for Environmental Protection [Ärztinnen und Ärzte für Umweltschutz]
- 1988-2001 Member of the Great Council
- 1997-2001 President of the Health Commission of the Great Council
- 2004 Election to the Executive Council
- 2005-2008 Head of the Department of Justice, which was dissolved on 31 December 2008
- 2009- Head of the Department of Presidential Affairs, which was created on 1 January 2009, and President of the Executive Council of the Canton of Basel-Stadt

PUBLIC OFFICES

Executive Council delegations during the 2009 – 2013 term of office

- External Relations and City Marketing Delegation
- Cultural Issues Delegation
- Personnel Issues Delegation
- Housing Delegation

Executive Council Steering Committees and Delegations in community corporations and mixed corporations during the 2009 – 2013 term of office

- CH Foundation
- German-French-Swiss Upper Rhine Conference
- German-French-Swiss Government Commission
- EURES-T Upper Rhine (Monitoring and Steering Committee)
- INTERREG Upper Rhine (Monitoring and Steering Committee)
- Conference of Cantonal Governments (Konferenz der Kantonsregierungen KdK)
- KdK City Commission (Kommission Städte der KdK)
- KdK Political Working Group Border Cantons Germany [Politische Arbeitsgruppe Grenzkantone Deutschland der KdK]
- North-West Switzerland Governing Conference [Nordwestschweizer Regierungskonferenz] (Working Committee)
- Regio TriRhena e.V. (Board)
- Regio Basiliensis (Board)
- Association of Swiss Cities [Schweizerischer Städteverband] (Board)
- Trinational Eurodistrict Basel TEB [Trinationaler Eurodistrict Basel TEB] (Board)
- Assembly of European Regions
- Regional Commission [Regiokommission GR]
- Tripartite Agglomeration Conference [Tripartite Agglomerationskonferenz TAK]
- Political Monitoring Group Spatial Concept Switzerland [Politische Begleitgruppe Raumkonzept Schweiz]
- IG Core Cities [IG Kernstädte]
- Metrobasel (Board)
- Worldexpo BS, GE, ZH (Board)
- Europainstitut Basel, Member of the Board of Trustees
- EuroAirport Basel-Mulhouse-Freiburg EAP (Executive Board Member)

Commissions and inspections during the 2009 – 2013 term of office

- Departmental Commission for Migration and Integration Questions MIK [Departementale Kommission für Migrations- und Integrationsfragen MIK]

Further mandates

- Foundation for Christian-Jewish Projects CJP [Stiftung für Christlich-Jüdische Projekte CJP]
- Infobest Palmrain (Supervisory Panel)

Jan Mous

PD Dr.

CEO of PharMida AG, Basel, and COO of Midatech Ltd., Oxford, UK.

EDUCATION

- 1980 PhD in biochemistry at the University of Leuven (Belgium)
- 1981-1984 post-doctoral stays at the Rega Institute in Leuven (1981) and at the Institute of Molecular Biology II of the University of Zürich (Switzerland, 1982-1984)
- 1993 *venia docendi* of the University of Basel (Switzerland

PROFESSIONAL CAREER

- 1985-2000 R&D manager at Hoffmann-La Roche's Pharma division in Basel (Switzerland), last position: senior VP, corporate Genomics
- 2000-2002 CSO of LION bioscience AG in Heidelberg (Germany) and non-executive director of LION bioscience Inc.(Boston, MA)
- 2003-2007 President & CEO of IntegraGen SA in Evry (France)
- 2008 independent consultant, MRM Consulting GmbH, in Giebenach (Switzerland).

Bert Müller

Bert Müller holds the Thomas Straumann-Chair for Materials Science in Medicine at the University of Basel, Switzerland. After receiving a diploma in mechanical engineering (1982), he worked as electrical fitter building large transformers in Berlin, Germany until 1984. Subsequently he studied physics and received the M.Sc. degree from the Dresden University of Technology and the Ph.D. from the University of Hannover, Germany in 1989 and 1994. From 1994 to 2001, he performed research in leading positions at the Physics Departments of the Paderborn University, Germany, of the EPF Lausanne, Switzerland and of the ETH Zurich, at the Swiss Institute for Materials Testing and Research and at the Materials Department of the ETH Zurich. Dr. Müller became a Faculty member of the Physics Department at ETH Zurich (venia legendi) in April 2001. From 2001 to 2006 he was the General Manager of the National Center of Competence 'Computer-aided and image-guided medical interventions' (www.co-me.ch) at the Department of Electrical Engineering of the ETH Zurich. After his election as Associate Professor at the Medical Faculty of the University of Basel and his appointment at the Surgery Department of the University Hospital Basel in September 2006, he founded the Biomaterials Science Center (www.bmc.unibas.ch). His interdisciplinary research team comprises doctoral students in medicine, dentistry, biomedical engineering, sport sciences, physics, chemistry and nanosciences.

JEAN BOSCO NDIHOKUBWAYO

Dr. Jean Bosco Ndihokubwayo is a Medical Doctor and a Clinical Pathologist (graduated at University of Aix Marseille II, School of Medicine, Marseille, France) and public health specialist (graduated at the Institute of Tropical Medicine, Antwerp, Belgium) qualified by over 20 years of achievements in Medical Laboratory Practice, patient safety, Academia, with current concentrations on infection control in the context of patient safety strengthening in the African region. Dr Ndihokubwayo is currently Programme Manager for the WHO-Regional Office for Africa Patient Safety and health technologies Programme. In this capacity, he coordinates both strategic management and external relations and business development for Patient Safety Programme in the African Region.

Dr Ndihokubwayo formally started Patient Safety Programme in the African Region by bringing patient safety matters to the 58th session of the Regional Committee held in Yaoundé, Cameroon in September 2008. The Regional Committee adopted document AFR/RC58/8 entitled "Patient safety in African Health Care Services: issues and solutions". This document provides an update on current knowledge on patient safety, outlines issues and challenges and proposes actions in order to improve patient safety in the African Region.

In order to address the challenges mentioned above, Dr Ndihokubwayo is developing, formalizing and strengthening a comprehensive regional patient safety program. He is also developing strategies to address and minimize patient safety risk through the provision of appropriate tools, evidence and organizational harmonization. In particular, since there is great need in patient safety policy setting at country level, Dr Ndihokubwayo is promoting the application of use of norms, standards, and guidelines for patient safety and quality of care that will enable countries to increase their ability to improve patient safety within their health systems.

During the 58th session of the Regional Committee held in Yaoundé, Cameroon, Dr Ndihokubwayo technically organized a ministerial signing ceremony where 26 Ministers of Health pledged and committed themselves to fight health-care associated infections, within the context of addressing the First Global Patient Safety Challenge, ''Clean Care is Safer Care''.

Prior to joining WHO, Dr. Ndihokubwayo was Dean of School of Medicine of Bujumbura, Burundi, where he was Associate Professor teaching Microbiology and Immunology. To prevent and fight health-care associated infections, Dr Ndihokubwayo promoted hand hygiene improvement among the health-care workers in Burundi. As Professor of Microbiology and Dean, Dr Ndihokubwayo introduced for the first time in the curriculum of the School of Medicine of Bujumbura, a special program on infection control. This Programme focuses on the prevention of health-care associated infections.

As an academician, Dr Ndihokubwayo has supervised a number of post-graduate student theses in the area of infection control including hand hygiene and antimicrobial resistance laboratory-based surveillance in the School of Medicine of Bujumbura, Burundi.

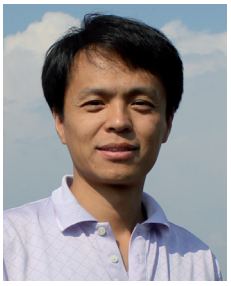
David Needham

Born in Oldham, nr Manchester, England, David Needham has been a Professor in the Department of Mechanical Engineering and Material Science at Duke University since 1987. He was recently awarded a 5 yr 35M DKK Niels Bohr Professorship by the Danish National Research Foundation (Grundforskningsfonden) of Denmark, and in January 2013 has taken up the professorship at the University of Southern Denmark (Syddansk Universitet, -- SDU). The vision is to create a world-class center for Single Particle Science and Engineering (SPSE) --an interdisciplinary research center concerned with experimental and theoretical research within the broad field of colloid and (bio)surface science. It is centered around a technique called micro-pipet manipulation that can form, study, and characterize materials and their interfaces as micro-particles of gas, liquid, and solid, including biological cells.

With an undergraduate degree in Industrial Chemistry (1975), he completed a PhD (1981), "Catalysis by Evaporated Films of Rare Earth Metals" in Physical Chemistry, University of Nottingham, with Daniel D. Eley, FRS. Motivated by his mother's bout with breast cancer in 1972, he decided to make a major switch of fields to that of lipid bilayer membranes, with the goal of working on cancer cells and their interfacial properties. "Learning about membranes whilst remaining productive"1 he did 2 post docs, --the first with Dennis Haydon FRS (black lipid films) at Cambridge University (1980-1983), and the second with Evan Evans at UBC, (Mechanochemistry of lipid membranes) (1983 – 1987). These physical-chemical and mechano-chemical studies provided the foundation for his current research at Duke in micro and nanoparticle engineering of lipid membranes, and other two-phase micro and nano systems. Although he has worked on the mechanical properties of cancer cells, he focused largely on the material science of lipid vesicle bilayers, and applied his expertise in membranes to invent and develop a thermally-sensitive, anti-cancer, liposome drug delivery system that is now in clinical trials for liver cancer and chest wall recurrence of breast cancer. Now at SDU, his priority for the next five years is to launch and establish the SPSE center, and also the design and testing of endogenous-inspired experimental therapeutics, focusing specifically on the design and testing of new drug delivery systems for cancer by reverse engineering nature's designs for nanoparticle fat transport and uptake by cancer cells. Other applications are in chemical and materials processing, the food industry, nano- and bio-technologies, and other advanced pharmaceutics.

Finally, in education, he is bringing together biology and medicine with the physical, chemical and pharmaceutical sciences, and engineering by reverse engineering problems nature, scientists and engineers have solved. The idea is to develop a greater understanding for students of the Natural Sciences and engineering for health, disease, and its treatment, and also to generate the innovation of new technologies and medicines. More broadly, he is exploring a paradigm shift that is more focused on "student-based learning".

He is developing new learning tools and instructional approaches that reveal the importance of an Experiment, Discover, Uncover pedagogy of learning, leading to new Knowledge, (EDU-K) --for the student. This EDU-K pedagogy is coupled with a content framework of design in the intellectual, emotional and motivational journey of life-long learners.



Guangjun Nie

Professor of Nanobiology and Nanomedicine, National Center for Nanoscience and Technology, PR China

EDUCATION/TRAINING

• 1996 Bachelor, Biology, Northeast Normal University, China
• 1999 Masters, Biochemistry and Molecular Biology, Jilin University, China
• 2002 Ph.D, Biophysics, Institute of Biophysics, Academia Sinica, Beijing
• 2007 Masters,Business Administration in Biotechnology Management, Simon Fraser University, Vancouver, Canada
• 2002-08 Postdoctoral Associate, Hematology and Cell Biology, Jewish General Hospital, McGill University, Canada

POSITIONS AND EMPLOYMENT

• 2008-present Professor of Nanobiology and Nanomedicine, National Center for Nanoscience and Technology, PR China
• 2002-08 Postdoctoral Research Associate, Jewish General Hospital, McGill University, Canada
• 2000 Jun-Dec Visiting Scientist, Institute of Food Research, Norwich, United Kingdom

Guangjun Nie is professor of Nanobiology and Nanomedicine, CAS Key Laboratory of Biological Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, CAS 100 Talents Scientist and Chief Scientist of National Basic Research Programs, Ministry of Science and Technology of China.
He has a long standing interest in cancer biology, blood physiology and pathophysiology of human disorders involving disregulation of redox balance and metal metabolism. His seminal works on the pathophysiological roles and expression regulation of mitochondrial ferritin highlighted the importance of the protein in physiology and in various diseases, including cancer, iron overload disorders and Parkinson's Diseases. The systemic studies on iron metabolism related works have generated a series of publications in hematology, biochemistry and cell biology journals (including Blood, JBC, Antioxidant Redox Signaling, Blood Cells, Molecules and Diseases and British Journal of Hematology).
More recently his major focus has been on the use of bio-inspired biomaterials and nanotechnology-enabled methods to generate multifunctional nanostructures to overcome the current medical barriers in tumor therapy. By introducing multifunctional nanostructures, specific regulating and targeting both tumor cells and their microenvironment have been achieved. By elaborated choosing various nanoproperties of nanostructures, Dr. Nie's laboratory designed various effective antitumor nanoparticles. He is leading a group of 20 scientists and initiated a key project on studying regulations of both tumor cells and their microenvironments, especially tumor associated macrophages and fibroblasts to inhibit tumor fast growth and their metastasis with nanotechnology-enable methods. His most recent research activities generated a group of interdisciplinary works in nanobiology and nanomedicine fields (including papers published in Angew Chem, JACS, Biomaterials, Nanoscale, Current Drug Metabolism, Small). He is an experienced supervisor of postgraduate students and collaborates widely both within China and internationally. His research is funded by the NSFC, CAS and MOST of China.

• Li Wan, **Guangjun Nie**, Jie Zhang, Yunfeng Luo, Peng Zhange, Zhiyong Zhang and Baolu Zhao, β-amyloid peptide increases levels of iron content and oxidative stress in human cell and C. elegans models of Alzheimer's disease, *Free Radical Biology & Medicine*, 2011, 50, 1, 122-129.
• Qinghua Miao, Dongxue Xu, Zhi Wang, Li Xu, Tiewei Wang, Yan Wu*, David B. Lovejoy, Danuta S. Kalinowski, Des R. Richardson*, **Guangjun Nie***, Yuliang Zhao, Amphiphilic hyper-branched copolymer nanoparticles for the controlled delivery of anti-tumor agents, *Biomaterials*, 2010, 31, 7364-7375.
• Wang, Y., LV, J., Ma, X., Wang, D., Ma, H., Chang, Y., **Guangjun Nie**, Jia, L., Duan, X. and Liang, X.J*. Specific hemosiderin deposition in spleen induced by a low dose of cisplatin: altered iron metabolism and its implication as an acute hemosiderin formation model. *Current Drug Metabolism*, 2010,11, 507-515.
• Yinghui Zhang, Marc Mikhael, Dongxue Xu, Yiye Li, Shan Soe-Lin, Bo Ning, Wei Li, **Guangjun Nie***, Yuliang Zhao, and Prem Ponka*, Lysosomal Proteolysis Is the Primary Degradation Pathway for Cytosolic Ferritin and Cytosolic Ferritin Degradation Is Necessary for Iron Exit, *Antioxidants & Redox Signaling*, 2010, 13:7, 999-1009.
• Soe-Lin, S, Mikhael, M, Apte, S, Kayembe, L, **Guangjun Nie**, Ponka, P. Both Nramp1 and DMT1 are necessary for efficient macrophage iron recycling, *Experimental Hematology*, 2010;38:609–617.
• Shi Z*, **Guangjun Nie**,* Duan, X, Rouault, T, Wu, W, Ning, B, Zhang, N, Chang, Y, Zhao, B. Neuroprotective mechanism of mitochondrial ferritin on 6-hydroxydopamine induced dopaminergic cell damage: implication for neuroprotection in Parkinson's disease. *Antioxidants & Redox Signaling*, 2010, 13:6, 783-796(*co-first authors).
• Xu J, Song J, Su J, Wei J*, Yu Y, Lv S, Li W, **Guangjun Nie***. A new human catalytic antibody Se-scFv-2D8 and its selenium-containing single domains with high GPX activity. *Journal of Molecular Recognition*, 2010; 23: 352–359 (*corresponding authors).
• Lu, Z*, **Guangjun Nie***, Li, Y, Soe-Lin, S, Tao, Y, Cao, Y, Zhang, Z, Liu, N, Ponka, P, Zhao, B. Overexpression of mitochondrial ferritin sensitizes cells to oxidative stress via an iron-mediated mechanism. *Antioxidant Redox Signaling* 2009, 11: 8, 1791-803 (*co-first authors).
• **Guangjun Nie**e, Guohua Chen, Alex D. Sheftel, Kostas Pantopoulos and Prem Ponka. In Vivo Tumor Growth is Inhibited by Cytosolic Iron Deprivation Caused by the Expression of Mitochondrial Ferritin, *Blood*, 2006, 108:2428-34.
• Zhongbi Lu, **Guangjun Nie**, Peter Belton, Huiru Tang and Baolu Zhao. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochemistry International*, 2006, 48:4, 263-74.
• M. Priwitzerova, **Guangjun Nie**, A. Sheftel, D. Pospisilova, V. Divoky, P. Ponka. Functional Consequences of the Human DMT1 Mutation on Protein Expression and Iron Uptake. *Blood*, 2005, 106(12):3985-7.
• **Guangjun Nie**, Alex Sheftel, Songwon Kim, Prem Ponka. Overexpression of Mitochondrial Ferritin Causes Cytosolic Iron Depletion and Changes Cellular Iron Homeostasis. *Blood*, 2005, 105:2161-7.

António Nunes

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EDUCATION AND PROFESSIONAL WORK EXPERIENCE

• Oct 2012-Present Biologist/ Medical Imaging Scientist, Institute for Biological and Medical Imaging (IBMI) of the Helmholtz Zentrum München (GmbH), Germany and in close collaboration with iThera Medical (GmbH), Munich, Germany. Project based on the development of preclinical and clinical applications for ground-breaking imaging sys-

tem, including develop, test and optimize agents for the targeted use of optoaccustic imaging. Reference: Prof. Vasilis Ntziachristos.

• Jan-Sept 2012 Post Doctoral Research Fellow, Nanomedicine Lab, UCL School of Pharmacy, UK. Project based on the characterization, biodistribution and imaging nanomaterials within the central nervous system (CNS), including: Fullerenes, Carbon Nanotubes, Carbon Nanohorns and Graphene. Reference: Prof. Kostas Kostarelos.

• Apr 2008-Jan 2012 PhD, UCL School of Pharmacy, UK. Entitled: Exploring carbon nanotubes pharmacology and transport to the brain. Supervision: Prof. Kostas Kostarelos and Dr Khuloud T. Al-Jamal.

Collaborator in several EU research projects, including: i) EU FP6 NINIVE (NMP4-CT-2006-033378): the development of non-invasive nanotransducer for in vivo gene therapy and ii) EU FP7 ANTICARB (HEALTH-F2-2008-201587): the improvement of oncology treatment options using systemic administration of carbon nanotubes-mediated antibody therapeutics.

• Sep 2001-De c2007 PharmD Degree in Pharmaceutical Science at University of Coimbra, Portugal.

PATENTS

• Process Of Cell Permeabilization Mediated By Nano-Antennas And/Or Electromagnetic Fields, V. Raffa, A.Ziaei, M.L.Baillif, V.Raffa, G.Ciofani, O.Vittorio, C.Riggio, A.Cuschieri, T.Pizzorusso, M.Costa, G.Bardi, L.Gherardini, K.Kostarelos, K.T.Al-Jamal, **A.Nunes**, S.Nitodas, T.Karachalios, PCT, Nov 17, 2009 (n°11066).

PUBLICATIONS

• Hybrid Polymer-Grafted Multiwalled Carbon Nanotubes for In vitro Gene Delivery.
A.Nunes, N.Amsharov, C.Guo, J.Van den Bossche, P.Santhosh, T.K.Karachalios, S.F.Nitodas, M.Burghard, K.Kostarelos and K.T.Al-Jamal. Small, 6(20):2281-91.

• Efficient receptor-independent intracellular translocation of aptamers mediated by conjugation to carbon nanotubes.
J.Van den Bossche, W.T.Al-Jamal, B.Tian, **A.Nunes**, C.Fabbro, A.Bianco, M.Prato and K.Kostarelos. Chem Commun (Camb.), 46(39):7379-81.

• Enhanced cellular internalization and gene silencing with a series of cationic dendron-multiwalled carbon nanotube:siRNA complexes.
K.T.Al-Jamal, F.M.Toma, A.Yilmazer, H.Ali-Boucetta, **A.Nunes**, M.A.Herrero, B.Tian, A.Eddaoudi, W.T.Al-Jamal, A.Bianco, M.Prato, K.Kostarelos. FASEB, 24(11):4354-65

• Polyamine functionalized carbon nanotubes: synthesis, characterization, cytotoxicity and siRNA binding.
P.Singh, C.Samorıi, F.M.Toma, C.Bussy, **A.Nunes**, K.T.Al-Jamal, C.Menard-Moyon, M.Prato, K.Kostarelos and A.Bianco. J Mater Chem, 21:4850-4860

• Length-Dependent Retention of Carbon Nanotubes in the Pleural Space of Mice Initiates Sustained Inflammation and Progressive Fibrosis on the Parietal Pleura.
F.A.Murphy, C.A.Poland, R.Duffin, K.T.Al-Jamal, H.Ali-Boucetta, **A.Nunes**, F.Byrne, A.Prina-Mello, Y.Volkov, S.Li, S.J.Mather, A.Bianco, M.Prato, W.MacNee, W.A.Wallace, K.Kostarelos, K.Donaldson. Am J Pathol, 178(6):2587-2600

• Functional motor recovery from brain ischemic insult by carbon nanotube-mediated siRNA silencing.
K.T.Al-Jamal, L.Gherardini, G.Bardi, **A.Nunes**, C.Guo, C.Bussy, M.A.Herrero, A.Bianco, M.Prato, K.Kostarelos and T.Pizzorusso. Proc Natl Acad Sci U S A, 108 (27):10952-10957

• Therapeutics, Imaging & Toxicity of Nanomaterials in the Central Nervous System.
A.Nunes, K.T.Al-Jamal and K.Kostarelos. J Control Release (DOI:10.1016/j.jconrel.2012.03.026).

• Application of Carbon Nanotubes in Neurology: Clinical Perspectives & Toxicological Risks.
A.Nunes, K.T.Al-Jamal, T.Nakajima, M.Hariz and K.Kostarelos. Arch Toxicol (DOI:10.1007/s00204-012-0860-0).

• Degree of Chemical Functionalization of Carbon Nanotubes Determines Tissue Distribution and Excretion Profile.
A.Nunes, K.T.Al-Jamal, L.Methven, W.T.Al-Jamal, S.Li, M.A.Herrero, H.M.M. ten Eikelder, S.J.Mather, M.Prato, K.Kostarelos and A.Bianco. Angew Chem Int Ed Engl (DOI:10.1002/anie.201201991).

• In vivo Degradation of Functionalized Carbon Nanotubes after Stereotactic Administration in the Brain Cortex.
A.Nunes, C.Bussy, L.Gherardini, M.Meneghetti, M.A.Herrero, A.Bianco, M.Prato, T.Pizzorusso, K.T.Al-Jamal and K.Kostarelos. Nanomedicine (Lond), (DOI:10.2217/NNM.12.33).

• Asbestos-like Pathogenicity of Long Carbon Nanotubes Alleviated by Chemical Functionalization.
H.Ali-Boucetta, **A.Nunes**, R.Sainz, M.A.Herrero, B.Tian, M.Prato, A.Bianco, and K.Kostarelos. Angew Chem Int Ed Engl, (DOI:10.1002/anie.201207664).

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PROFESSIONAL PREPARATION

• 1999 B.Sc., Physics and Biology, Hebrew University, Jerusalem, Israel

• 2003 Ph.D., Molecular Biophysics and Biochemistry, Columbia University, New York USA

• 2006 Postdoctoral, Computational Biology, Columbia University, New York, USA

• 2007 Postdoctoral, Biochemistry, Tel Aviv University, Tel Aviv, Israel

APPOINTMENTS AND POSITION

• 11/2007-present Member, Institute of Nanotechnology and Advanced Materials, Bar Ilan University, Israel

• 11/2007-present Senior Lecturer, Faculty of Life Sciences, Bar Ilan University, Israel

• 11/2007-present Program for Computational Biology, Bar Ilan University, Israel

• 11/2007-present Program for Biophysics, Bar Ilan University, Israel

• 08/2003-01/2006 Research Fellow, Center for Computational Biology and Bioinformatics, Columbia Univeristy, NY, USA

• 02/2006-8/2007 Safra Fellow, Tel Aviv University

• 1/1/2010-present Founder, Biolojic Design Ltd.

Wolfgang J. Parak

• 1989-1997 General Physics studies at the Technische Universität München, D

• 1995-1997 Diploma thesis ("Diplomarbeit") at the Technische Universität München, Germany, at the Institute for Biophysics (chair: Prof. Dr. Erich Sackmann) in the group of Prof. Dr. Hermann Gaub with the topic "set-up and characterisation of a LAPS-Sensor for non-invasive measurements of membrane potentials"

• 1997-1999 Graduate student at the Ludwig Maximilians Universität München, Germany, at the Institute of Applied Physics (chair: Prof. Dr. Hermann Gaub with the topic "cell-semiconductor-hybrids"

• 2000-2002 Postdoc at the Department of Chemistry at the University of California at Berkeley, California, USA, in the group of Prof. Paul Alivisatos with the field of work of "biological applications of colloidal nanoparticles"

• 2003-2006 Leader of a Junior Research Group (Emmy-Noether fellowship of the German Research Foundation (DFG), equivalent to

Assistant Professor), hosted at the Ludwig Maximilians Universität München, Germany, at the Institute for Applied Physics (chair: Prof. Dr. Hermann Gaub) and at the Center for Nanoscience

• 2005 Temporary position as Associate Professor for Physical Chemistry at the Department of Chemistry and Pharmacy at the Ludwig Maximilians Universität München, Germany for the Summer Semester

• since 2007 Full Professor (chair) for Experimental Physics at the Philipps-University of Marburg, Germany

• 2009 "Nanoscience" - award 2008 from the Association of Nanotechnology-Centres Germany (AGenNT)

• since 2010 Associate Editor for ACS Nano from the American Chemical Society

• 2011 ranked #59 in Top Materials Scientists of the pastdecade by Essential Science Indicators (http://science.thomsonreuters.com/products/esi/)

• 2012 Awarded Chinese Academy of Sciences Visiting Professorship for Senior International Scientists

Anil K. Patri

Ph.D., Deputy Director

Dr. Patri joined the Nanotechnology Characterization Laboratory (NCL) as a Senior Scientist in February 2005. He directs the physico-chemical characterization and standardization of nanomaterial for the NCL and actively collaborates with NIST and FDA.

Dr. Patri received his B.Sc., from Osmania University and M.Sc., in organic chemistry from Aligarh Muslim University, India. He then worked for two years as a lecturer in chemistry in India and then obtained his Ph.D. in organic chemistry from the University of South Florida under the guidance of Prof. George Newkome. After a one-year post-doctoral training with Dr. Donald Tomalia at the University of Michigan, he joined the Center for Biologic Nanotechnology as a staff scientist. In this position, he developed dendrimer based nanomaterial for targeting, imaging and drug delivery applications.

Zoe Pikramenou

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QUALIFICATIONS

• 1983-1987 B.Sc., Chemistry, University of Athens, Athens, Greece

• 1988-1993 Ph.D., Chemistry, Michigan State University, E. Lansing, Michigan, USA

CAREER TO DATE

• 1988-1993 Michigan State University, Teaching and Research Assistant, Prof. D. G. Nocera

• 1993-1995 Université Louis Pasteur, Postdoctoral Researcher, Prof. J.-M. Lehn, France

• 1995-2000 University of Edinburgh, Lecturer in Chemistry

• 2000 Lecturer in Chemistry, University of Birmingham

A period of 4-month maternity leave in 2001

• 2004 Senior Lecturer in Chemistry, University of Birmingham

• 2006 Reader in Supramolecular Photochemistry, University of Birmingham

Two periods of 4-month maternity leave in 2007, 2010

RESEARCH INTERESTS

The research programme includes inorganic chemistry, photophysics and nanoscience in the design of new functional molecular probes for: a) the monitoring of key biomolecular events that tackle detection and therapeutic issues in healthcare technologies and b) the study of energy conversion processes in supramolecular assemblies for optical devices and solar cell applications.

Noble metal and silica nanoparticles are used as scaffolds to carry luminescent and MRI active molecular metal probes based on lanthanide and transition metals in cells for multimodal imaging. Our approach allows control of probe design using ligand and coordination chemistries and allows selective coating of the particle with targeting vectors. We use optical imaging to monitor cellular uptake and nuclear targeting of the functional nanoparticle in cancer cells and platelets in blood: imaging of single nanoparticles provides the potential of monitoring of cellular events in the nucleus that will be affected by delivery of a drug on the nanoparticle scaffold; the nanoparticle controlled release in platelets may allow imaging of the actin cytoskeleton that underpins platelet function in blood clotting. We develop techniques of efficient coating of luminescent probes on the nanoparticle that enable their imaging in blood flow in the microvasculature of biological tissues and in microchannels for imaging of flow patterns.

SELECTED PUBLICATIONS

• DAVIES, A., LEWIS, D. J., WATSON, S. P., THOMAS, S. G. & PIKRAMENOU, Z. 2012. pH-controlled delivery of luminescent europium coated nanoparticles into platelets. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 1862-1867.

• LEWIS, D. J., DORE, V., GOODWIN, M. J., SAVAGE, A. C., NASH, G. B., ANGELI, P. & PIKRAMENOU, Z. 2012. Luminescent ruthenium(II) tris-bipyridyl complex caged in nanoscale silica for particle velocimetry studies in microchannels. *Measurement Science & Technology*, 23, 084004, 1-9.

• LEWIS, D. J., MORETTA, F., HOLLOWAY, A. T. & PIKRAMENOU, Z. 2012. Evaluation of quinoline as a remote sensitiser for red and near-infrared emissive lanthanide(III) ions in solution and the solid state. *Dalton Transactions*, 41, 13138-13146.

• LEWIS, D. J., GLOVER, P. B., SOLOMONS, M. C. & PIKRAMENOU, Z. 2011. Purely Heterometallic Lanthanide(III) Macrocycles through Controlled Assembly of Disulfide Bonds for Dual Color Emission. *Journal of the American Chemical Society*, 133, 1033-1043.

• SAVAGE, A. C. & PIKRAMENOU, Z. 2011. Peptide coated gold nanoparticles that bind lanthanide ions. *Chemical Communications*, 47, 6431-6433.

• LEWIS, D. J., BRUCE, C., BOHIC, S., CLOETENS, P., HAMMOND, S. P., ARBON, D., BLAIR-REID, S., PIKRAMENOU, Z. & KYSELA, B. 2010. Intracellular synchrotron nanoimaging and DNA damage/genotoxicity screening of novel lanthanide-coated nanovectors. *Nanomedicine*, 5, 1547-1557.

• RUSTON, L. L., ROBERTSON, G. M. & PIKRAMENOU, Z. 2010. Luminescence Screening Assays for the Identification of Sensitizers for Lanthanides Based on the Controlled Formation of Ternary Lanthanide Complexes with DTPA-Bisamide Ligands. *Chemistry-an Asian Journal*, 5, 571-580.

• FAIZ, J. A., KYLLONEN, L. E. P., CONTRERAS-CARBALLADA, P., WILLIAMS, R. M., DE COLA, L. & PIKRAMENOU, Z. 2009. Photoinduced energy transfer across non-covalent bonds in the nanoscale: cyclodextrin hosts with enhanced luminescent properties for guest communication. *Dalton Transactions*, 3980-3987.

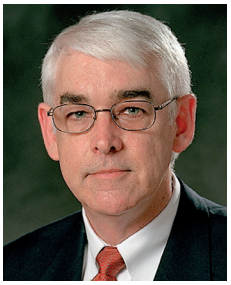
• FAIZ, J., PHILIPPOPOULOS, A. I., KONTOS, A. G., FALARAS, P. & PIKRAMENOU, Z. 2007. Functional supramolecular ruthenium cyclodextrin dyes for nanocrystalline solar cells. *Advanced Functional Materials*, 17, 54-58.

• GLOVER, P. B., BASSETT, A. P., NOCKEMANN, P., KARIUKI, B. M., VAN DEUN, R. & PIKRAMENOU, Z. 2007. Fully fluorinated imidodiphosphinate shells for visible- and NIR-Emitting lanthanides: Hitherto unexpected effects of sensitizer fluorination on lanthanide emission properties. *Chemistry-a European Journal*, 13, 6308-6320.

• MALLON, C. T., FORSTER, R. J., MCNALLY, A., CAMPAGNOLI, E., PIKRAMENOU, Z. & KEYES, T. E. 2007. Surface-immobilized pyridine-functionalized gamma-cyclodextrin: Alkanethiol co-adsorption-induced reorientation. *Langmuir*, 23, 6997-7002.

Adriele Prina-Mello

Dr. Adriele Prina-Mello is a CRANN Investigator, a Senior Research Fellow of the School of Medicine and a part-time lecture at Trinity College Dublin, the vice-chair of the Nanodiagnostic working group of the European Technology Platform of Nanomedicine, a Nanosafety Cluster member and recently national coordinator of the NANoREG project on regulatory testing of nanomaterials.
Dr Prina-Mello has extensively published his work in biomedicine, nanotechnology, nanotoxicology and nanomedicine research area. Dr Prina-Mello main research interest is on the development of future applications of nanoparticles and nanomaterials in the Biomedical and Bioengineering field.
Dr Prina-Mello is involved in developing and advancing several multidisciplinary research projects between University, Research Hospital and Industry partners for future applications in medicine and nanotechnology industry. Dr. Prina-Mello is involved in several EU FP7 funded projects: Deputy Coordinator of NAMDIATREAM (NMP), dissemination coordinator of MULTIFUN (NMP), and TCD partner of Celtic Alliance for NanoHealth (INTERREG) and Science Foundation Ireland CRANN Pathfinder project.



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Biology Program
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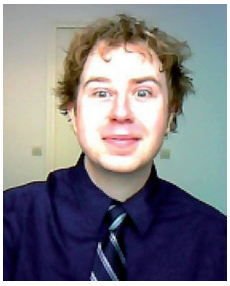
Robert K. Prud'homme is a professor in the Department of Chemical and Biological Engineering at Princeton University and Director of the Engineering Biology Program. He received his BS at Stanford University and his PhD from the University of Wisconsin at Madison under Professor Bob Bird. He has served on the executive committees of the American Institute of Chemical Engineers Materials Science Division and the U.S. Society of Rheology and was the President of the U.S. Society of Rheology. He has served as the chair of the Technical Advisory Board for Material Science Research for Dow Chemical Company, which directs Dow's materials research programs, and he was on the Board of Directors of Rheometric Scientific Inc., the leading manufacturer of rheological instrumentation. He also served on the Nanotechnology Scientific Advisory Committee for BASF, which provided guidance for future trends in nanotechnology for the company. His awards include the NSF Presidential Young Investigator Award, Princeton School of Engineering and Applied Science Outstanding Teaching Award, the Sydney Ross Lectureship at RPI, the Bird,Stewart and Lightfoot Lecturer at the University of Wisconsin, the Dinesh Shah lectureship at the University of Florida, and the Midland Macromolecular Institute Visiting Professor in Midland Michigan. He has been the organizer and Chair of the Gordon Conference on Ion Containing Polymers, and the Society of Petroleum Engineers Forum on Stimulation Fluid Rheology, in addition to organizing numerous sessions at AIChE, ACS, and SOR meetings. He directed the Princeton-University of Minnesota-Iowa State NSF NIRT Center on nanoparticle formation. His research interests include rheology and self-assembly of complex fluids. Systems of interest are biopolymer solutions and gels, surfactant mesophases, and polymer/surfactant mixtures. The goals of the studies are to understand how weak molecular-level interactions can be used to tune macroscopic bulk properties and phase behavior. Application of the work is directed at nanoparticle formation for the drug delivery, controlled release, targeting, and imaging.

Michael Richter

Michael Richter was born in Ludwigshafen/Rh. in 1972 and studied chemistry at the Universities of Mainz and Freiburg i. Br., where he obtained his diploma in 2000. In 2005 he obtained his doctoral degree from the University of Leipzig studying the chemical modification of aldo-/keto-reductases. Afterwards he returned as a Postdoc to the University of Freiburg i. Br., where he worked at the Institute of Pharmaceutical Chemistry on thiamindiphosphate-dependent enzymes, alcohol dehydrogenases and oxidative phenol coupling in fungi. 2009 he joined Empa St. Gallen and is currently head of the Biocatalysis group within the Laboratory for Biomaterials. His personal interest is applied enzyme technology and the investigation of new enzymes inferred from biosynthetic pathways for diverse applications. He considers biocatalysis as a distinct complementary chemical discipline.

Bernd Riebesehl

Dr. Bernd Riebesehl is Principal Fellow & Technical Project Leader in the Pharmaceutical Development Parenteral & Topical Dosage Forms of Novartis Pharma AG, Basel, Switzerland since 2008.
At Novartis he is leading the Parenteral Technology Platform Nanomedicine and the technical development of several parenteral drug products.
He completed his thesis in Pharmaceutical Technology at the Technical University of Braunschweig.
1992 he started his industrial career at Lilly Forschung GmbH in Hamburg leading several teams for preformulation, early phase development and formulation development. In his role as Research Advisor in Pharmaceutical R&D he led several initiatives enabling the formulation of poorly soluble drugs. In 2007 he became Director of Pharmaceutical Development at Speedel Experimenta AG, Basel.



Douglas K. R. Robinson

Managing Director of TEQNODE SARL, Paris
www.teqnode.com
Douglas K. R. Robinson is an expert in innovation studies, mapping of science and technology fields, ELSA and constructive technology assessment (including scenarios and roadmapping).

Originally trained as a physicist and space scientist, he earned his PhD in Constructive Technology Assessment of Nanotechnology as part of the Dutch TA-NanoNed nanotechnology consortium under the guidance of Professor Arie Rip and in combination with the FRONTIERS and Nano2Life networks of excellence. After postdoctoral research in management of innovation, and a 2 ½ year position as Technical Analyst at the Institute of Nanotechnology (UK) he decided to locate himself permanently in Paris and to apply his expertise through his own small firm.
In May 2011 he created a contract research consultancy with Prof. Rip called TEQNODE Limited located in Paris, France, which provides a platform for applied R&D in national and international projects, with clients including the French Ministry of Research and Higher Education and the OECD.
TEQNODE provides strategic intelligence and future-oriented analysis on emerging science and technology fields through contract research and consultancy. With a focus on early stage and emerging fields, where high uncertainty reigns and expectations about

the promising technology are abundant, TEQNODE combines nnovation models to inform analysis and future-oriented assessments of potential innovations to characterise opportunities, challenges and projections of emerging technologies and their embedment in markets and society.
TEQNODE also supports researchers and firms in new and emerging fields of technology and innovation with Societal aspects, Ethics, Governance and Product development aspects, effectively the elements that allow the effective translation of research into applications in society.
At present he has returned to academia as a part-time Post-Doctoral researcher in IFRIS-LATTS (Paris) to gain experience in quantitative analysis of emerging interdisciplinary fields of technology and to contribute his expertise in qualitative analysis of emerging field of science, technology and innovation domains.
Douglas has a number of publications in high-ranked academic peer-reviewed journals including Research Policy and Technology Forecasting and Social Change, and was guest editor of the July 2012 issue of Technology Analysis and Strategic Management journal.

Eder Lilia Romero

Eder Lilia Romero was educated at University of La Plata, Argentina where she obtained her M.A. in Biochemistry and PhD in Exact Sciences (1996). Following a post-doctoral research in Groningen University, The Netherlands under the supervision of Prof. Gerrit Sherphof, she returned to Argentina in 1998. She is a Researcher at the National Council of Scientific and Technological Research (CONICET), Associate Professor of Chemistry at the Department of Science and Technology, National University of Quilmes (UNQ, 2008), and full Professor at the Villa Mercedes National University in San Luis, Argentina (UNViMe,2013), where she is responsible for the first Bioengineering career incorporating Nanotechnology. From 2007 she leads the Nanomedicine Research Program (NRP) in the UNQ. Since 2008 is a member of the Advisory Committee of the Argentinean Foundation for Nanotechnology (FAN). Since 2010, she is funding member and president of the Argentinean Association for Nanomedicines (Nanomed-ar). She has been responsible for the first second and third Nanomedicine Schools in Latino America (2008, 2010, 2012) and Editorial Board Member of ISRN Pharmaceutics (ISRN), Biosafety (OMICS Group) and Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier), and is a consulting expert for local pharmaceutical companies.
Our research interest is focused in developing passively targeted nanomedicines for topical or mucosal delivery of siRNA, anti-parasitic and anti-mycotic agents, anti-inflammatory agents, and non parenteral vaccination strategies.

Barbara Rothen-Rutishauser

Prof. Dr. Barbara Rothen-Rutishauser has received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology (ETH) in Zurich. From 1996 to 2000 she held a post-doctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences at the ETH where she developed and characterised cell culture models for drug transport studies. In 2000 she joined Prof. Peter Gehr's research group at the Institute of Anatomy, University of Bern, Switzerland. During the period of her research B. Rothen-Rutishauser has become an expert in the field of cell-nanoparticle interactions in the lung, with a special focus on lung cell culture models and various microscopy techniques such as laser scanning and transmission electron microscopy. In addition, she has overlooked the establishment of a wide-variety of commonly used and specialist cell biology assay methods within her research group. Since 2011 she is an independent group leader at the Respiratory Medicine, Department of Clinical Research and Bern University Hospital, Switzerland, and since 1st of July 2011 she is the new chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. Alke Fink.

Kumiko Sakai-Kato

Ph.D.

Section Head, Division of Drugs, National Institute of Health Sciences, Ministry of Health, Labour and Welfare

Kumiko Sakai-Kato received her B.S. and M.S. degrees from the University of Tokyo. She developed her carrier as a research scientist at a pharmaceutical company. She received her Ph.D. degree in analytical chemistry at the University of Tokyo in 2004. After postdoctoral work of the Japan Society for Promotion of Science, she became an assistant professor at Musashino University. In 2008, she became a section head of Division of Drugs at the National Institute of Health Sciences.
She is responsible for the research on evaluation method for highly functionalized medicines, such as DDS drugs and nonomedicines. Her present major work is the development of an evaluation strategy of nanomedicines from the standpoint of quality, efficacy and safety. She works as a rapporteur of the Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products which discusses principles for the pharmaceutical development, and non-clinical and early clinical studies of block-copolymer micelles. She is also engaged in the preparation of the Japanese Pharmacopoeia.

Hélder Santos

Born 27 January 1981 in Dalvares–Tarouca, Portugal
Highest Academic degree: D.Sc. Tech. (Chem. Eng.)
Present position: Adjunct Professor (Docent) & Principal Investigator (group leader) in Pharmaceutical Nanotechnology, and Academy of Finland Research Fellow / Associate Researcher
Professional address: Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), FI-00014 University of Helsinki
Professional Phone: +358-9-191-59661; Fax: +358-9-191-59144.
www.helsinki.fi/~hsantos; Email Address: helder.santos@helsinki.fi

Dr. Hélder A. Santos is a physical chemist specialized in electrochemistry, biological mimetic models and soft interfaces. In 2003, he obtained his MSc in Chemistry from the Department of Chemistry, University of Porto (Portugal). In 2007, he obtained his DSc in Chem. Eng. from the Laboratory of Physical Chemistry and Electrochemistry, Helsinki University of Technology (Finland). In 2007, Dr. Santos joined the University of Helsinki, Division of Pharmaceutical Technology, as a Postdoctoral Fellow. In 2009, he was appointed Academy of Finland Postdoctoral Research Fellow.
Currently, he is an Adjunct Professor (Docent) in Pharmaceutical Nanotechnology, an Academy of Finland Research Fellow (Associate Researcher) and Principal Investigator/Group Leader at the Division of Pharmaceutical Technology, University of Helsinki. In 2012, Dr. Santos received the ERC starting grant for 2013-2017 worth of 1.5 M€.
Dr. Santos' research is focused on nanobiomaterials, including nanoporous silica/silicon materials, liposomes and solid lipid nanoparticles for biomedical nano/micro-technological applications, controlled drug delivery, nanomedicine, diagnostics and therapy

(e.g., diabetes/obesity, cancer and cardiovascular diseases). He is the author/co-author of more than 50 refereed articles, including 9 reviews (> 350 citations, h-index = 11), 6 book chapters and more than 67 conference proceedings/abstracts. He has supervised several undergraduate students and ca. 15-MSc students. He supervised/co-supervised 3-PhD students and is currently the supervisor of 2-MSc students, 8-PhD students and 3 post-doctoral researchers. He has also given more than 37 invited/selected talks in national/international meetings/conferences/lectures.
In 2010, he received the "Talent Prize in Science" awarded by the Portuguese Government (TV-broadcasted for more than 15 million people). Recently, he was awarded with the Young Researcher's Prize 2013 worth of 10 000 € by the Faculty of Pharmacy, University of Helsinki.
Dr. Santos is also an active referee for about 34 international journals and evaluator for 4 international research foundations (The Leverhulme Trust, UK; Research Foundation Flanders, Belgium; The Swiss National Science Foundation, Switzerland; and The Netherlands Organisation for Scientific Research (NWO), The Netherlands). Dr. Santos is also an Editorial Board Member for the journals of Drug Delivery Letters, Biomatter, International Journal of Nanotechnology and Nanoscience, Pharmaceutical Nanotechnology, World Journal of Pharmacology. He is also Associate Editor for Versita in the Chemistry field, the Editor of the recently launched Mesoporous Biomaterials journal and the Editor of a book entitled "Porous Silicon for Biomedical Applications" (in preparation), Woodhead Publishing.
With only 32-years old, Dr. Santos' research career and development portfolio since 2009 totals ~3 M€ awarded by several projects from the European Research Council, the Academy of Finland, and the University of Helsinki Research Funds, among others.

Angela Schipanski

Angela Schipanski was born in 1981 in Germany. After finishing her school education in Ilmenau (Germany) she studied Biology at the University of Würzburg (Germany). She graduated with a Diploma in Neurobiolgoy in 2007. She did her PhD at the Department of Neuropathology at the University Medical Center Hamburg-Eppendorf (UKE)(Germany) studying the mechanisms of neuronal death in FENIB (familial encephalopathy with neuroserpin inclusion bodies), a unique conformational dementia. During this time, Angela was an associated member of the research training group 1459 „Sorting and interactions between proteins of subcellular compartments" of the German Research Foundation. After successfully defending her PhD in 2011 she started in 2012 as a postdoc in the field of medical technology and engineered nanomaterials (ENMs) at the Swiss Federal Institute for Materials Science and Technology (Empa) in St. Gallen (Switzerland).

Ruth Baumberger Schmid

Year of birth 1952
Nationality Swiss
Position Chief Business Developer
Institute SINTEF Materials and Chemistry / Synthesis and Properties

EDUCATION

Diplomierter Naturwissenschafter (Dipl. Natw.), Eidgenössische Technische Hochschule (ETH) Zürich, 1975; Doktor der Naturwissenschaften (Dr. Sc. Nat.), Eidgenössische Technische Hochschule (ETH) Zürich, 1979; Highschool teacher in chemistry (Befähigungsausweis für das höhere Lehramt, Fach Chemie), ETH Zürich, 1977

EXPERIENCE

- 1980 Postdoctoral research at the Institute of Organic Chemistry, NTH
- 1981-1994 Research Scientist at SINTEF Applied Chemistry
- 1989-1991 Lecturer in physical organic chemistry at NTH
- 1994-1997 Senior Research Scientist at SINTEF Applied Chemistry
- 1997-2003 Research Director at SINTEF Applied Chemistry
- 2003-2004 Senior Research Scientist at SINTEF Applied Chemistry
- 2004-2011 Research manager at SINTEF Materials and Chemistry, Department of Synthesis and Properties, Research Team Polymer Particles and Surface Chemistry
- 2010 - Lecturer in Nanomedicine at NTNU
- 2011 Senior Research Scientist at SINTEF Materials and Chemistry, Department of Synthesis and Properties, Research Team Polymer Particles and Surface Chemistry
- 2011 - Chief Business Developer at SINTEF Materials and Chemistry

MAIN FIELDS OF COMPETENCE

- Scientific competence: Particle technology, encapsulation of solids and liquids, surface modification of polymers and composites, interactions between polymer surfaces and biological materials, targeted and controlled release, biodegradable polymers, biomaterials, nanomedicine, medical technology, organic chemistry
- Business Development: Development of SINTEF's strategy in Life Sciences including Biotechnology, SINTEF's strategy in Medical Technology, a technology platform to prepare nano- and microparticles and -capsules based on the miniemulsion process for a broad variety of applications
- Management: Research Management, project management

PROFESSIONAL MEMBERSHIPS

- Member of the American Chemical Society
- Member of the Controlled Release Society (Board of Directors 2009 – present, Secretary 2012-2013)
- Member of the European Technology Platform in Nanomedicine
- Member of the External Advisory Board of the ERA-Net EuroNanoMed
- Vice Chair and member of the Management Committee of the COST Action TD1004

PUBLICATIONS

39 scientific publications, 16 patent and patent applications, 44 oral presentations, 12 poster presentations, 13 webinar, mass media and popular science publications.

SELECTED PUBLICATIONS

1. H. Johnsen & **R. Schmid**, J. Microencapsulation 24, 731-742 (2007). "Preparation of polyurethane nanocapsules by miniemulsion polyaddition."
2. **R. Schmid** & H. Johnsen, 34th Annual Meeting & Exposition of the Controlled Release Society, 7.-11.7.2007, Long Beach, CA, USA. "Biodegradable and biocompatible nanocapsules and particles prepared by the miniemulsion polymerisasjon."
3. S. Kubowicz, P. Stenstad, H. Johnsen & **R. Schmid**, 38th Annual Meeting & Exposition of the Controlled Release Society, 31.7.-3.8.2011, National Harbour, Maryland, USA. "Protein-Coated Biodegradable Poly(butyl-2-cyanoacrylate) Nanoparticles with Stealth Surface Properties."
4. **R. Schmid**, P.M. Stenstad & Y. Mørch, "Ultrasound bubbles stabilized with multifunctional nanoparticles for combined diagnostics and therapy", International Workshop COST Action TD1004, 17.-18.2.2012, Torino, Italy.
5. S. Armada, **R. Schmid**, W. Equey, I. Fogoaga & N. Espallargas, J Thermal Spray Techn. 22, 10-17 (2013); DOI: 10.1007/s11666-012-9847-x (2012). "Liquid-Solid Self-Lubricated Coatings."

Simó Schwartz

Dr Simó Schwartz Jr (1967th, Barcelona) obtained his Medical degree in 1991 at the Faculty of Medicine of the Autonomous University of Barcelona, where he also got his PhD in 1996 working on oncogenic signalling pathways in prostate cancer. He was a research fellow in the New York Univ. Medical Center in 1993, working on molecular mechanisms related to Ras/Raf activation in transgenic mice. In 1996 he moved to California as postdoctoral research at the Burnham Institute for Biomedical Research, where he work intensively in the molecular pathways involved in the development of colorectal cancer. In 2004, Dr Schwartz start several collaborations with biotech companies in the field of diagnostic and prognostic biomarkers and new therapeutic targets in colorectal cancer. The success of these collaborations lead to 12 patents transfered to leading companies of the biotech and pharma sectors. In 2010 he was appointed as Director of Nanomedicine at the CIBBIM-Nanomedicine of the Vall d'Hebron Hospital Research institute (VHIR) and coordinator of its strategic action plan for R+D, which is focused on the research of new biomedical nanotechnology-based applications. In particular, new drug delivery systems, image based diagnostic systems and preclinical validation of therapeutic conjugates and bio-nanosensors, mainly in the areas of oncology and rare diseases. He is also member of the Science Committee of the Vall d'Hebron Research Institute. and member of the Science Advisory Board of Oryzon Genomics, and Co-founder of ARGON Pharma a Spanish leading biotech company. He also leads several national and international projects involving SME's in which animal models are being used for preclinical validation of new therapies directed against cancer. Since 2011, Dr Schwartz Jr acts as the Associate Director of the "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers a total of 50 research groups of national excellence in the field of nanotechnology and nanomedicine.

Giacinto Scoles

Adjunct professor – University of Udine
Distinguished scientist at ELETTRA Synchrotron Light Source Laboratory in Triest
P.I. of an Advanced Grant from the ERC
Donner Professor of Science, Emeritus, Princeton University
Distinguished Adjunct Prof. of Biology, Temple University - Philadelphia

PERSONAL STATEMENT

GIACINTO SCOLES' scientific career has spanned an unusually long length of time and an equally unusually broad range of subjects. The general philosophy was to exploit new physical ideas and novel instrumentation to solve outstanding problems in chemistry and materials science before, and biology and medicine now. Much before the coming of age of Nanotechnology and Nanoscience, particular emphasis was given to the behavior of nano systems and materials. His half-century-long research career in five lines:

- late '50s Mass spectroscopy
- '60s Transport properties and intermolecular forces
- '70s Intermolecular forces, Crossed molecular beam scattering
- '80s Noble gas clusters
- '90s Surface science, Fluid Helium clusters
- 2000s Manipulation of biomolecules, Biomolecular interaction at the nanoscale
- 2010s - Nanomedicine

POSITIONS AND HONORS

- 2011 to date: Adjunct Professor, University of Udine, Faculty of Medicine, Department of Biological and Medical Sciences, Ospedale Universitario Santa Maria della Misericordia Building #13, Udine , Italy.
- 2011 to date: Distinguished scientist at ELETTRA Synchrotron Light Source Laboratory in Trieste (It).
- 2011 to date: Holder of an Advanced Grant from the ERC within the Program IDEAS at the Univ. of Udine
- 2008 to date: Donner Professor of Science, Emeritus, Princeton University, Princeton, NJ 08544, USA and Distinguished Adjunct Prof. of Biology, Temple University, Philadelphia, PA, (USA).
- 2003-2010: Professor of Biophysics at the Scuola Internazionale Superiore di Studi Avanzati (SISSA) Miramare (Trieeste) Italy;
- 2009: Senior Consultant to the Inter. Center for Science and High Technology of the United Nations Industrial Development Organization (ICS-UNIDO) responsible for the Nanotechnology and Nano Drug Delivery Group;
- 2005-2009: Scientific Coordinator of LANADA the Laboratory for NAno Diagnostic, Drug Delivery and Analysis of CBM The Consortium for Biomolecular Medicine in Trieste (Italy).
- 2003-2009: Collaborator of ELETTRA, Sincrotrone Trieste S.C.p.A. Basovizza (Trieste), Italy;
- 1987-2008: Donner Professor of Science at Princeton University Chemistry Department and Princeton Materials Institute;
- 1971-1986: Prof. of Chemistry and Physics Univ. of Waterloo, Waterloo, Canada;
- 1982-1985: Director of the Center for Molecular Beams and Laser Chemistry, University of Waterloo.(Canada);
- 1977-1979: Professor of Solid State Physics, University of Trento, Italy;
- 1974-1975: Acting Director, in its founding year, of the Guelph-Waterloo Centre for Graduate Work in Chemistry.
- 1968-1971: Assoc. Prof., Physics Dept., University of Genova, Genova, Italy;
- 1964-1968: Assist. Prof., Physics Dept., University of Genova, Genova, Italy;
- 1961-1964: Research Associate, Kamerlingh-Onnes Laboratory, University of Leiden, Leiden, The Netherlands
- 1960-1961: Assist. Prof., Physics Dept., University of Genova, Genova, Italy.

HONORS AND AWARDS

- 2006 Benjamin Franklin Medal in Physics (with J.P.Toennies) from the Franklin Institute.
- 2006 Research Prize of the Chemistry Faculty of the University of Bochum
- 2003 Creativity Award from the NSF 2003-5
- 2004 Texas A&M University, Frontiers in Chemical Research Lecturer
- 2004 Moscowitz Lecturer at the University of Minnesota
- 2003 Distinguished Visiting Professor, University of Florida, Gainesville.
- 2003 Earle K. Plyler Prize for Molecular Spectroscopy from the American Physical Society (with Kevin K. Lehmann).
- 2002 Peter Debye Award in Physical Chemistry from the American Chemical Society
- 2001 H. E. Gunning Lecturer, Dept. of Chem., University of Alberta
- 2000 Elected Foreign Member of The Royal Netherlands Academy of Arts and Sciences
- 2000 Honorary Science Doctorate from the University of Waterloo
- 1999 Samuel M. McElvain Lecturer, University of Wisconsin–Madison

1997 Elected Fellow of The Royal Society (United Kingdom)

- 1996 Recipient of an Honorary Doctorate in Physics from the University of Genoa
- 1995 Recipient of a Senior Fellowship of the Alexander von Humboldt Foundation
- 1995 Recipient of the 1995 Lippincott Award of the Optical Society of America, the Coblentz Society, and the Society for Applied Spectroscopy
- 1986 Senior Killam Fellowship.

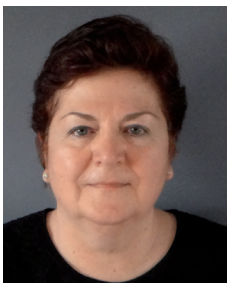
RESEARCH SUPPORT

Giacinto Scoles has been recently granted an advanced ERC grant (2011, MONALISA QUIDPROQUO, MOlecular NAnotechnology for LIfe Science Applications: QUantitative Interactomics for Diagnostics, PROteomics and QUantitative Oncology) of nearly 3M€ over 5 years for an ambitious collaborative research project. The focus

and the goal of his research is to introduce innovative devices and protocols (based on micro/nano-fluidics and on the nano-mechanical response of bio-molecular nano-strucures) to carry out precise, quantitative and low cost measurements on large, predetermined diagnostically relevant, subsets of the proteome obtained from very small samples in samples produced by a very small number of cells or within single cells with potential capability of measuring its interactions (Interactomics). By means of these measurements he hopes to make new inroads into quantitative diagnostics and disease monitoring.
Prof. Scoles is partner of an italian project recently granted by AIRC (Italian Association for Cancer Research) which aims at generating an innovative nanotechnology-based intrinsically high throughput platform to carry out real-time, highly sensitive, and inexpensive quantification of circulating biomarkers for cancer risk assessment (project title: "Application of Advanced Nanotechnology in the Development of Innovative Cancer Diagnostics Tools). Within this project, Scoles will introduce novel strategies to improve sensitivity and specificity of protein-protein (such as the antigen/antibody) recognition in all the diagnostic systems adopted by the rest of the team. During his tenure at Princeton Scoles had, at steady state, three major grants: one from the NSF, one from DOI, and one from AFOSR for a total of about half a million dollar per year from 1993 to 2003. Furthermore Scoles was one of the main PIs who obtained from the NSF the original MRSEC grant (15 M$ over 5 years). In Italy, Scoles could count on a starting package to equip SENIL of about 200,000 euros from Elettra and an operating budget of 30,000 euros each per year from SISSA and ELETTRA. Finally, SENIL also enjoyed support from the Italian Institute of Technology for about 50,000 euros per year from 2006 to 2010. Finally, at CBM during the last three years has administered two major grants one CIPE grant from the Ministry of Italian Research for 2 million euros over 4 years to support the work of Dr. Krol at CBM. This grant was originally obtained by Prof. E. Di Fabrizio before he moved to University of Catanzaro. Finally, from 2006 until 2009 Scoles also administered a group grant from the European commission of 2 million euros for Nanotechnological Infrastructure (BINASP).

Joachim Seelig

Since 1972 Joachim Seelig carried out research, first, as Assistant Professor and since 1982 as Full Professor of Structural Biology at the Biozentrum of the University of Basel. He was one of the founding members of the research institution and was its chairman from 1997 to 1999 and from 2000 to 2009. Joachim Seelig developed special biophysical methods for detailed measurements of the structure and the thermodynamic behavior of cell membranes. For his research he was awarded with the Cloëtta Prize and the Julian Sturtevant Award for Outstanding Contributions to Experimental Thermodynamics. Furthermore, Joachim Seelig has been a member of numerous scientific associations including the Royal Netherlands Academy of Arts and Science as well as the president of the Swiss Foundation for Excellence and Talent in Biomedical Research.



Hripsime Shahbazian

Mrs. Hripsime Shahbazian holds a MSc. in Medical Physics and a BSc in Molecular Physics. She worked as a Research Assistant in Physics Department at Carleton University while working towards her Master of Science degree. She joined Health Canada in 1988 as a Technology Assessor at the Medical Devices Bureau (MDB) and from 1991 to 1998 she was acting in different managerial roles within the Bureau. While at the MDB she contributed to the development and finalization of the new Medical Devices Regulations. Mrs. Shahbazian chaired and participated in various working groups involved in planning and implementation of the new regulations. In 1998 Mrs. Shahbazian joined the Office of Science within the Therapeutic Products Directorate (TPD), at the Health Products and Food Branch (HPFB) as an Associate Manager. She is currently a Senior Science Advisor in the Office of Science. Her duties include management of the activities of Scientific/Expert Advisory Committees Committees, Scientific/Expert Advisory Panels, Reconsiderations and Second Level of Appeals. Mrs. Shahbazian is one of the key members working on the development and implementation of nanotechnology related activities at Health Canada. She chairs the Directorate (TPD) and Branch (HPFB) Working Groups on Nanotechnology and coordinates Nanotechnology related International activities for regulated health products for the Branch. She is a member of the Health Portfolio Nanotechnology Working Group composed of key officials across the department, coordinating departmental approach to science, policy and research needs for nanotechnology. Mrs. Shahbazian was a member of the Health Portfolio team that developed the Policy Statement on Health Canada's Working Definition for Nanomaterial.

Andrey Solokov

Phone	+47 93 26 25 74
Date of birth	04.01.1981
E-mail	Sokolov.andrej@gmail.com

WORK EXPERIENCE

• 2007-2008 Anesthesiologist; Research Institute of Surgery named by A.V. Vishnevsky, Moscow, Russia; Providing of anesthesia for patients with cardio-vascular diseases (atherosclerosis of carotid artery, ischemic heart disease, aortal malformations). Duties at the department of anesthesia and intensive care and providing of anesthesia for the patients with acute diseases.
• 2007-2008 Product manager, anesthesiology and urology; «Rean-med» company, Moscow, Russia; Communication with head quarter in Europe and translation of educational materials, advertisement, and scientific articles from English to Russian. Communication and meetings with opinion leader in urology, physicians at hospitals and presentation of new products. Participation in a business meeting with international partners. Participation in a scientific conferences and product demonstration to potential clients.
• 2006-2008 Project manager; Publishing group «Geotar-med»; Developing of publishing plan for specialty «Cardiology» and «Nephrology». Meetings with authors and coordination of writing and publishing processes. Successful management and coordination of the «National guideline in cardiology» and «National guideline in kidney disease»

EDUCATION

• 2008-2012 PhD-program, Institute of Immunology, University of Oslo
2 • 007-2008 Postgraduate education in anesthesiology and intensive care, Research Institute of Surgery named by A.V. Vishnevsky
• 2004-2007 Postgraduate education in anesthesiology and intensive care, I.M. Sechenov Moscow Medical Academy
• 2000-2004 General medicine, I.M. Sechenov Moscow Medical Academy

EDUCATIONAL COURSES

• 2012 Medical practice at the Department of Gastrosurgery, Oslo University Hospital
• 2010 Introduction to statistical methods, The Medical Faculty, UiO
• 2009 Flow cytometri i medisinsk forskning og diagnostikk, The Medical Faculty, UiO
• 2008 Course in Laboratory Animal Science, Norwegian School of Veterinary Science

• 2006 Medical practice at the University Hospital Rikshospitalet in according to program of President scholarship. Basic training at the Department of anesthesiology and Intensive care and participation in work of transplantation team.

RESEARCH EXPERIENCE

• 2008-2012 PhD program at the University of Oslo. Have participated in research project in the field biomaterials (immune response to implantation), inflammatory disorders (sepsis-model), and complement activation (at the patients under liver resection and colon cancer resection).
• 2004-2007 Research work at the Department of Organ Transplantation, Russian Research Center of Surgery. Evaluation of inflammatory response during liver and kidney transplantation
• 2006-2007 Research practice at the Department of anesthesiology, Rikshospitalet. Evaluation of hemodynamic disorders at the patients under coronary surgery, statistical analysis of data

AWARDS

• 2012 Best poster presentation on translational research, European Congress on Biomaterials, Dublin, 2011
• 2006 National award for 1 year reserch training in European University
• 2005 Diploma for the Best student scientific work from Russian Academy of Medical Science, 2005
• 2004 Best oral presentation, European Student Congress, Berlin

LIST OF SCIENTIFIC PUBLICATIONS

1. Halvorsen, P. S., Sokolov, A., Cvancarova, M. et al. Continuous cardiac output during off-pump coronary artery bypass surgery: pulse-contour analyses vs pulmonary artery thermodilution. Br.J.Anaesth. 2007;99:484-492.
2. Johannessen E, Krushinitskaya O, Sokolov A et al. Toward an injectable continuous osmotic glucose sensor. J Diabetes Sci Technol. 2010 Jul 1;4(4):882-92
3. Sokolov A, Hellerud B.C, Pharo A, Johannessen E.A, Mollnes T.E. Complement activation by candidate biomaterials of an implantable microfabricated medical device. J Biomed Mater Res B Appl Biomater 2011;98B(2):323-9.
4. Sokolov A., Hellerud B.C., Lambris J.D., Johannessen E.A., Mollnes T.E. Activation of polymorphonuclear leukocytes by candidate biomaterials for an implantable glucose sensor. J Diabetes Sci Technol., November 2011, Volume 5, Issue 6: pages 1490-1498
5. Barratt-Due A., Johansen H.T., Sokolov A., Thorgersen E.B., Hellerud B.C., Reubsaet J.L., Seip K.F., Tønnessen T.I., Lindstad J.K., Pharo A., Castellheim A., Mollnes T.E., Nielsen E.W. The role of bradykinin and the effect of the bradykinin receptor antagonist icatibant in porcine sepsis. Shock 2011 Nov;36(5):517-23
6. Sokolov A, Hellerud BC, Johannessen EA, Mollnes TE. Inflammatory response induced by candidate biomaterials of an implantable microfabricated sensor. J Biomed Mater Res A. 2012 May;100(5):1142-50
7. Kvarnström A, Sokolov A, Swartling T, Kurlberg G, Mollnes TE, Bengtsson A. Alternative pathway activation of complement in laparoscopic and open rectal surgery. Scand J Immunol. 2012 Jul;76(1):49-53
8. Barratt-Due A, Sokolov A, Gustavsen A, Hellerud BC, Egge K, Pischke SE, Lindstad JK, Pharo A, Castellheim A, Thorgersen EB, Mollnes TE. Polyvalent immunoglobulin significantly attenuated the formation of IL-1β in Escherichia coli-induced sepsis in pigs. Immunobiology. 2012 Aug 9. [Epub ahead of print] PubMed PMID: 22947599
9. Sokolov A, Hellerud BC, Tønnessen TI, Johannessen EA, Mollnes TE. Activation of coagulation and platelets by candidate membranes of implantable devices in a whole blood model without soluble anticoagulant. J Biomed Mater Res A. 2013 Feb;101(2):575-81

Andrei P.Sommer

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University of Ulm
Albert-Einstein-Allee 47
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Germany
andrei.sommer@uni-ulm.de
samoan@gmx.net

ACHIEVEMENTS IN SCIENCE AND MEDICINE

Principle for the treatment of Alzheimer's disease with light and drugs
Method to kill cancer cells and microorganisms with light and drugs
Method to improve sperm performance for in vitro fertilization
Method to treat peripheral neuropathy with light

• 1999 – University of Ulm:
Senior Scientist, Institute of Micro and Nanomaterials
Senior Scientist, Central Institute of Biomedical Engineering

• 1992 – 1998 Philipps University:
PhD Physical Chemistry, Dentistry (Minor) 1998
Diploma Physics 1992

RESEARCH FOCUS

Biomaterial design: predicting biocompatibilities via biomimetic modelling
Controlled release: nanoscale drug delivery systems triggered by light
Cell metabolism: light activated uptake and clearance programs
Culture devices: strategies to improve cell performance

PUBLICATIONS

As first author >100

Stephan Stern

Ph.D., DABT, Senior Scientist

Dr. Stern is actively involved in applied nanomaterial research, and has published numerous articles on nanomaterial toxicology and pharmacokinetics in leading scientific journals. In his position at the Nanotechnology Characterization Laboratory (NCL), Dr. Stern is involved in the design and implementation of pharmacology and toxicology studies used to characterize nanoparticle disposition and assess nanoparticle biocompatibility. Data generated is used to support IND regulatory filings and aid in environmental risk assessment. Additional responsibilities include representing NCL at local and national nanotechnology meetings and the education of study personnel regarding nanoparticle safety issues.

Prior to joining the NCL, Dr. Stern was a Post-Doctoral Fellow at the University of North Carolina - Chapel Hill in the Division of Drug Delivery and Disposition, and Curriculum in Toxicology. In this position, his research focused on examining the role of intestinal metabolism in modulating the gastrointestinal toxicity of chemotherapeutic agents. His areas of expertise include biochemical toxicology of the liver and kidney, analytical methodology and drug metabolism/pharmacokinetics. He received his B.S. degree in biochemistry from the University of Rochester and his Ph.D. in toxicology from the University of Connecticut at Storrs. Dr. Stern is a Diplomate of the American Board of Toxicology.

Gert Storm

Professor Gert Storm studied biology at the Utrecht University, The Netherlands. He graduated in 1983. He obtained his Ph.D. degree in 1987 at the Dept. of Pharmaceutics of the same university. His research interests are in the fields of biopharmaceutics and drug targeting. In 1988-1989 he was a visiting scientist at Liposome Technology Inc. in Menlo Park, USA, and visiting assistant professor at the School of Pharmacy, UCSF, San Francisco. In 1990-1991 he was senior research scientist at Pharma Bio-Research Consultancy B.V. in Zuidlaren, The Netherlands. During this period he contributed to the design, co-ordination and evaluation of clinical pharmacological studies. In September 1991 he took up his position at the Utrecht University. In 1999, he was appointed adjunct professor at the Royal School of Pharmacy, Copenhagen. From July 2009 on, he is Honorary Professor in Biomacromolecular Drug Delivery at the University of Copenhagen. In 2000, he was appointed as professor (Targeted Drug Delivery) at Utrecht University. From 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente. Furthermore, he is active at the University Medical Center Utrecht (UMCU) within the CBOI institute (Centre for Image-Guided Oncological Interventions).
He is author/co-author of more than 400 original articles, reviews and book chapters, in the field of advanced drug delivery/drug targeting, and theme (co-)editor of Advanced Drug Delivery Reviews and the book 'Long Circulating Liposomes. Old Drug, New Therapeutics'. He was co-ordinator of an Integrated Project (FP6) on targeted nanomedicines (MediTrans) based on the collaboration of 30 european partners and funded by the EC and industry. He is program director of the program Drug Delivery embedded within the recently approved New Nano Initiative (NanoNextNL) strongly sponsored by the dutch government and industry. He is also principal investigator of a national industry-academia partnership (HIFU-CHEM) studying the clinical application of MRI-guided high-intensity focused ultrasound (HIFU) to improve cancer chemotherapy with temperature-sensitive targeted nanomedicines. He is course director of the GUIDE/UIPS/LACDR Course on Advanced Drug Delivery & Drug Targeting, co-sponsored and accredited by EUFEPS and the GALENOS Network, and held in The Netherlands. He is on the Board of Scientific Advisors (BSA) of the Controlled Release Society (CRS). He is on the Scientific Board of the spin-off company Enceladus Pharmaceuticals BV (Amsterdam). He is involved in organizing conferences in the field of advanced drug delivery, e.g. chairman of the ESF-UB Conference "Nanomedicine: Reality Now and Soon", held 23-28 October 2010 in San Feliu de Guixols, Spain. He is member of the editorial (advisory) board of a variety of scientific journals. He is on the board of the Dutch Society for Gene Therapy. He was involved in the foundation and is currently on the board of the European Society for Nanomedicine (ESNAM/CLINAM) and The Netherlands Platform for Targeted Nanomedicine (NPTN).
contact: g.storm@uu.nl

Erik Stroes

Prof Dr ESG, MD
Chair Department of Vascular medicine, AMC, Amsterdam

EDUCATION/TRAINING

- Master: 1990 Rotterdam
- PhD: 1997, Utrecht
- Clinical Specialization: Internal medicine UMCU, 1998
- Subspecialization: Vascular Medicine, AMC, 2001

POSITIONS AND HONORS POSITIONS AND EMPLOYMENT

- 1997-1999 Fellow department of Nephrology, UMCU
- 1999-2001 Utrecht Staff member department of internal medicine
- 2001-2009 UMCU, Utrecht Head outpatient clinic, dept of vascular medicine, AMC
- 2010 - Chair department of Vascular medicine, AMC, Amsterdam

Other Experience and Professional Memberships

- Member Vascular Biology working group
- Chair Dutch Atherosclerosis society
- Board member Section Vascular Medicine (NIV)
- Member basic science council Arteriosclerosis Thrombosis Vascular biology
- Former chair of the Internist Vascular Society (IVG)

HONORS AND AWARDS

- Vascular biology science price 1998

SELECTED PEER-REVIEWED PUBLICATIONS

Selected from last 4 years, out of 196:

1. van Leuven SI, van Wijk DF, Volger OL, de Vries JP, van der Loos CM, de Kleijn DV, Horrevoets AJ, Tak PP, van der Wal AC, de Boer OJ, Pasterkamp G, Hayden MR,Kastelein JJ, **Stroes ES**. Mycophenolate mofetil attenuates plaque inflammation in patients with symptomatic carotid artery stenosis. *Atherosclerosis*. 2010 Feb 6.
2. Vergeer M, Brunham LR, Koetsveld J, Kruit JK, Verchere CB, Kastelein JJ, Hayden MR, **Stroes ES**. Carriers of loss-of-function mutations in ABCA1 display pancreatic beta-cell dysfunction. *Diabetes Care*. 2010 Apr;33(4):869-74.
3. Visser ME, Akdim F, Tribble DL, Nederveen AJ, Kwoh TJ, Kastelein JJ, Trip MD, **Stroes ES**. Effect of apolipoprotein-B synthesis inhibition on liver triglyceride content in patients with familial hypercholesterolemia. *J Lipid Res*. 2010 May;51(5):1057-62.
4. **Stroes ES**, Kastelein JJ, Bnardeau A, Kuhlmann O, Blum D, Campos LA, Clerc RG, Niesor EJ. Dalcetrapib: no off-target toxicity on blood pressure or on genes related to the renin-angiotensin-aldosterone system in rats. *Br J Pharmacol*. 2009 Dec;158(7):1763-70.
5. Franssen R, Sankatsing RR, Hassink E, Hutten B, Ackermans MT, Brinkman K, Oesterholt R, Arenas-Pinto A, Storfer SP, Kastelein JJ, Sauerwein HP, Reiss P, **Stroes ES**. Nevirapine increases high-density lipoprotein cholesterol concentration by stimulation of apolipoprotein A-I production. *Arterioscler Thromb Vasc Biol*. 2009 Sep;29(9):1336-41.
6. Mingozzi F, Meulenberg JJ, Hui DJ, Basner-Tschakarjan E, Hasbrouck NC, Edmonson SA, Hutnick NA, Betts MR, Kastelein JJ, **Stroes ES**, High KA. AAV-1-mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. *Blood*. 2009 Sep 3;114(10):2077-86.
7. **Stroes ES**, Nierman MC, Meulenberg JJ, Franssen R, Twisk J, Henny CP, Maas MM, Zwinderman AH, Ross C, Aronica E, High KA, Levi MM, Hayden MR, Kastelein JJ,Kuivenhoven JA. Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. *Arterioscler Thromb Vasc Biol*. 2008 Dec;28(12):2303-4.
8. Kastelein JJ, Akdim F, **Stroes ES**, Zwinderman AH, Bots ML, Stalenhoef AF, Visseren FL, Sijbrands EJ, Trip MD, Stein EA, Gaudet D, Duivenvoorden R, VeltriEP, Marais AD, de Groot E; ENHANCE Investigators. Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med*. 2008 Apr 3;358(14):1431-43.
9. Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JA, Ince C, Holleman F, Diamant M, Heine RJ, Hoekstra JB, Kastelein JJ, **Stroes ES** Vink H. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. *Diabetes*. 2006 Apr;55(4):1127-32

RESEARCH SUPPORT

Ongoing Research Support:

- Dutch Heart Foundation: HDL and regulation of steroid synthesis
- Dutch Heart Foundation: testing of HDL-protective capacity in humans
- STREP-EU: HDL-omics approach assess HDL-increasing strategies in humans
- CTMM: Glycocalyx as predictive tool in humans (work package in large CTMM application) - Industrial:
- Merck: Educational grant for development of 3T carotid MRI as surrogate marker for CV-disease - ISIS: Educational grant for application of antisense strategies in animal models

Completed Research Support

- Dutch Kidney foundation: Career stimulation program: Gene therapeutic approaches to improve shunt survival in hemodialysis
- ZON NW: gene therapy in patients with LPL deficiency
- Dutch Heart foundation: glycocalyx in health and disease
- Dekker fellowship: endothelial function as a tool to predict vascular risk

(co-)promotorships:

- 2002-2010: 11 co-promotorships
- 2010 – ongoing: 10 PhD students

RESEARCH INTEREST:

Over 2 decades now, Stroes has focused at the role of the vessel wall in atherogenesis development. In the late nineties, modulation of endothelial nitric oxide synthase (co-factor suppletion, gene therapy) was a major topic for research, leading to the first demonstration of the relevance of nitric oxide synthase uncoupling in humans with dyslipidemia.

Since his transition to the AMC, Stroes have focused at lipid disorders in relation to atherogenesis. He has participated in numerous lipid lowering trials using surrogate markers such as intima media thickness (ENHANCE study) and flow mediated dilation. More recently, 3T-MRI has been added as surrogate marker for vascular disease progression.

In addition, novel gene defects contributing to lipid disorders (HDLc, hypertriglyceridemia) have been pursued by collecting autosomal dominant families with these disorders.

In parallel, Stroes has been involved in the development of novel therapeutic moieties for dyslipidemia, such as Lipoprotein lipase gene therapy, apoB antisense, reconstituted HDL-infusion and other novel compounds. Stroes has published more than 195 papers in peer reviewed journals. He currently chairs the department of vascular medicine at the AMC, Amsterdam. The Netherlands.

Rudolf W. Strohmeier

Deputy Director-General of DG RTD since 2010, responsible for Research Programmes.

After studies of Law and Economics at the University of his home town Würzburg and that of Bonn, he started his career as teaching assistant at the Department of Civil and Commercial Law at the University of Würzburg in 1979. In 1980, he joined the Bavarian Liaison Office to the Federal Government in Bonn. Following a 2-years detachment to the Development Policy department of the European Commission, he established the Bavarian Information Office in Brussels and became its first Director.

From 1987, he served in the three Cabinets of Commissioner Peter Schmidhuber (Macroeconomics and Regional Policies, later Budget and Budget Control). In 1995, Agriculture Commissioner Franz Fischler appointed him Deputy Head of his Cabinet.

Nominated Head of Division in DG Agriculture and Rural Development in 2000, he joined the Cabinet of Commission President Prof. Romano Prodi in 2003 as Adviser i.a. for industrial, agricultural and environmental policies. From 2005-2010, he served as Head of Commissioner Viviane Reding's 2nd Cabinet (Telecommunication and Media).

Robert Sum

- 1991 Diploma in Physics at University of Basel
- 1995 PhD in Condensed Matter Physics with Prof. Dr. H.-J. Güntherodt, University of Basel
- 1997 Founder of Nanosurf AG together with Dr. Lukas Howald, and Dominik Brändlin.
- 1997-2003 working as Head of Sales & Marketing and Chairman of the board of Nanosurf AG
- 2003-2009 CEO of Nanosurf AG
- Since 2009 Head of Marketing & Businessdevelopment and Member of the Board of Directors of Nanosurf AG

Wojciech Swieszkowski

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fax.: + 48 22 234 87 50
e-mail: wojciech.swieszkowski@inmat.pw.edu.pl
www.bio.materials.pl

EDUCATION

- 1989-1994 M.Sc., Bialystok University of Technology, Poland
- 1997 - 2000 PhD, Warsaw University of Technology, Poland

2011 Habil., Warsaw University of Technology, Poland

RESEARCH AND PROFESSIONAL EXPERIENCE

- 1994-1997 Assistant, Scientific-didactic worker, Bialystok University of Technology, Faculty of Mechanical Engineering
- 1996-2000 Postgraduate Student (PhD), Warsaw University of Technology, Faculty of Power and Aeronautical Engineering
- 2000-2004 PostDoc, Biomaterial Laboratory, Faculty of Aerospace Engineering, Delft University of Technology, The Netherlands
- 2004- Senior Researcher, Assoc. Professor, Division of Materials Design, Faculty of Materials Science and Engineering, Warsaw University of Technology, Poland

RESEARCH TOPICS

biomaterials, including biomaterial synthesis, cell-biomaterial interactions, smart biomaterials for tissue regeneration, drug delivery, advanced design, fabrication and fabrication of scaffolds

PUBLICATIONS

- 100 publications in national and international journals and proceedings
- Index H=9, no of citations: 326
- co-author of 6 book chapters
- participation in more than 40 intentional and national conferences
- invited lectures/key lectures in Poland and abroad, i.e. Mayo Clinic w USA, AMC Amsterdam, the Netherlands, KU Leuven, Belgium, Helsinki University, Finland, NIMS, Japan
- 3 patent

AWARDS

- laureate of a 2nd degree Group Prize from the Rector of the Warsaw University of Technology for scientific achievement (1997)
- laureate of a 2nd Prize of dr. Komora (2000)
- laureate of a 1st Prize of Prime Minister of Poland (2001)
- laureate of a 2nd Prize of European Society of Biomechanics for the best presentation (2004)
- laureate of a 2nd degree Prize from the Rector of the Warsaw University of Technology for scientific achievement (2012)

MEMBERSHIPS

- membership in Polish Biomechanics Society
- member of European Society of Biomechanics and European Society of Biomaterials

FUNDING

Selected national grants:

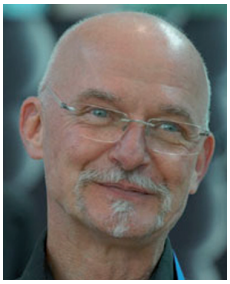
- Hydrogel/titanium implants for shoulder joint replacements
- Materials for improved wear resistance of total artificial joints

• Retrieval analyses of orthopedic implants
• Drugs delivery implants
• Scaffolds for cartilage regeneration
• Bioimplants for bone regeneration for oncological patients
• A mathematical model of aliphatic polyesters degradation as an efficient tool for preclinical evaluation of biodegradable tissue engineering implants
• Hybrid growth factors delivery system supporting bone tissue regeneration

Selected international grants:
• KMM-NoE (FP6- MAT-NoE) - Knowledge-Based Multicomponent Materials For Durable And Safe Performance
• CELLFORCE (FP6- IST&NMP-STREP) - CellForce: Development of a single cell based biosensor for subcellular on-line monitoring of cell performance for diagnosis and healthcare
• MC EST: JOIN(ed)T (MC FP6) - Joined Education for Tissue Engineering: a multidisciplinary approach to regenerate joints
ExActResoMat (FP6 IP for SME) - External Activation of Resorbable Materials
• COST533 (COST Action FP6) - Biotribology: Materials for improved wear resistance of total artificial joints
• COST537 (COST Action FP6) Core laboratories for the improvement of medical devices in clinical practice from the failure of the explanted prostheses analysis
• "In vivo bone engineering via combining a novel composite scaffold technology with a growth factor potentiating collagen/heparan sulphate" (Bilateral grant with Singapore)

Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University, co-sponsored by the Bay Zoltán Applied Research Nonprofit Ltd. in Budapest, Hungary. He also has teaching or guest professor affiliations at the following institutions: Institute of Pathophysiology, Semmelweis University; Department of Nanobiotechnology and Regenerative Medicine, Faculty of Health Science, Miskolc University; and Faculty of Pharmaceutical Sciences and NanoScience Center, University of Copenhagen, Denmark. He regularly teaches biology, immune biology and nanomedicine. He obtained M.D. in 1978 at Semmelweis University, and then held various scientific positions in Hungary and abroad, including the Institute of Hematology in Budapest, Christchurch University (Christchurch, New Zealand), ETH (Zurich, Switzerland), University of Arizona (Tucson, Arizona), Harvard University (Boston, MA), National Cancer Institute at NIH and the Walter Reed Army Institute of Research (Bethesda, MD, USA). His research over 34 years on various themes in hematology, membrane biology and immunology resulted some 90 scientific papers, 12 book chapters, 2 patents, a book "The Complement System: Novel Roles in Health and Disease" (Kluwer Academic Press, 2004) and a topical issue of "Critical Reviews in Therapeutic Drug Carrier Systems". Two fields stand out where he has been most active: liposomes and the complement system. He is best known for spearheading the concept that complement activation underlies numerous liposomal- and other nanodrug-induced hypersensitivity (anaphylactoid) reactions, called complement activation-related pseudoallergy (CARPA). Along with numerous social commitments in Hungary and abroad, he is a founder and scientific director of an immune toxicity CRO in Hungary (SeroScience Ltd).



Thomas Teuscher

Thomas Teuscher is a physician and public health specialist with extensive professional experience in developing countries and in public private partnerships.

Thomas worked in remote rural areas in Africa for over 20 years. First as a pediatrician in Gabon, then as a public health practitioner in Togo, later as Director of the Ifakara Health Research and Development Centre in Tanzania, and finally as General Manager of the ECOWAS Health Research Consortium in Côte d'Ivoire. Under Thomas' direction, the Ifakara Research and Development Centre provided the first testing ground in Africa for a disease-modulating malaria vaccine and also tested the efficacy of deploying intermittent preventive treatment through the Expanded Immunization Programme to protect infants from the consequences of malaria infections. Thomas is a founding member of the Mapping Malaria Risk in Africa, or MARA, a collaboration that generated the first evidence-based malaria risk map for Africa.

Dr Thomas Teuscher joined the Roll Back Malaria Cabinet Project as a Senior Adviser in 2000. During his tenure, Thomas helped to facilitate RBM's transition from a partnership ,built on the strength of loose ties' to a well-structured, accountable, and internationally recognized body for the coordination of global and regional malaria control efforts; today he is the Partnership's Deputy Executive Director.

Thomas has played a key role in strengthening RBM's governance and decision-making processes, contributing to the establishment of formal RBM Partnership mechanisms such as the RBM Board, Constitution and By-Laws. Thomas also helped to set increasingly ambitious objectives and priorities for the Partnership, particularly by facilitating the development of the Global Strategic Plan (GSP) and Global Malaria Action Plan, which strengthened the Partnership's shift away from an exclusive focus on vulnerable populations to the adoption of the wider goal of universal coverage for all populations at risk. More recently, Thomas worked on expanding the Partnership beyond Africa by forging strategic alliances with malaria control coalitions in Asia and the Americas. He has also served as the research focal point within the RBM Secretariat.

Thomas holds a Doctor in Medicine (MD), and Master's of Science degrees in Clinical and Tropical Medicine and in Human Nutrition from the London School of Hygiene and Tropical Medicine. Thomas has published over 40 articles in international peer-reviewed scientific journals focussing on generating evidence that informs public health policy, planning and programme implementation; most recently Thomas published on Improving Livelihoods Beyond 2015: Linking Malaria Control with Sustainable Development. Global Health and Diplomacy, Fall 2012 (page12) http://onlinedigeditions.com/publication/?i=127661. Thomas is a former commercial pilot and keen mountaineer.

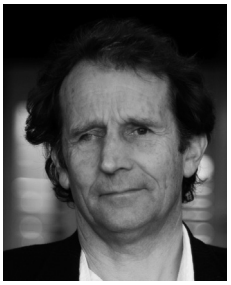
Carsten Timpe

Carsten Timpe, Ph.D., born in Hannover/Germany, studied Pharmacy at the Technische Universität Braunschweig. He completed his Ph.D. thesis at the Philipps University of Marburg/Germany in Pharmaceutical Chemistry in the working group of Prof. Klaus Hartke. He joined Hoffmann La Roche AG in Basel in 2012 as a group head/ Senior Principal Scientist in early drug development projects. Prior to Roche he worked five years as a Senior Fellow and project leader at Novartis AG in Basel in drug development of poorly soluble and highly potent compounds. Further stations of his professional career were Eli Lilly in Hamburg/Germany as Principal Research Scientist until February 2007 in the early drug formulation development area and prior to Lilly he was working at Jenapharm/

Schering as group leader in oral solid dosage form development. Dr. Timpe is inventor/co-inventor of several international formulation patents (i.e. for a pulsatile capsule formulation, a buccal tablet, solid dispersions) and invited speaker to several drug delivery conferences in Europe and an active member of the APV focus group "Drug Delivery" in Germany. His focus is currently poorly soluble drugs and oral nanoparticulate drug delivery.

Panagiotis Trohopoulos

Dr med Panagiotis (Panos) N. Trohopoulos is a Distinction of Excellence Greek (Ellin) Medical Doctor and his Specialty is Cardiologist (E-mail: panagiotis.trohopoulos@cosmophos.com). He is the Founder / Owner / Managing Director of the CosmoPHOS Ltd which is an Innovative Nanomedicine SME established in Thessaloniki, Greece (Ellas), and is focused on the Research & Development of Novel Nanomedicine Products for the Diagnosis, Targeted Therapy, and Therapy Monitoring of Atherosclerotic Coronary Artery Disease (CAD) of the heart causing the myocardial infarctions (heart attacks), and of Atherosclerosis in general. Dr med Trohopoulos is also the Founder and the Scientific / Exploitation / Strategic Coordinator of the Large-Scale EU FP7 NMP CosmoPHOS-nano Project with total budget 12,9 Million Euros, funded by the European Union under the FP7 Programme / NMP Theme with 8,5 Million Euros, and co-funded by All Project Participants with 4,4 Million Euros. CosmoPHOS-nano is a five-year Project started on March 1, 2013 and will be concluded on February 28, 2018. CosmoPHOS-nano is a Multidisciplinary Project consisting of 19 World-Class Participants (including 13 Universities and Research Foundations and 6 Companies, from 11 European Countries, Japan, and the USA) and it is the Global Largest Translational Nanomedicine R&D Project aiming to develop a Radical Innovative Theranostic "Smart" Nanomedicine Product, the CosmoPHOS System, for the Molecular Imaging, Targeted Therapy, Real-time Therapy Monitoring, and Follow-up Therapy Monitoring of Atherosclerotic Coronary Artery Disease (CAD) of the heart, which is the leading cause of human death and morbidity worldwide. The CosmoPHOS-nano Project is foreseen to conduct during the final project year, a First-in-man Phase-I Clinical Trial in CAD patients to study the safety and tolerability of the CosmoPHOS System for human use.



Hans Van der Voorn

Hans van der Voorn is the Executive Chairman and CEO for Izon Science Ltd, based in New Zealand. He originally trained as an engineer and previously founded and developed an energy company. Hans was one of the founders of Izon in 2005 and became its fulltime CEO in 2007. He has been the inventor on several Izon patents and has a particular interest in developing high quality and reliable nano-measurement capabilities for biomedical use.
Izon Science has a nanopore based measurement and analysis platform it calls TRPS for Tunable Resistive Pulse Sensing. Its qNano instruments are of particular interest to nanomedicine researchers and developers. Izon's aim is for TRPS to become the world's leading measurement platform for nanomedicines needing to migrate from research laboratories to clinical use. The core capabilities of izon's TRPS system include particle number, real number based size distribution, and more recently, particle by particle charge distribution. Hans' van der Voorn's view is that transparency and objective repeatability of the measurements is necessary for nanomedicines needing approval, and that this is now very achievable.

Peter van Hoogevest

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Telephone +49 621 53819-63
E-mail: pvanhoogevest@phospholipid-institute.com

PROFESSIONAL EDUCATION

• **University of Basel (Switzerland):** 1994 Degree as "Privat Dozent" (Adjunct Professor) in Pharmaceutical Sciences. Title of "Habilitationsschrift": "Recent Advances in the Industrial Research and Development of Liposomal Drugs".
• **State University of Utrecht (The Netherlands):** 1984 Ph.D. degree. Faculty of Mathematics and Natural Sciences; Title of Ph.D. Thesis: "Permeability properties of Band 3- and Glycophorin-containing Model membranes", Promoters: Prof. Dr. B. de Kruijff and Prof. Dr. J. de Gier, Department of Biochemistry.
- 1979 Pharmacist certificate.
- 1978 Masters degree. Faculty of Mathematics and Natural Sciences, Main subject: Pharmaceutical Sciences, Ancillary subject: Biochemistry.
- 1975 Certificate B.S. Faculty of Mathematics and Natural Sciences Main subject: Pharmaceutical Sciences.

WORK EXPERIENCE

• Lipoid GmbH, Ludwigshafen, Germany, January 2012-now: Head Scientific Department
• Phospholipid Research Center Heidelberg, Germany, January 2012-now: Managing Director
• Phares Drug Delivery AG, Muttenz, Switzerland, December 2000-November 2011: Managing Director, Chief Operations Officer (COO), Member of the Board of Directors.
• ADD Advanced Drug Delivery Technologies AG, Muttenz, Switzerland, 1998-2000: CEO, Co-founder, Delegate and Member of the Board of Directors.
• Novartis Ltd., Basel, Switzerland, 1996-1998: Laboratory Head, Sterile Dosage Forms.
• Ciba Ltd., Basel, Switzerland, 1994-1996: Section Head Early Development Area/Sterile and Semi-Solid Dosage Forms.
• Chiron Corporation, USA, 1993-1994: Visiting scientist at the Vaccine Research and Development Department at The Biocine Company a Joint Venture between Ciba-Geigy and Chiron (California, USA).
• Ciba-Geigy Ltd., Basel, Switzerland, 1989-1993: Section Head Biotech Products of Sterile and Semi-Solid Dosage Forms Area, Pharmaceutical Development, Pharmaceuticals Division; 1985-1989: Section Head Liposomal Dosage Forms of Novel Dosage Forms Area, Pharmaceutical Development, Pharmaceuticals Division.; 1984-1985: Section Head Protein Formulation, Biovet Group, Animal Health, Agrochemical Division.

Danny van Hoorn

• JUL 2012- Present: OctoPlus, Leiden, NL
Program manager; Spearheads important client accounts for OctoPlus regarding complexity and size. Provides leadership to the internal and external (client) project teams with respect to content, timelines, quality, budget and sales.

• MAR 2010 – JUN 2012: Astellas Pharma, Leiderdorp, NL
Associated Project Director; As such full responsibility for all operational activities for multiple early and later stage global urology projects (non-clinical to Phase III). Multidisciplinary interaction on global level with leads for clinical, non-clinical, CMC, medical, reg-

ulatory and clinical pharmacology to enable timely finalization of activities within budget. Review of all clinical and regulatory documentation. In core team setting provide advice on strategy. Good understanding and approach to different cultures US, Asia and EU.

Successfully introduced and lead the change management in Astellas for a large global project for which the daily operation of 800 (senior) managers and functional lead were influenced on a daily basis (including the CEO and leadership team). This enabled a smooth transition towards the newly desired situation for all global regions.

AREAS OF FULL RESPONSIBILITY

• Project management of complex global drug development projects at different stages of development (non-clinical to phase III) in global matrix organization.
• Budget forecasting, budget actual including periodic overviews.
• Build and maintain the global drug development planning.
• Facilitate, chair and document global project team meetings, regulatory bodies. and other meetings important to the progress of the projects.
• Review and comments on of drug development documents such as global master plan non-clinical, CMC, Clinical (Study design, protocols, study results), Regulatory documents (IMPD/IND/IB/GRSD/PIP/WR, submission preparation of eCTD).
• Global communication towards and with the different core team and extended team members covering all disciplines of drug development in US, ASIA and EU.
• Discuss and review strategy for the drug development projects.

Additional
Participated in a large global team configured a global project management tool (planning, timelines, resources, budget and portfolio management. Training -1- influencing with and without formal power (PMI); -2- Excellent Managers: What They do to be Highly Successful (PMI)
• DEC 2006- MAR 2010 : Xendo and Xendo CTM, Leiden, NL
Program manager/leader, manager business development and grant manager; As such have successfully lead numerous projects and programs from non-clinical to post-marketed products in the pharmaceutical and biotech industry.
• JUL 2005- DEC 2006 :Crucell, Leiden, NL
Project manager and grant manager; As such lead several vaccine projects to develop a (better)cure against Tuberculosis, Malaria and Ebola with the intention that the product cost would be around 1-3 dollar cent and thus enable use in the third world.
• 2003- 2005: SmartVax, Lelystad, NL
Chief Science Officer (CSO), Project leader; As such responsible for the development of new technologies enabling efficient and animal friendly mucosal induction of polyclonal antibodies in cow' milk by means of targeted vaccination against several infections disease targets. The resulting product is a nutraceutical.
• 1997-2003: Numico Research (now Danone), Wageningen, NL
Junior Scientist

EDUCATION

• 1999-2003: Ph.D., Free University Medical Center Amsterdam (VUMC)
- Completed dissertation with working title: ''Nutrition and multiple organ dysfunction''
Current methodology of pre-operative fasting prior major surgery was shown to hamper post-operative patients recovery and also increased the risk of complication compared to specialized nutritional components. The final results of this thesis research was a product to benefit patients recovery after major surgery. This product has shown to lessen the post-operative oxidative stress level, increase post-operative energy levels, maintains muscle strength, decreased length of hospital stay and less post-operative complications.
- Presented at several conferences both oral and poster.
- Relevant course work post Ph.D. : About 20-30 training sessions covering Project Management (PMI, Lindsey McKenna), Change management (PMI, Lindsey McKenna), Global Cultural training to increase understanding and effectiveness of interaction with other cultures, GMP, GLP and GCP training courses.
- Masterclass Biobusiness (2004\5; 1 year); MBA program focused on all bio-business aspects of the bio(tech) industry

REYHANEH VARSHOCHIAN

15 Koohpayeh, Zaferanieh St., Tehran, Iran
+982122436305
varshochian@razi.tums.ac.ir

EDUCATION

• PhD student, Pharmaceutics, Tehran University of Medical Sciences, Tehran, Iran (May 2013)
- Concentration: Protein delivery by polymeric nanoparticles
- Dissertation: Preparation and evaluation of polymeric nanoparticles containing bevacizumab
•PharmD, Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran (2002-2008)
- Dissertation: Reduction of percutaneous absorption of toxic chemicals by dendrimers

AWARDS AND HONORS

• Number one student in the national Board exam (Pharmaceutics) across Iran and awarded by Iran Ministry of Health and Medical Education (2010)
• Number one student in the national PhD entrance exam (Pharmaceutics) across Iran (2008)

PUBLICATIONS AND PERESENTAIONS

• Goodarzi N, Varshochian R, Kamalinia G, Atyabia F, Dinarvand R, A review of polysaccharide cytotoxic drug conjugates for cancer therapy, Carbohydrate Polymers, 92 (2), 1280–1293, 2013
• Kamalinia G, Varshochian R, Goodarzi N, Atyabia F, Dinarvand R, Bioconjugation of cytotoxic drugs to peptides, proteins and aptamers as novel nanostructures for enhanced anticancer drug delivery, Nanotechnology (7): Diagnostics and Therapeutics,PP: 265-290, 2012
• Moghimi HR, Varshochian R, Kobarfard F, Erfan M, Reduction of percutaneous absorption of toxic chemicals by dendrimers, Cutaneous and Ocular Toxicology, 29 (1), 34-40, 2010 [PharmD thesis]
• Varshochian R, Dinarvand R, Jeddi-Tehrani M, Mahmoudi AR, Atyabi F, Sabzevari A, Riazi –Esfahani M, The protective effect of albumin on bevacizumab activity and stability in poly(D,L-lactic-co-glycolic) acid nanoparticles intended for retinal and choroidal neovascularizations treatment, [Submitted in January 2013]
• Abstract in 39th Annual Meeting & Exposition of the Controlled Release Society, Québec, Canada. (CRS 2012)
• Abstract in International Conference on Nanotechnology in Medicine, UK. (NanoMED 2012)
• Abstract acceptance in 8th PBP World Meeting Istanbul, Istanbul, Turkey. (PBP 2012)
• Abstract in the 2nd Symposium on Innovative Polymers for Controlled Delivery, Suzhou, China. (SIPCD 2012)
• Poster in the 13th Iranian Pharmaceutical Sciences Conference, Isfahan, Iran (IPSC 2012)
• Poster in the 5th Iranian Controlled Release Conference, Mashhad, Iran (ICRC 2011)
• Oral presentation in the 12th Iranian Pharmaceutical Sciences Conference, Zanjan, Iran (IPSC 2010)
• Poster in the 11th Iranian Pharmaceutical Sciences Conference, Kerman, Iran (IPSC 2008)

Viola Vogel

Viola Vogel is a Professor in the Department of Health Sciences and Technology (D-HEST) at the ETH Zürich, where she heads the Laboratory of Applied Mechanobiology. She received her doctorate from Frankfurt University after completing her graduate research at the Max-Planck Institute for Biophysical Chemistry, and was then a postdoctoral fellow at the University of California, Berkeley. Professor Vogel started her academic career in the Depart-

ment of Bioengineering at the University of Washington, Seattle (1991-2004), and was there the Founding Director of the Center for Nanotechnology (1997-2003) before she moved to the ETH in 2004. Major awards include the Otto-Hahn Medal from the Max-Planck Society (1988), Feodor-Lynen Fellowship Humboldt Foundation (1989-1990), "FIRST" Award from the National Institutes of Health (General Medicine) (1993), Research Award from the Philip Morris Foundation (2005), Julius Springer Award for Applied Physics (2006), the ERC Advanced Grant from the European Research Council (2007), the International Solvay Chair in Chemistry Brussels (2012), and an Honorary Doctorate from the University of Tampere Finland (2012).

Yuri Volkov

Prof. Yuri Volkov received his MD from the 1st Moscow Medical University and subsequently a PhD in biomedical sciences at the Institute of Immunology, Moscow. He has been working at the Department of Clinical Medicine, Trinity College Dublin since 1995 and he is presently Professor and Chair of Molecular and Translational Medicine and Director of Research at the School of Medicine, TCD. His research interests include nanomedicine and biomedical applications of nanotechnologies, molecular mechanisms of immune system functioning in health and disease, cell adhesion and migration in inflammation and cancer, intracellular signalling and cytoskeletal dynamics, advanced cell and molecular imaging. Prof. Volkov is a Principal Investigator at the Trinity College's Institute of Molecular Medicine and the Centre for Research on Adaptive Nanostructures and Nanodevices. He coordinates a large-scale EU FP-7 funded Consortium NAMDIATREAM (www. namdiatream.eu), which unites the expertise of 22 European academic, research, clinical and industrial partners towards the development of nanotechnology-enabled toolkits for early diagnostics and treatment monitoring of major types of cancer.

Andreas Wagner

Dr Andreas Wagner is currently the Head of Liposome Technology at Polymun Scientific GmbH. He has significant expertise in the field of liposomal formulation and process development. He studied Biotechnology in Vienna, Austria and earned his Master and Ph.D. degrees in the field of biotechnology and biopharmaceutical technology/liposomology at the Institute of Applied Microbiology supervised by Prof. Hermann Katinger and Prof Karola Vorauer-Uhl. Dr Andreas Wagner is listed as inventor on several patents, like the liposome technology and some product patents of liposomal formulations. Furthermore, he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies. Since 2001, he built up the liposome technology unit at Polymun Scientific GmbH. Polymun Scientific GmbH is a private Austrian company offering contract development and manufacturing of biopharmaceuticals as well as development and production of liposomal as well as lipid nanoparticle formulations. Its patented liposome technology allows efficient manufacturing of constantly high quality in small and large scale. Polymun is an EMEA-certified manufacturer conducting several own R&D projects. For more information, please visit www.polymun.com

Heinrich Walt

Heinrich Walt (HW) is a tumor scientist who got his degrees (master diploma and Ph.D.) at the Universities of Zurich and Fribourg, Switzerland. After a postdoc phase performing cancer related projects at the Institutes of Anatomy and Pathology of the University of Zurich, he built up a Research Division of Gynecology at the Department of Gynecology, University Hospital Zurich. In 1993 he received his title as a professor from the medical faculty of the University of Zurich. Actually he is Group Leader of Oral Oncology Research, University Hospital Zurich, Department of Cranio-Maxillo-Facial and Oral Surgery. Based on several sabbaticals HW has strong scientific connections to the Jackson Laboratory (TJL), Bar Harbor, Maine, USA. In 2006 he was appointed Adjunct Professor at TJL. From 2003-2008 HW served as president of the Swiss Society for Oncology. Since 2009 he is president of an association for managing a new Internet platform about cancer http://www.oncocampus.ch/. Towards the end of 2009 he became a board member of the IT'IS foundation which is related to research in Information Technologies in Society.
Since 2011 HW serves as president of an organisation, called European Platform for Photodynamic Medicine (EPPM) http://www.eppm-photomedicine.org/. This organisation is involved in the promotion of photodiagnosis and photodynamic therapy and includes nanomedicine as well. It fosters the formation of consortia for EU-projects. The official Journal of EPPM is Photodiagnosis and Photodynamic Therapy, where HW represents an associated editor.

Frank F. Weichold

M.D., Ph.D.

Dr. Weichold is director for the Office of Regulatory Science and Innovation (ORSI) as well as the Office of Critical Path and Regulatory Science Initiatives at the FDA in the office of the Chief Scientist and the Office of the Commissioner for the Food and Drug Administration. The expertise he brings to the FDA builds on his ability to advance, coordinate, and integrate the scientific resources of the Agency addressing mission critical regulatory responsibilities in a global environment.

Dr. Weichold's experience includes execution of strategic and operational initiatives across the sciences' value chain. Dr. Weichold has led the development of international collaborations and public private partnerships for discovery and early development, implemented global operating and development models, and executed large scale business model transformations. He has accumulated more than a decade of industrial research and medical product development experience while leading teams in Clinical Pharmacology, DMPK, as a Director at MedImmune LLC, Gaithersburg, Maryland. Prior, he directed research and clinical development of vaccines at the Aeras Foundation (founded by The Bill and Melinda Gates Foundation).

As a tenured Professor in the University of Maryland system, he developed and managed independent research programs and trained graduate students. He also held faculty positions at the University of Maryland Biotechnology Institute to study signal transduction pathways that affect immune responses, and at the Humboldt University, Berlin (Germany) to teach and study microbial immune modulation. During the five years of postdoctoral education, Dr. Weichold worked at the National Institutes of Health in Bethesda, Maryland, first at the LTCB (NCI) where he researched immune pathologies in HIV infection, then at the Hematology Branch of the NHLBI where bone marrow pathologies, transplantation immunology and gene therapy were the focus of his studies.

Iris Welten

Iris Welten has been CEO of BaselArea since December 2011. She has over 16 years' experience in various international companies in the US, Europe and Asia. She has held senior positions in listed companies such as Deutsche Telekom, Clariant and Swiss Life. Most recently, Iris Welten was a member of the extended executive committee of a pharmaceutical wholesaler, where she was responsible for Business Development, Communications and Quality Control. Since 2005 she has also been a lecturer for the Master's degree in Corporate Finance at the University of Applied Sciences and Arts of Northwestern Switzerland (FHNW). And since 2012 she has been a member of the Life Sciences Commission of the Chamber of Commerce of Basel-Stadt and Baselland. Iris Welten was born and brought up in Munich, where she studied Communication Sciences, Business Administration and Market Psychology. She has dual German-Swiss citizenship and has lived in the region with her Swiss husband for 12 years.

Klaus-Michael Weltring

Dr. Klaus-Michael Weltring is a molecular biologist by training with a PhD and a Habilitation degree from the University of Münster. Since 2001 he is the managing director of bioanalytik-muenster responsible for the development of the Münster region into a leading nanobioanalytic location at the European level. He has set-up a local network of researchers from different disciplines and SMEs and organizes the marketing of the region at international events and fairs. Between 2003 and 2008 he was the deputy-coordinator of the Nano2Life Network of Excellence and leader of the "ELSA" Board in this network. Until recently he co-managed the Nanomedicine Round Table and the EuroNanoBio project (FP7 CSA projects). Since 2009 he is a member of the Executive Board of the ETP Nanomedicine leading the ELSA Advisory Group of this platform. Currently he is the Chief Scientific Officer of the Nano-Bioanalytik-Zentrum and manages a ZIM NEMO Network of 12 local companies, which develop new and certified methods for characterization of Nanomaterials in consumer products and biological systems.

Wolfgang Wenzel

Wolfgang Wenzel studied physics at the University Bochum starting in 1983. As a Fulbright fellow he moved to Ohio State University (Columbus, Ohio, USA) in 1985 where he graduated 1989 with a Ph.D. in physics. He stayed as a postdoctoral fellow in the laboratory of Nobel Laureate Ken Wilson until his return to Germany in 1992, where he joined the department of physics of Dortmund University. In 2001 he became a group leader for computational nanophysics at the newly founded Institute for Nanotechnology at the Research Center Karlsruhe, one of Germany's national laboratories which in 2009 merged with Karlsruhe University to form the Karlsruhe Institute of Technology.

The Wenzel group works on the development of predictive simulation methods to accurately describe slow processes in various scientific fields: these include the POEM (protein optimization with energy methods) for biomolecular structure simulation, including protein folding, docking and structure prediction; and FlexScreen high-throughput in-silico screening approach for drug development and efficient simulation techniques for the description of nano-materials (http://int.kit.edu/nanosim)

PUBLICATIONS

• Kokh, D.B. and Wenzel,W., Flexible Side Chain Models Improve Enrichment Rates in In Silico Screening. J. Med. Chem, 51: 5919-5931 (2008)

• Behrens, S., A. Heyman, Maul R, Essig S., Steigerwald, S., Quintilla, A., Wenzel W. , Burck, J., Dgany, O., Shoseyov, O. , Constrained Synthesis and Organization of Catalytically Active Metal Nanoparticles by Self-Assembled Protein Templates. Advanced Materials. 21(34): 3515-19 (2009)

• Singh, A., Tolev, M., Meng, M., Klenin, K., Plietzsch, O., Schilling, C.I., Muller, T., Nieger, M., Bräse, S., Wenzel, W. and Richert, C. (2010) Branched DNA that Forms a Solid at 95 °C, Angewandte Chemie (International Ed) (in press).

• Kim, H.G., Ahn, J.W., Kurth, I., Ullmann, R., Kim, H.T., Kulharya, A., Ha, K.S., Itokawa, Y., Meliciani, I., Wenzel, W., Lee, D., Rosenberger, G., Ozata, M., Bick, D.P., Sherins, R.J., Nagase, T., Tekin, M., Kim, S.H., Kim, C.H., Ropers, H.H., Gusella, J.F., Kalscheuer, V., Choi, C.Y. and Layman, L.C. (2010) WDR11, a WD Protein that Interacts with Transcription Factor EMX1, Is Mutated in Idiopathic Hypogonadotropic Hypogonadism and Kallmann Syndrome, American Journal of Human Genetics, 87, 465-479.

• Walther, Torsten H., Gottselig, C., Grage, Stephan L., Wolf, M., Vargiu, Attilio V., Klein, Marco J., Vollmer, S., Prock, S., Hartmann, M., Afonin, S., Stockwald, E., Heinzmann, H., Nolandt, Olga V., Wenzel, W., Ruggerone, P. & Ulrich, Anne S. (2013). Folding and Self-Assembly of the TatA Translocation Pore Based on a Charge Zipper Mechanism. Cell 152, 316-326.

Marieluise Wippermann

CEO TECOmedical AG, Sissach, Switzerland
E-mail: wippermann@tecomedical.com

• Since 2000 CEO TECOmedical AG, CH
• 1997 – 2000 Managing Director CH-Werfen Group, Spain
• 1988 – 1997 Vice President International Nichols Institute, USA
• 1983 – 1988 Head of development and production, Eurodiagnostics, The Netherlands
• 1983 Management Degree, School of economics, Basel, CH
• 1979 – 1983 Head of development and production - Bühlmann Laboratories AG, Switzerland
• 1976 – 1978 Research scientists, Institute of Biochemistry, University of Hamburg, Germany
• 1973 – 1976 Research scientists, Dep. of Internal Medicine, University of Zurich, Switzerland
• 1973 Degree , Chemistry Engineer

Ron Wolbert

MSc.

Eichenweg 9, A-2130 Hüttendorf,
Mob.: +43-650-6666.981 Carasova
E-mail: Ron.Wolbert@Live.com
Birth-date: 23-10-1960 Nationality: Dutch
Birthplace: Stad Delden Languages: English, German, Dutch.

EDUCATION

• 1973-1977 Middle school
• 1977-1979 High school
• 1979-1983 Sec. Education -Biochemistry & CT
• 1983-1987 Ir. Molecular Biology/- Bio-Chemistry, Agricultural University, Depart. of Biochemistry
• 1987-1993 University-didactic &- Biochemical Process-technology &- Downstream processing (Prof Trampe)
• 1993-2001 Face to face, SPIN selling & Counsellor Sales
• 1998 Psychological-sales training, ISA

• 2001 Business school English training for the European position
• 1998-2009 Practical & Theoretical Management training
• 2007 MBA-A.

WORKING EXPERIENCES

• 1987-1993 Agricultural University Study and R&D, Research with different technologies and member of Biochemistry faculty
• 1993-1998 - Pharmacia Biotech BN/ Amersham Pharmacia Key Account Manager., Sales and advisory of Pharmacia's product lines at key customers
• 1998- 1/2005 Biacore International Ltd Sales Manager, BN & EU Sales and marketing, employing, founding Benelux office and generating new business in area Life Sciences, Pharma, Biotechnology and Food
• 1-1-2006 Establishment of Lauden Holding Ltd Director/ owner Outsourcing obtained knowledge and created network to external companies for business development and market implementation of their products.

The following companies were/ are using Lauden's services:
• 2-12/2005 Microscreen Ribo-Technologies Ltd Sales Manager, EU & Global.Sales and Business Development for implying a new impulse for generating revenues in area Life Sciences, Water Quality and Food
• 2006- 11/2009 RnA Ltd CEO/ Business Development. Corporate Imaging, Sales, Business Development and marketing activities for a total new product concept (suspension array) in Food quality-safety control for Multi Analytes analyses.
• 12-2009-6-2011 Tecna S.r.l. and other companies Area Manager, EU Sales and Business Development in Food and Biotechnology markets.
• 6-2011 till now Scienion AG Sales Manager DACH/ EU, Sales Manager for DACH and Eastern Europe on Micro Array Multiplexing for IVD market segment.

HOBBY

Beekeeper, cycling, walking gardening.

Ada Yonath

Born in Jerusalem, Israel, Prof. Ada Yonath studied at the Hebrew University, earned Ph.D. degree from Weizmann Institute of Science (WIS) and completed her postdoctoral studies at Carnegie Mellon University and MIT in USA. In the seventies she established the first laboratory for protein crystallography in Israel, which was the only laboratory of this kind in the country for almost a decade, and then spent a Sabbatical leave in Chicago University. Currently she is the WIS Kimmel Professor of structural biology and the Director of the Kimmelman Center for Biomolecular Structure and Assembly. In parallel, during 1986-2004 she directed the Max-Planck-Research-Unit for Ribosome Structure in Hamburg, Germany.
She has been and is studying protein biosynthesis, focusing on ribosomes, the universal cellular "factories" translating the genetic code into proteins. Owing to their fundamental significance for life, ribosomes are targeted by many antibiotics and she is investigating their action alongside mechanisms acquiring antibiotics resistance. Thus revealing routes for structure-based drug improvements and design.
She is a member of US National Academy of Sciences; the American Academy of Arts & Sciences; Israel Academy of Sciences & Humanities; European Academy of Sciences & Art; Korean Academy for Science & Technology; European Molecular Biology Organization (EMBO) and the International Academy of Astronautics.
She holds honorary doctorates from almost all Israeli Universities; KEK, Japan; Oslo U, Norway; Fujian U, China; NYU and Mt. Sinai Universities, NYC; Hamburg University; Patras U. Greece; Oxford and Cambridge Universities, UK.
Her awards include the 1st European Crystallography Prize; the Israel Prize; the Paul Karrer Gold Medal; the Israel EMET award; the Rothschild Prize; Louisa Gross Horwitz Prize of Columbia U, NY; the Paul Ehrlich-Ludwig Medal; Linus Pauling Gold Medal; Anfinsen Prize; Wolf Prize; Massry Award; UNESCO/L'Oreal Award for Women in Science; Albert Einstein World Award for Excellence; Erice Peace Prize; DESY pin; Exner medal; Nobel Prize for Chemistry; the Indian PM Gold medal; President of Panama Award; Maria Sklodowska-Curie Medal of the Polish Chemical Society; Cite of Florence Prize; Datta Medal.

Rolf M. Zinkernagel

Professor Emeritus of Experimental Immunology

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Born and raised in Basel, Rolf Zinkernagel studied at the Medical School of the University of Basel, obtaining his MD degree in 1968, and graduating to become a surgeon.
After working as a surgeon for a little over a year, Rolf Zinkernagel chose to focus on immunological research, undertaking a postgraduate course in experimental medicine at the University of Zurich; and subsequently spending two years in the Institute of Biochemistry of Lausanne working on immunity against infections. From 1973-1975 he became a PhD student, and later a post-doctoral researcher, at the John Curtin School of Medical Research of the Australian National University, Canberra, where Peter Doherty and he made seminal observations on how cytotoxic T cells recognize virus infected cells in an infected host (Nobel Prize for Physiology or Medicine 1996). Rolf Zinkernagel moved to the Scripps Clinic and Research Foundation in La Jolla, USA from 1975 to 1979, where he studied T cell maturation and development of the T cell repertoire, dependent on the transplantation antigen expression in the thymus. In 1980 he joined the Department of Pathology, University of Zurich, as an associate professor where, together with Hans Hengartner, he has been studying immune protection and immunopathology caused by virus infections. Over the past 25 years within the Experimental Pathology group, and after 1992 the Institute of Experimental Immunology, he studied the role of antigen dependent beneficial immune protection or detrimental immunopathology, and compared these mechanisms with theories of immunological memory and immunological tolerance. He has retired from the University in Spring 2008.
Besides his interest in solving uncertainties and discrepancies in immunology, Rolf Zinkernagel tries to further biomedical research and its application in Zurich, in Switzerland and Europe. He has scientifically supported gene technology and animal experimentation in various biotech- and pharmaceutical companies in Switzerland and Europe, was a member of the Swiss Science Council and of the European Research Council and is a member of the Executive Council of the International Union of Immunological Societies. He has also helped to popularize science in tabloid newspapers.

EDUCATION

• Mathematisch-Naturwissenschaftliches Gymnasium, Basel, Matura, 1962
• Faculty of Medicine, University of Basel, 1962-1968 National Board Examination, 1968, M.D. Thesis, 1970, E.C.F.M.G. (USA), 1968
• Course in Tropical Medicine, Tropical Institute, University of Basel, 1967-1968
• Postgraduate course in Experimental Medicine, Faculty of Medicine, University of Zürich, 1971
• Ph.D. Thesis, 1975. Australian National University, Canberra, Aus

PROFESSIONAL RECORD

• Externship, Glen Cove Community Hospital, Glen Cove, Long Island, N.Y., USA, 1966
• Internship, Surgical Department, Clara-Spital, affiliated to the Facility of Medicine, University of Basel, 1969

• Postdoctoral Fellow, Laboratory for Electron Microscopy, Institute of Anatomy, University of Basel, 1969-1970
• Postdoctoral Fellow, Institute of Biochemistry, University of Lausanne, Switzerland, 1971-1973
• Visiting Fellow, Department of Microbiology, The John Curtin School of Medical Research, Australian, National University, Canberra, Australia, 1973-1975
• Associate (Assistant Professor), Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, California
• Associate Member (Associate Professor), Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California, 1976 - 03/1979
• Adjunct Associate Professor, Department of Pathology, UCSD, 1977-1979
• Member (Full Professor), Department of Immunopathology, Scripps Clinic and Research Foundation, 1979
• Associate Professor, Department of Pathology, University of Zürich, University Hospital, Zürich 01/10/79 - 14/10/88
• Full Professor, Department of Pathology, University of Zürich, University Hospital, Zürich 15/10/88 - 29/02/2008
• Head, Institute of Experimental Immunology, Zürich 16/04/92 - 29/02/2008

CURRICULA VITAE OF POSTER-SUBMITTERS

Walla Alelwani

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Mobile Number: +44 (0) 7838738167
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Born: 30/07/1982, Nationality: Saudi

EDUCATION

• 2011-2015 (expected date) University of Nottingham
PhD of Philosophy
• 2009-2010 University of Nottingham
MSc Nanoscinces with merit
• 2001-2005 Om Alqura University
BSc Chemistry with merit

WORK EXPERIENCE

• 2006-2008 Alnoor specialist hospital, Lab technician in the clinical chemistry department

Current research: I am a researcher PhD student in the University of Nottingham at school of pharmacy under the division of drug delivery and tissue engineering collaborated with bimolecular biology division. In brief, my project aims to design a drug based Nanoparticles approach that targeted specific cells cause allergic diseases.

Languages: English: Full professional proficiency. Arabic: Native

TECHNICAL SKILLS

• Cells Culture
• Degranulatin assay (β-hexosaminidase assay)
• Dynamic Light Scattering (DLS)
• Zeta Potential
• Fluorescence Microscopy
• Basophils Activation test (BAT)
• Confocal Microscopy
• MTS viability assay
• LDH cytotoxicity assay
• Classical uptake study
• Polymerase Chain Reaction (PCR)
• Agarose Gel electrophoresis
• HistaFlow
• Flow Cytometery
• Nanoparticles prepration

REFERENCE

Franco Falcone, Associate professor, The school of Pharmacy, Division of Molecular and Cellular Scinces , Boots Science Building, Since Road, Nottingham, United Kingdom, NG7 2RD
Tel: +44(0)1158466073, Franco.Falcone@nottingham.ac.uk

Bahman Anvari

PROFESSIONAL PREPARATION

• 1985, B.A., Biophysics, University of California, Berkeley
• 1988, M.S., Biome. Eng., California State University, Sacramento
• 1993, Ph.D., Bioengineering, Texas A&M University

APPOINTMENTS

• 2006-Present Professor (with tenure), Dept. Bioeng., University of California, Riverside
• 2008-Present Participating Faculty, Division of Biomedical Sciences, UC Riverside
• 2008-Present Cooperating Faculty, Department of Mechanical Engineering, UC Riverside
• 2003-2006 Associate Professor (with tenure), Dept. Bioeng., Rice University
• 1998-2003 Assistant Professor, Dept. Bioengineering, Rice University
• 1997-1998 Research Assist. Prof., Dept. Eng., Harvey Mudd College, Claremont, CA
• 1995-1997 Whitaker Foundation Res. Fellow, Dept. Eng., Harvey Mudd College,
• 1993-1995 Postdoctoral Fellow, Beckman Laser Institute, UC Irvine

RECENT RELEVANT PUBLICATIONS (FROM A LIST OF 76 PEER-REVIEWED JOURNAL PUBLICATIONS)

1. S. Gupta, M. R. Chatni, A. L. N. Rao, V. I. Vullev, L. V. Wang, and B. Anvari, "Virus-mimicking nano-constructs as a contrast agent for near infrared photoacousic imaging," *Nanoscale*, 5: 1772-1776, 2013.
2. B. Jung, and B. Anvari, "Synthesis and characterization of bovine serum albumin-coated nanocapsules loaded with indocyanine green as potential multi-functional nano-constructs," *Biotechnology Progress,* 28: 533-539, 2012.
3. B. Jung, A. L. Rao, and B. Anvari, "Optical nano-constructs composed of genome-depleted brome mosaic virus doped with a near infrared chromophore for potential biomedical applications," *ACS Nano*, 22: 1243-1252, 2011.
4. B. Bahmani, S. Gupta, S. Upadhyayula, V. I. Vullev, and B. Anvari, "Effect of polyethylene glycol coatings on uptake of indocyanine green loaded nanocapsules by human spleen macrophages in-vitro," *Journal of Biomedical Optics*, 16: 051303, 2011.
5. J. Yu, D. Javier, M. A. Yaseen, N. Nitin, R. Richards-Korutm, B. Anvari, and M. S. Wong, "Self-assembly synthesis, tumor cell targeting, and photothermal capabilities of antibody-coated indocyanine green nanocapsules," *Journal of American Chemical Society*, 132: 1929-1938, 2010.
6. M. A. Yaseen, J. Yu, B. Jung, M. S. Wong, and B. Anvari, "Biodistribution of encapsulated indocyanine green in healthy mice," *Molecular Pharmaceutics*, 6: 1321-1332, 2009.

HONORS

Fellow, American Institute for Medical and Biological Engineering (AIMBE) (2008-)
Fellow, American Association for the Advancement of Science (AAAS) (2010-)

EDITORIAL BOARDS

Associate Editor, Annals of Biomedical Engineering (2004-)
Editorial Board Member, Journal of Biomedical Optics (2011-)

ILLUSTRATIVE AWARDS

NSF Grant CBET-9634110 (1996-1999) (PI); The Whitaker Foundation Biomedical Engineering Research Grant (1996-2000) (PI); NIH-R15 Grant (1997-2000 (PI); Texas Higher Education Coordinating Board Grant (1997-2000) (PI); NIH-R01AR047996 Grant (2001-2006) (PI); NIH-R01-DC02776 Grant (2001-2012) (Co-PI); NSF Grant CBET-0522862 (2005-2009) (PI); NSF Grant CBET-1144237 (2011-2014) (PI).

Chantal Appeldoorn

PhD

Chantal studied Organic Chemistry at Leiden University. In 1998 she started her PhD at the Department of Biopharmaceutics within the Leiden/ Amsterdam Center of Drug Research. Within this UNYPHAR project (a collaboration between Yamanouchi Pharmaceutical (currently Astellas) and the Universities of Groningen, Leiden and Utrecht) she worked on her thesis entitled "Generation of P-selectin inhibitors for anti-atherothrombotic therapy". After a short postdoc period at the same department focusing on the preparation of new P-selectin ligands for the prevention and visualization of atherosclerosis, she moved to Utrecht University in 2002. At the Department of Medicinal Chemistry she worked within the EU financed POLYCARB project synthesizing various monomeric, dendrimeric and polymeric carbohydrates as anti-adhesion

compounds against several bacterial infections.
As of 2005 she has been working at to-BBB technologies, currently as a senior scientist. to-BBB is a clinical stage biotechnology company located in Leiden focusing on enhanced drug delivery across the blood-brain barrier. The company is developing novel treatments for devastating brain disorders, such as brain cancer, neurodegenerative diseases and lysosomal storage diseases, by combining existing drugs with the G-Technology®, to-BBB's proprietary brain delivery platform. Its lead product 2B3-101 (brain-targeted liposomal doxorubicin) is currently in a phase I/IIa clinical trial, in patients with primary and metastatic brain tumors.

Leila Arabi

Pharm.D.,Ph.D. candidate

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Born 08/07/1985 Mashhad, Iran

RESEARCH INTERESTS

Establishing of targeted drug and gene delivery systems using nanotechnology methods and materials, Isolation and characterization of cancer stem cells, Targeting tumor cells, Cancer stem cells and tumor microenvironment, Nanomedicine, Gene delivery using non-viral vectors (liposomal and cationic polymer (polyethyleneimine: PEI) based gene transfer system, Therapeutic implications of non-coding RNA, Primary culture of patient derived malignant effusions as specific models for in vitro assays and personalized medicine.

EDUCATION

• since August 2012: 1-year internship at the Molecular Pathology department of the Institute for Pathology (University Hospital Basel). involved in the projects such as the "Molecular regulation of cancer stem cells in gastrointestinal neoplasia", "Anticancer-treatment effects in vitro using real-time impedance measurements on patient-derived samples", "Prognostic and therapeutic implications of the oncogenic miR-17-92 cluster in osteosarcoma", " Evaluation of putative amplification target genes (Vinculin and KCNMA1) in prostate and breast cancer"
• since 2009: Ph.D. candidate of Pharmaceutical Nanotechnology: School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. **Ranks first** in Ph.D. based on overall scores (18.90 out of 20)
Ph.D. Theses Title: "*in vitro* and *in vivo* targeted drug delivery to cancer stem cells isolated from murine colon carcinoma by doxorubicin nanoliposomes conjugated with CD44, CD133, EpCAM monoclonal antibodies".under the supervision of Dr Mahmoud Reza Jaafari.
• 2003-2009: Pharm.D. School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. **Ranks second** in Pharm.D. based on overall scores (18.05 out of 20)
Pharm.D. Theses Title: "Preparation and characterization of nanoliposmes modified with lipopolymers constructed from polyethyleneimine (10 kDa) and bromoalkane derivatives, as a non-viral gene transfer system".under the supervision of Dr Malaeke Nikuei, Dr Mohammad Ramezani.

HONORS AND AWARDS

• Fellowship of CLINAM foundation for The European Summit for Clinical Nanomedicine 2013 (6th CLINAM 2013) Basel- Switzerland
• Award for poster presentation in Biovalley Life Science Week- 25th September- 2012- Basel- Switzerland
• Ranks first among candidates, Iranian National "pharmaceutical Nanotechnology" Ph.D. comprehensive Exam based on examination and interview 2011
• Ranks first among candidates, Iranian National "pharmaceutical Nanotechnology" Ph.D. Entrance Exam based on entrance examination and interview 2008
• Ranks first in Ph.D. based on overall scores (18.90 out of 20)
• Ranks second in Pharm.D. based on overall scores (18.05 out of 20)
5% upper first in national comprehensive exam of basic sciences.

REFERENCES

Prof. Luigi Terracciano, Molecular Pathology Division, Institute of Pathology,University Hospital Basel, CH, lterracciano@uhbs.ch
Christian Ruiz, Ph.D., Molecular Pathology Division, Institute of Pathology,University Hospital Basel, CH, christian.ruiz@unibas.ch

Konstantinos Avgoustakis

Associate Professor
Department of Pharmacy, University of Patras, Patras 26500, Greece
Tel.: ++2610-9696330
Fax: ++2610—997607
Email: avgoust@upatras.gr

Konstantinos Avgoustakis obtained his Diploma in Pharmacy from the Aristotle University of Thessaloniki, Greece in 1985 and his Ph.D. in Pharmaceutics/Quality Control of Medicines, from the same University in 1991. He received a scholarship for Ph.D. studies abroad (1989-1992) from Greek State Scholarships Foundation and he obtained in 1992 a Ph.D. degree in Pharmaceutical Technology from King's College, University of London, UK. In 1993-1994 he served as research assistant in the Institute of Radioisotopes in National Centre for Scientific Research "Demokritos". Since 1994 he has joined the Department of Pharmacy in University of Patras (Greece), where he teaches subjects related to Pharmaceutics and Drug Delivery. His research interests lie on the controlled, targeted drug delivery using engineered nanoparticles based on biodegradable polymers and copolymers and magnetic hybrid inorganic/organic nanocarriers, on the development of novel prophylactic or therapeutic vaccines based on biodegradable and biocompatible, polymeric nano- and micro-particles and on the development of formulations for the efficient delivery of drugs with limited aqueous solubility. He is the author/co-author of 47 articles in peer-reviewed journals and 1 article in biomaterials encyclopedia. He has been Guest Editor for the special issue of "European Journal of Pharmaceutics and Biopharmaceutics" on "engineered polymers and polymeric systems in controlled drug delivery and targeting" (2009). He is also the author (inventor) of 1 European patent. His published research has received over 800 citations (h index 14). He has participated in 9 research programs in collaboration with academic and industrial organizations (in 7 as coordinator). He serves as reviewer for major pharmaceutical and nanoscience/nanotechnology journals and he is Assistant Editor of "Current Nanoscience" and member of the Editorial Board for the "Open Drug Delivery Journal" and for the "Journal of Excipients and Food Chemicals".

Thierry Bastogne

Associate Professor in the Université de Lorraine
Prime d'Excellence Scientifique (rang A) since 2012
Phone: +33 383 684 473
Email: thierry.bastogne@univ-lorraine.fr

Born July, 1968

DEGREES AND TITLES

• Habilitation à Diriger des Recherches, Experimental Modeling of Interconnected Systems - Applications in
Systems Biology, Université Henri Poincaré, Nancy, 2008
• Doctor of Philosophy in System Control Engineering, Université Henri Poincaré, Nancy, 1997

RESEARCH INTEREST

Topic: Modeling and control of tumors with nanoparticles activated by ionizing and non-ionizing radiations. My research activity is shared between two scientific projects:

• Research leader of project team "Biologie intégrative : cybErnétique des thérApies par rayonneMent" at
CRAN (Centre de Recherche en Automatique de Nancy), Univ. de Lorraine - CNRS UMR 7039

• Member of the INRIA team BIGS (BIology, Genetics and Statistics), INRIA Nancy Grand-Est

MAJOR ACHIEVEMENTS (Research & Education)

• More than 60 international publications in peer-refereed journals and conferences

• Thematic mobility and Visiting positions:
- Centre de Lutte Contre le Cancer de Lorraine (Centre Alexis Vautrin, Nancy) (2005-2006)
- Centre de Recherche Public de la Santé, NorLux Neuro-Oncology Laboratory (Luxembourg), S. Niclou (2011-2012)

• Coordinator and member of European & National scientific projects in nano-medicine & systems biology:
- Nano-Xrays (2011-2014) Nanoparticles-based X ray-induced photodynamic therapy in glioblastoma multiforme, INCa.
- PDTX (2010-2013) Active Nanoplatforms for Photodynamic Therapy, ANR.
- Target-PDT (2009-2013) Photodynamic Therapy using photosensitizer-doped targeted organic nanoparticles, FP7 ERA-NET EuroNanoMed: European Innovative RTD Projects Proposals in Nanomedicine
- Nano-VTP (2008-2011) Nanoparticles for Imaging and Vascular Photodynamic Treatment of Brain Tumors, ANR

FIVE RECENT PUBLICATIONS

[1] H. Benachour, T. Bastogne, M. Toussaint, Y. Chemli, A. Sève, C. Frochot, F. Lux, O. Tillement, R. Vanderesse, and M. Barberi-Heyob. Real-time monitoring of photocytotoxicity in nanoparticles-based photodynamic therapy: A modelbased approach. PLoS ONE, 7(11):e48617, 2012.

[2] H. Benachour, A. Sève, T. Bastogne, C. Frochot, R. Vanderesse, J. Jasniewski, I. Miladi, C. Billotey, O. Tillement, F. Lux, and M. Barberi-Heyob. Multifunctional peptide-conjugated hybrid silica nanoparticles for photodynamic therapy and MRI. Theranostics, 2(9):889–904, 2012.

[3] S. Dobre, T. Bastogne, C. Profeta, M. Barberi-Heyob, and A. Richard. Limits of variance-based sensitivity analysis for non-identiability testing in high dimensional dynamic models. Automatica, 48(11):2740–2749, 2012.

[4] R. Keinj, T. Bastogne, and P. Vallois. Tumor growth modeling based on cell and tumor lifespans. Journal Theoretical of Biology, 312:76–86, 2012.

[5] J. Mriouah, C. Boura, M. Thomassin, T. Bastogne, B. Faivre, and M. Barberi-Heyob. Tumor vascular responses to antivascular and -angiogenic strategies: looking for suitable models. Trends in Biotechnology, 30(12):649–658, September 2012.

Sidsel Bering Olsen

PhD student
Group for Molecular Oncology
Institute for Molecular Medicine, University of Southern Denmark
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Nationality: Danish
Language skills: Danish (Mother tongue), English

EDUCATION

• Aug. 2007 - Jun. 2010 Bachelor student of Biochemistry and Molecular Biology at the University of Southern Denmark, Denmark

• Jun. 2010 Ba. Scient from the University of Southern Denmark in Biochemistry and Molecular Biology; Grade: 12

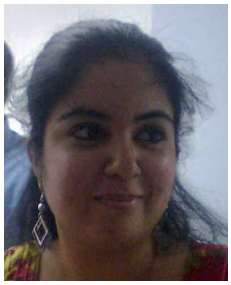
• Aug. 2010 - Oct. 2012 Master student of Biochemistry and Molecular Biology at the University of Southern Denmark

• Aug. 2011 - Oct. 2012 Master student at the University of Southern Denmark, Institute for Molecular Medicine, Molecular Oncology, Odense, Denmark; Grade: 12

• Apr. 2012 Received funding from Kræftens Bekæmpelse

Annabelle Bertin

Annabelle Bertin studied organic and inorganic chemistries at the University of Versailles (UVSQ), then specializing in polymer chemistry and physico-chemistry at the University Pierre et Marie Curie, Paris (UPMC), receiving a master degree (DEA) in 2004. In 2007, she completed a PhD in chemistry at the University of Strasbourg (ULP) / Institute of Physics and Chemistry of Materials of Strasbourg (IPCMS), France, on the topic of dendron-based materials for biomedical applications. Afterwards, she spent two years as a postdoctoral fellow in the department of Colloid Chemistry at the Max Planck Institute of Colloids and Interfaces (MPIKG) in Golm, Germany, where she worked on bioinspired polymers and composite materials. She then joined for one year the team of Prof. Percec at the University of Pennsylvania, USA, where her research topic dealt with biodegradable Janus dendrimers based on carbohydrates and amino acids as drug delivery systems. In 2011, she joined the Federal Institute for Materials Research and Testing (BAM) in Berlin as a postdoctoral fellow and became the same year project leader in the division "Polymers in Life Sciences and Nanotechnology". Her main research interests are devoted to the synthesis of polymers and polymer-stabilized nanoparticles for biomedical applications in the area of gene delivery and diagnostic imaging.



POOJA BHATIA

Research Scholar at IITDelhi & Project Officer, BCIL
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EDUCATIONAL QUALIFICATION

Am Masters in Biotechnology and currently pursuing research in the area of nanomedicine research and its management in India, as a part of my doctorate research at Indian Institute of Technology, Delhi.

WORK EXPERIENCE

Have been working in the field of IP management and Technology transfer for the last 6 years. I am currently managing Stanford India Biodesign Program funded by Department of Biotechnology for overall strategic IP management right from searching to filing to developing IP policy.

PUBLICATIONS

Review Paper

Jain. A, Bhatia.P and Chugh. A (2012) "Microbial Synthetic Biology for human therapeutics", Syst Synth Biol. DOI: 10.1007/s11693-012-9092-0

Book chapters

1. Chugh A, Bhatia P and Jain A (2012) "Synthetic Biology for Biodrugs And Designer Crops And The Emerging Governance Issues" S2B2 (Springer Publications) (submitted).

Conference Presentations

Bhatia.P and Chugh. A "Nanotechnology in medicine: IPR, Safety and Regulatory issues" "Poster Presentation at the Healthcare India 2012, APA International Congress on Advances in Human Healthcare Systems, New Delhi", February 20-23, 2012.

Chugh A and Bhatia P, "Role of marine bioprospecting contracts in developing access and benefit sharing mechanism for marine tradition knowledge holders in the pharmaceutical industry" selected for the "Oral Presentation at the World Conference on Marine Biodiversity 2011, Aberdeen", September 26-30, 2011.

Sven Even Borgos

Sven Even Borgos has an undergraduate degree (MSc) in Physics and Mathematics, specialized in Biophysics and Medical Technology from NTNU (The Norwegian University of Science and Technology). He earned his PhD (also at NTNU) in molecular genetics, specifically genetic engineering of antibiotic-producing Streptomyces bacteria for biosynthesis of antibiotic derivatives with enhanced pharmacological properties, and the characterization of these compounds by LC-MS and NMR. He has worked with chemical analysis within SINTEF since 2006, while doing a part time post doc. in prokaryotic systems biology up to 2011. His current main focus is MS analyses on biological and nanoparticle systems.

Flávia Chiva Carvalho

Ph.D., M.Sc., B.Sc. Pharm Sci
Post-doctoral researcher, School of Pharmaceutical Sciences, UNESP, Brazil (2012-date)

Date of birth: 24th December, 1980

QUALIFICATIONS

B.Sc. (2005), M.Sc. (2009) and Ph.D. (2012) Pharmaceutical Sciences, UNESP, Brazil. Sandwich Ph.D., School of Pharmacy, Queen's University, Belfast, UK, 2011

PUBLICATIONS

1. Carvalho et al., J Pharm Sci 99(5): 2367-74, 2009
2. Carvalho et al., Eur J Pharm Biopharm, 84(1):219-27, 2012
3. Carvalho et al., J Pharm Pharmacol 62: 430-439, 2010
4. Carvalho et al., Chromatographia 69(2): 207-211, 2009
5. Carvalho et al., J Biomedical Nanotech 8(2) 280-289, 2012
6. Carvalho et al., Drug Develop Ind Pharm, In press, 2012
7. Carvalho et al., Curr Pharm Anal, In press
8. Carvalho et al., Braz J Pharm Sci 46(1): 1-17, 2010
9. Cazedey et al., Braz J Pharm Sci 45(4) 759 - 766, 2009
10. Balcárkováa et al., J Pharm Res 5(6), 2012
11. Carvalho et al., Exploring the Nanotechnology-Based Drug Delivery Systems for AIDS Treatment, In: KASENGA, F. H. (Org.). Understanding HIV/AIDS Management and Care - Pandemic Approaches in the 21st Century., Rijeka: InTech, 2011. p. 367-384.

AWARDS

Honorable Mention in the poster presentation of XIX Argentinean Pharmaceutical Congress of South-American Pharmaceutical Federation (FEFAS), 2009.

SUPERVISIONS AND EXAMINER FOR UNDERGRADUATE DEGREE COURSES

Supervised 1 B.Sc. Pharm. undergraduate Pharmacy degree at UNIARA, Brazil. External supervisor of 1 M.Sc. student and 3 B.Sc. Pharm. undergraduate Pharmacy degree at UNESP, Brazil. Acted as examiner of three B.Sc. Pharm. Undergraduate Pharmacy degree candidates at the School of Pharmacy, UNESP, Brazil.

PRESENTATION IN RESEARCH CONFERENCES

Speech presentations at Int. Workshop on Immune System, Dietary Factors and Nanotechnology, Araraquara, 2012; IV Braz. Conference on Rheology, Rio de Janeiro, 2008. Poster presentations at Colloids and Nanomedicine, Amsterdam, 2012; I Int. Meeting of Pharmaceutical Care, Brazil, 2010; XX and XIX Conference of South-American Pharmaceutical Federation, San Juan, 2009; 36th Annual Meeting & Exposition of the CRS, Copenhagen, 2009; 2nd Int. Symposium on Adv. Materials and Nanostructure, São Carlos, 2007.

RESEARCH FUNDING

Collaborator of the research project of the university-industry partnership between UNESP and Ferring Pharmaceuticals (2012-date).

Ad-hoc Journal Reviewer: J Pharm Sci, RBCFBA, Braz J Pham Sci.

Claudia Cella

Claudia Cella received her Master degree in Pharmaceutical Chemistry and Technology from the University of Pavia, Italy, in 2008, with a thesis about the development of a nanoparticle vehicle based on palmitate chitosan for an antifungal drug, clotrimazole. For the following 3 years, she pursued a school of specialization in Biomedical Research at the Mario Negri Institute for Pharmacological Research, Pharmacokinetics and Clinical Chemistry Laboratory, Renal Medicine Department, where she gained very good knowledge about analytical techniques, such as HPLC and ELISA, and their application in data collection in pharmacological clinical trials. Since October, 2012, she started her PhD course at the European School of Molecular Medicine (SEMM), Medical Nanotechnology curriculum, in the Advanced Biomaterials Platform at Fondazione Filarete, where she is working on the development of micro- and nano-polimeric systems for drug and gene delivery.

Vivek Chandra

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UNDERGRADUATE QUALIFICATION

MBBS, 2008, HIMS Dehradun, HNB Garhwal University, India

POSTGRADUATE QUALIFICATION

MD(Internal Medicine), 2011, University of Buckingham, UK

EMPLOYMENT

- December 2008 to January 2009 : Lecturer of Medicine, Purvanchal Institute of Dental Sciences, Gorakhpur, India.
- January 2009 to October 2010: Clinical Fellow, Ealing Hospital NHS London,UK
- February 2009 onwards : Sub Editor, West London Medical Journal, London,UK (Hon. International Chair, Editorial Board)

Presently: Consultant Physician at Private Clinic, Gorakhpur, India

RESEARCH

- Chandra V, Singhal A "Effectiveness of Visualisation Techniques in Primary Hypertension", Indian Council of Medical Research (ICMR)
- Chandra V, Prakash A " Composition of Gallstone and its prevalence in Uttranchal", Indian Council of Medical Research (ICMR)

AUDIT

- Audit of Medical Records for Medicine at Ealing Hospital NHS Trust, London.
- Audit on Management of Cardiac Failure at Ealing Hospital NHS Trust.
- Audit of Adenoma Detection at Colonscopy at Ealing Hospital NHS Trust.

PUBLICATIONS & CONFERENCE PRESENTATIONS

- British Infection Society Annual Meet, London
- Chandra V, "Hepatic Dysfunction in Diabetes", WLMJ
- Chandra V, Arnold A, "Hepcidine in Innate Immunity", WLMJ
- Jackson L, Chandra V, Michie C, "Hemivertebrae- Are they always symptomatic?",WLMJ
- International Congress of Cardiovascular Diseases, Mumbai, India
- Nanosensors & Artificial Kidney, World Congress of Nephrology, Singapore

• Indian Society of Nephrology, BHU, Varanasi, India

SKILLS COURSES ATTENDED

• Adult Basic Life Support Resuscitation Training Certificate
• Confidentiality, Access to Records & Data Protection for Health Professionals Course
• Hands-On Training Session, ESGE/OMED, European Society of Gastro Endoscopy, London.
• Various nano sensors KTN meets, NPL, London.

CONFERENCES ATTENDED

• Paediatrics update GP Teaching, Ealing Hospital NHS Trust.
• Infectious Diseases Multidisciplinary Workshop, Ealing Hospital NHS Trust.
• Diabetes and Endocrinology Study Day, Ealing Hospital NHS Trust.
• IS/AMM 13th Annual Scientific Meeting, SOAS, University of London.
• British Thoracic Society Winter Meeting, Westminster London.
• GASTRO 2009 UEGW/WCOG, ExCel London, UK.

MEMBERSHIP

• Permanent Member of The Royal Society of Medicine, London, UK.
• Indian Medical Association,India
• Indian Society of Gastroenterology, India
• British Society of Nanomedicine,UK
• Nano sensors KTN, UK
• Association of Physicians of India(API), India

Fu-Hsiung Chang

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EDUCATION

• 1989 Ph.D., Endocrinology Program, Department of Physiology University of California, San Francisco, U.S.A.
• 1982 M.S., Graduate Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan
• 1977 B.Ed., Department of Health Education, National Taiwan Normal University, Taipei, Taiwan

PROFESSIONAL EXPERIENCE

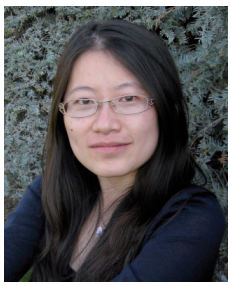
• 1992-2004 Department of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan
• 1989-199 Research Fellow, Eleanor Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center, Denver, Colorado, U.S.A.

RESEARCH FIELD

(1) Development of High-throughput Systems for Functional Genomics
(2) Application of Nanotechnology in Targeted Medicine

RECENT PUBLICATIONS

• Chang, C.-F., Chen, C.-Y., **Chang, F.-H**., Tai, S.-P., Chen, C.-Y., Yu, C.-H., Tseng, Y.-B., Tsai, T.-H., Liu I.-S., Su, W.-F. & Sun, C.-K (2008) Cell tracking and detection of molecular expression in live cells using lipid-enclosed CdSe quantum dots as contrast agents for epi-third harmonic generation microscopy, *Optics Express*, 16, 9534-9548.
• Tsai, C.-P., Chen, C.-Y., Hung, Y., **Chang, F.-H**. & Mou, C.-Y. (2009) Monoclonal antibody-functionalized mesoporous silica nanoparticles (MSN) for selective targeting breast cancer cells, *J. Material Chem*., 19, 5737–5743.
• Huang, H.-C., Chang, P.-Y., Chang, K., Chen, C.-Y., Lin, C.-W., Chen, J.-H., Mou, C.-Y. & **Chang, F.-H**. (2009) Formulation of novel lipid-coated magnetic nanoparticles as the probe for in vivo imaging, *J. Biomed. Sci*. 16, 86.
• Hui, Y.-Y., Zhang, B., Chang, K., Chang, Y.-C., Chang, C.-C., Chang, H.-C., Hsu, J,-H., Chang, K. & **Chang, F.-H**. (2010) Two-photon fluorescence correlation spectroscopy of lipid-encapsulated fluorescent nanodiamonds in living cells, *Optics Express*, 18, 5896–5905.
• Chang, C.-F., Chen, H.-C., Chen, M.-J., Liu, W.-R., Hsieh, W.-F., Hsu, C.-H., Chen, C.-Y., **Chang, F.-H**., Yu, C.-H. & Sun, C.-K. (2010) Direct backward third-harmonic generation in nanostructures, *Optics Express*, 18, 7397-7406.
• Lin, C.-Y., Liu, T.-M., Chen, C.-Y., Huang, Y.-L., Huang, W.-K., Sun, C.-K., **Chang, F.-H**. & Lin. W.-L. (2010) Quantitative and qualitative investigation into the impact of focused ultrasound with microbubbles on the triggered release of nanoparticles from vasculature in mouse tumors, *J. Control. Release*, 146, 291-298.
• Lin, C,-Y., Huang, Y.-L., Li, J.-R., **Chang, F.-H**. & Lin, W.-L. (2010) Effects of focused ultrasound and microbubbles on the vascular permeability of nanoparticles delivered into mouse tumors, *Ultrasound Med. Biol*., 36, 1460-1469.
• Lin, W.-J., Chen, T.-D., Liu, C.-W., Chen, J.-L. & **Chang, F.-H**. (2010) Synthesis of lactobionic acid-grafted-pegylated-chitosan with enhanced HepG2 cells transfection, *Carbohyd. Polym*., 83, 898-904.
• Chung, Y.-C., Wei, M.-F., **Chang, F.-H**. & Young, T.-H. (2011) PEGylated guanidinylated polyallylamine as gene-delivery carrier, J. Biomat. *Sci., Polym*. E. 22, 1829-1843.
• Lin, C.-Y., **Chang, F.-H**., Chen, C.-Y., Huang, C.-Y., Hu, F.-C., Huang, W.-K., Ju, S.-S. & Chen, M.-H. (2011) Cell therapy for salivary gland regeneration, *J. Dent. Res*., 90, 341-346.
• Hsieh, W.-J., Liang, C.-J., Chieh, J.-J., Wang, S.-H., Lai, I.-R., Chen, J.-H., **Chang, F.-H**., Tseng, W.-K., Yang, S.-Y., Wu, C.-C. & Chen, Y.-L. (2012) In vivo tumor targeting and imaging with anti-vascular endothelial growth factor antibody-conjugated dextran-coated iron oxide nanoparticles. *Int. J. Nanomed*., 7, 2833-2842.
• Chen, C.-L., Kuo, L.-R., Lee, S.-Y., Hwu, Y.-K., Chou, S.-W., Chen, C.-C.,**Chang, F.-H**., Lin, K.-H., Tsai, D.-H. & Chen, Y.-Y. (2012) Photothermal cancer therapy via femtosecond-laser-excited FePt nanoparticles. *Biomaterials*, 34, 1128-1134.



Cheng Chen

DFG-Centre for Functional Nanostructures, Karlsruhe Institute of Technology (KIT), Wolfgang Gaede Str. 1a, 76131 Karlsruhe, Germany
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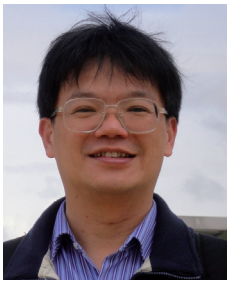
EDUCATION

• 04. 2011-onwards PhD student, DFG-Centre for Functional Nanostructures, Karlsruhe Institute of Technology, Germany
• 10. 2008-07. 2010 Exchange Student in Inorganic Chemistry, College of Chemistry and Molecular Engineering, Peking University, China
• 09. 2007-07. 2010 Master in Materials Physics and Chemistry, College of Chemistry and Chemical Engineering, Inner Mongolia University, China
•09. 2003-07. 2007 Double Bachelor's Degree in Materials Chemistry and English, Inner Mongolia University, China

PUBLICATIONS

1. **C. Chen**, I. Ahmed and L. Fruk, Oxidizing Properties of Copper Nanoparticles Stabilized by Dopamine Derivatives, submitted.
2. **C. Chen** and L. Fruk*, Functionalization of Maleimide-Coated Silver Nanoparticles through Diels-Alder Cycloaddition, RSC Adv., 2013, 3, 1709-1713.
3. F. Gao, C. F. Lv, J. X. Han, X. Y. Li, Q. Wang, J. Zhang*, **C. Chen**, Q. Li, X. F. Sun, J. C. Zheng, L. R. Bao, X. Li, CdTe-Montmorillonite Nanocomposites: Control Synthesis, UV Radiation- Dependent Photoluminescence and Enhanced Latent Fingerprint Detection, J. Phys. Chem. C, 2011, 115, 21574-21583.
4. **C. Chen**, L. D. Sun, Z. X. Li, L. L. Li, J. Zhang*, Y. W. Zhang, C. H. Yan*, Ionic Liquid-Based Route to Spherical NaYF4 Nanoclusters

with the Assistance of Microwave Radiation and Their Multicolor Upconversion Luminescence, Langmuir, 2010, 26, 8797-8803.
5. Z. X. Li, L. L. Li, H. P. Zhou, Q. Yuan, **C. Chen**, L. D. Sun, C, H. Yan*, Colour Modification Action of An Upconversion Photonic Crystal, Chem. Commun., 2009, 6616-6618.



Peilin Chen

Research Center for Applied Sciences
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PROFESSIONAL APPOINTMENTS

• 2010-present Research Fellow, Research Center for Applied Sciences, Academia Sinica
• 2012 Chief Executive Officer, Thematic Center for Optoelectronics, Research Center for Applied Sciences, Academia Sinica
• 2010-2012 Deputy Director, Research Center for Applied Sciences, Academia Sinica
• 2005-2010 Associate Research Fellow, Research Center for Applied Sciences
• 2001-2005 Assistant Research Fellow, Research Center for Applied Sciences
• 1999-2001 Postdoc, Department of Chemistry, University of California, Berkeley

EDUCATION

• 1998 Ph.D, Department of Chemistry, University of California, Irvine
• 1990 graduated from Department of Chemistry, National Taiwan University

AWARDS AND HONORS

• 2009 Career Development Award, Academia Sinica, Taiwan
• 2007 Research Award for Junior Research Investigators, Academia Sinica, Taiwan
• 2007 Ta-You Wu Memorial Award, National Research Council, Taiwan

RESEARCH INTERESTS

1)Super-resolution microscopy
2)Biointerfaces
3)Nanomaterials

María de la Fuente

María de la Fuente has a degree in Pharmacy and obtained her PhD degree in 2006 at the University of Santiago de Compostela (Spain), in the field of nanomedicine and nanotechnology. She was a visiting scientist at several institutions, namely the University of Angers (France), the University of Valladolid (Spain), the University of Kuopio (Finland), and the Institute of Biomedical Research "Alberto Sols" in Madrid (Spain). She also worked as a post-doctoral research fellow at the UCL-School of Pharmacy, London (UK), Department of Pharmaceutical & Biological Chemistry (2007-2010), and at the University of Santiago de Compostela, Department of Pharmaceutical Technology, Nanobiofar group (2010-2012). She currently holds a research position ("Miguel Servet" program) at the Health Research Institute - Clinical University Hospital of Santiago de Compostela (Spain), Translational Medical Oncology group.
María is the author of more than 20 scientific articles published in internationally recognized journals and book chapters, two international patents, and a number of presentations at international congresses. She has been participating in national and international research projects with a translational perspective. Her research is mainly focussed on the engineering, development, characterization, and preclinical evaluation of novel nanocarriers for the delivery of APIs, biotech drugs and gene therapies to cancer cells. The final aim is to develop new therapeutic strategies to battle cancer and to improve the current outcome of metastatic disease.

Laura De Miguel

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27 years, Spanish

SCIENTIFIC EDUCATION AND EXPERIENCE

• 2009- present PhD in Drug Delivery: Galien Institute, UMR-CNRS 8612, School of Pharmacy, University Paris-Sud XI, Châtenay-Malabry, France. (Oral defense: July 2013). Research Supervisor: Pr. G. Ponchel Design of different multifunctional bone targeted nanoparticles derived from poly (benzyl) glutamate for bone cancer applications. Supervision of 4 Master II Students
• 2011-2012 Part-time temporary lecturer and research assistant (ATER) at Galien Institute, UMR-CNRS 8612, School of Pharmacy, University Paris-Sud XI.
• 2008-2009 Master of Research II in Pharmaceutical Technology: School of Pharmacy, University Complutense of Madrid, Spain. Score: 97/10. Research Supervisor: Pr. J. Torrado. Insulin containing microspheres with modified release: design, physico-chemical characterisation and in vivo efficacy.
• 2003- 2008 Master of Pharmacy: School of Pharmacy, University of Navarre, Spain. Score > 9/10. Finalist of the best degree award.
• 2007 2 month awarded research fellowship at Center of Molecular Biology Severo Ochoa(CBMSO),CSIC. Supervisor: Dr F. Wandosell. Treatment of ovarian tumour cells TOV-112D with estradiol.

PUBLICATIONS

• Poly (γ-benzyl-glutamate) -PEG-alendronate multivalent nanoparticles for bone targeting applications. Laura de Miguel, Magali Noiray, Georgiana Surpateanu, Bogdan Iorga and Gilles Ponchel (in preparation)
• Effect of PLGA hydrophilia on the drug release and the hypoglucemic activity of different insulin-loaded PLGA microspheres: Presmanes C, de Miguel L, Espada R, Alvarez C, Morales E, Torrado JJ. J Microencapsul, 2011

POSTER COMMUNICATIONS

5 poster communication at national or international congresses.

Xavier de Mollerat du Jeu

Xavier de Mollerat du Jeu, Ph.D. is a Senior Staff Scientist and lead the R&D transfection group at Life Technologies, in Carlsbad, California, working on creating and improving siRNA in vivo/in vitro delivery methods for both research and therapeutic applications. Xavier is also working on identifying new DNA delivery approaches for primary cells (neurons, Blood cells). Xavier studied molecular biology and plant physiology at the University of Montpellier II in France, and received his Ph.D. in human genetics in 2003 from Clemson University in South Carolina. His thesis work involved identifying the gene(s) responsible for Split Hand/Split Foot Malformation 3 (SHFM 3). His post-doctoral fellowship research was in the laboratory of Dr. Michael G. Rosenfeld at UCSD, where he studied the roles of microRNAs in pituitary gland development. He joined Invitrogen (Life Technologies) in 2005.

María Victoria Defain

María Victoria Defain Tesoriero received a Biochemistry degree of the University of Buenos Aires in 1994. She has worked as resident in the "Dr. R. Gutiérrez Children´s Hospital" and she was resident chief in 1998. Nowadays she is working in National Institute of Industrial Technology (INTI, Buenos Aires Argentina) in R&D of Controlled Release System and Nanomedicines. She has published some articles in these fields. She is studying the last year of PhD in Universidad Nacional de Quilmes, Programme of Nanomedicine Research (PNM). Her PhD research focused on the development of targeted nanomedicines across the oral mucosa (delivery of macromolecules).

Fabio Del Ben

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Mobile +39-334-7549576
E-mail fdbf13@gmail.com

Date of birth: 13 November 1986

EDUCATION AND TRAINING

• 2013 PhD Student, Nanotechnology, Graduate School of Nanotechnology, University of Trieste, Italy
• 2012 Post-doctoral Research Fellow, Stem Cells and Myocytes biology,Brigham and Women's Hospital - Harvard Medical School, Boston, MA (USA)
• 2011 Medical Degree (MD), Thesis title: "Effects of cellular therapy on cardiac progenitor cells"., University of Udine, Italy
• 2005 Diploma of Liceo Scientifico, Focus on Math, Physics and Computer Science (PNI course), Liceo Scientifico "M.Grigoletti", Pordenone, Italy
• 2004 Diploma of 5th year of Pianoforte, Classical music, Conservatorio "G.Tartini", Trieste, Italy
• 2003 Diploma of musical theory and solfeggio, Conservatorio "G.Tartini", Trieste, Italy

WORK EXPERIENCE

2004-now Musician, all kind of Keyboard (Piano, Pipe Organ, Electronic) for special events.
2009 - 2011 Librarian, University of Udine, Italy

Thomas Démoulins

institute of Virology and Immunoprophylaxis
Sensemattstrasse 293, CH-3147 Mittelhäusern, Switzerland
E-mail address: thomas.demoulins@ivi.admin.ch

RESEARCH EXPERIENCE

• 2010-2013 Post-doctoral Fellow, Project: «Highly efficient new generation synthetic RNA-replicon based vaccine», IVI, Mittelhäusern, Switzerland
• 2007-2010 Post-doctoral Fellow, Project: «Role of pDCs in the initiation of immune response during early SIV infection», CEA/IMETI/SIV, Fontenay-aux-Roses, France
• 2003-2007 Post-doctoral Fellow, Project: «Impact of type I IFNs on T cell development», Unité INSERM U743, CHUM, Montréal, Canada
• 1999-2003 Ph.D. Thesis, Project: «Immunobiology of Multiple Sclerosis: an auto-immune disease of the central nervous system», CEA/SNV, Fontenay-aux-Roses, France

RELEVANT SCIENTIFIC PUBLICATIONS

• **Démoulins T.**, et al., Alginate-coated chitosan nanogel capacity to modulate the effect of TLR ligands on blood dendritic cells., *Nanomedicine: NBM* 2013; xx:1-12, http://dx.doi.org/10.1016/j.nano.2013.01.002
• **Démoulins T.**, et al., Poly (I:C) induced immune response in lymphoid tissues involves three sequential waves of type I IFN expression, *Virology* 2009; 386(2): 225-36
• **Démoulins T.**, et al., Reversible blockade of thymic output: an inherent part of TLR ligand-mediated immune response, *The Journal of Immunology* 2008; 181(10): 6757-69
•**Démoulins T.**, et al., A biased Vα24+ T-cell repertoire leads to circulating NKT cell defects in a multiple sclerosis patient at the onset of his disease, *Immunology Letters* 2003; 90(2-3): 223-28
• **Démoulins T.**, et al., The same TCR (N)Db(N)Jb junctional region is associated with several different Vβ13 subtypes in a multiple sclerosis patient at the onset of the disease, *Neurobiology of Disease* 2003; 14(3): 470-82
• **Démoulins T.**, et al., Preferential Survival of an MBP-Specific T Cell Clone in an HLA-DR2 Multiple Sclerosis Patient, *NeuroImmunoModulation* 2002; 10: 1-4

Diti Desai

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Phone: +358 465205520
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EDUCATION

• Pursuing Ph.D. (July 2010 till date) - Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, India
• Visiting Ph.D student - Laboratory of Physical Chemistry, Dept. of Natural Sciences, Åbo Akademi University, Turku, Finland
• Master of Pharmacy (2008-2010) - Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, India. Percentages- 74.92%, Class- First Class, Distinction
• Bachelor or Pharmacy (2004-2008) - ROFEL Shri G.M.Bilakhia College of Pharmavy, Veer Narmad South Gujarat University, Surat, India. Percentages- 76.40%, Class- First Class, Distinction

ACADEMIC ACHIEVEMENTS

• CIMO-India Finland Fellow (10/2011-01/2012; 01/2013-06/2013)
• Otto A. Malm Foundation Stipender (11/2012-12/2012)
• Received INSPIRE-DST Fellowship from Department of Science and Technology, INDIA for pursuing Ph.D.
• University Research Fellowship by The Maharaja Sayajirao University of Baroda for pursuing doctoral studies.
• Scholarship from "Themis Medicare Education Fellowship" for meritorious student in Second and Final B.Pharm.

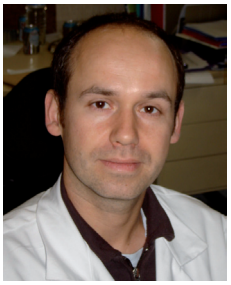
PUBLICATIONS

• Shape engineering vs organic modification of inorganic nanoparticles as a tool for enhancing cellular internalization. *Nanoscale Research Letters*, 2012, 7, 358
• Development and Validation of Stability-Indicating HPLC Method for Solifenacin Succinate: Isolation and Identification of Major Base Degradation Product, *Acta Chromatographica*, 2012, 1-16
• Determination of Felodipine and Enalapril in binary mixture using second derivative spectrophotometric, first derivative of the ratio-spectra UV methods and High-Performance Liquid Chromatography method. *Contemporary Investigations and Observations in Pharmacy*, 2012, 1(1), 31-39
• Development and validation of Stability-Indicating HPTLC method of Solifenacin succinate, *Asian Journal of Pharmaceutical and Bio-*

medical Research, 2011, 1(3), 310-16
• Simultaneous Estimation of Abacavir Sulfate and Lamivudine in their Combined Dosage Form by First Derivative Zero-Crossing and Ratio First Derivative Spectroscopic methods, *Indo American Journal of Pharmaceutical Sciences*, 2011, 1(1), 26-33.

REFERENCES

• Dr. Jessica Rosenholm, Docent, Principal Investigator (BioNanoMaterials research group), Laboratory of Physical Chemistry, Department of Natural Sciences, Abo Akademi University Porthansgatan 3-5, FIN-20500 Turku, Finland.
• Prof. Sadhana Rajput, Professor, Pharmacy Department, Faculty of Technology & Engineering, The Maharaja Sayajirao University of Baroda, Kalabhavan, Vadodara-390 001, Gujarat, INDIA.



Laurent Devel

Professional address: CEA, Direction des Sciences du Vivant (DSV), Institut de Biologie et Technologies (iBiTec-S), Service of Molecular Engineering of Proteins (SIMOPRO), CE de Saclay, Bat 152, Gif/Yvette 91191 France; E-mail: laurent.devel@cea.fr
Tel: 33-1-69089565; Fax: 33-1-69089071
Date of Birth: 02-06-1972

QUALIFICATIONS AND PROFESSIONAL APPOINTMENTS

• 2000 PhD in Organic and Molecular Chemistry (University P. M. Curie, Paris VI)
• 2001 Post-doctoral Fellow (University of Birmingham, UK)
• 2002 Post-doctoral Fellow (CEA, France)
• 2003 CEA Researcher, Service of Molecular Engineering of Proteins (SIMOPRO)

RESEARCH ACTIVITIES

Within the group of Vincent Dive, Dr Devel's research mainly focused on the development of potent inhibitors of zinc-proteases for therapeutic applications, diagnosis and molecular imaging. Development of Matrix MetalloProtease (MMP) inhibitors is the most long-term project that has been developed by Dr. Devel starting almost ten years ago in the frame of different European Projects dealing with the development of selective MMP inhibitors for cancer applications. Discovery of MMP-12 selective inhibitors (see Devel et al. 2006, 2010 and 2012) has redirected the interest of our group towards cardiovascular field, as MMP-12 is considered as an anti-target in cancer. MMP-12 is a zinc Matrix MetalloProtease also called macrophage elastase that is secreted by macrophage and over expressed in various pathologies such as atherosclerosis. Our group recently demonstrated in a mice model of atherosclerosis with established plaques, that RXP470.1, one of our highly selective inhibitor, fully blocked further plaque growth and prevent plaque rupture (Johnson et al. ATVB 2011). In the frame of the National Institutes of Health (NIH) funding, our group is currently developing novel MMP-12-specific tracers for imaging vessel wall inflammation in atherosclerosis. Moreover our tream belongs to the Laboratory of Excellence in Research on Medication and Innovative Therapeutics (LERMIT). Within this laboratory, we have initiated collaboration with the groups of Elias Fattal and Sylviane Lesieur with the aim to identify novel nanosystems for the diagnosis and therapy of atherosclerosis. Similar objectives, but with different nanoparticle systems and different targeting ligands are also pursued with the support of the European consortium "Nano_Athero".

MAIN PUBLICATIONS

• Development of selective inhibitors and substrate of matrix metalloproeinase-12. **Devel, L.**, Rogakos, V., David, A., Makaritis, A., Beau, F., Cuniasse, P., Yiotakis, A. and Dive, V. *J. Biol. Chem.* (2006) 281, 11152-11163.
• Insights from selective non-phosphinic inhibitors of MMP-12 tailored to fit with an S1' loop canonical conformation. **Devel, L**, Garcia S, Czarny B, Beau F, LaJeunesse E, Vera L, Georgiadis D, Stura E, Dive V. *J Biol Chem*. (2010), 285, 35900-9.
• A selective matrix metalloproteinase-12 inhibitor retards atherosclerotic plaque development in apolipoprotein E-knockout mice. Johnson JL, **Devel, L**, Czarny B, George SJ, Jackson CL, Rogakos V, Beau F, Yiotakis A, Newby AC, Dive V. *Arterioscler Thromb Vasc Biol*. (2011), 31, 528-35.
• Simple pseudo dipeptides with a P2' glutamate: a novel inhibitor family of MMPs and other metzincins. **Devel, L**, Beau F, Amoura M, Vera L, Cassar-Lajeunesse E, Garcia S, Czarny B, Stura EA, Dive V. *J Biol Chem*. (2012), 287, 26647-56.

Hans Deyhle

Goldauerstrasse 37, 8006 Zuerich
Tel. +41 (0)44 3610416
email: hans.deyhle@unibas.ch

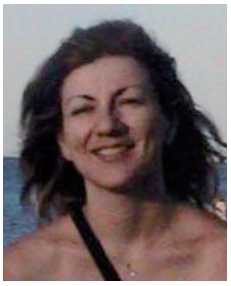
• 2007 Diploma in experimental physics at the Swiss Federal Institute of Technology Zürich; Diploma thesis on artificial muscles based on electrically activated polymers at the Biomaterials Science Center (University of Basel, Switzerland)
• 2007-2009 scientific collaborator at School of Dental Medicine (University of Basel, Switzerland), in the field of dental re-mineralization and imaging by X-ray absorption tomography
• 2009-2012 PhD thesis at the Biomaterials Science Center (University Basel) on the topic of small angle X-ray scattering applied to human teeth
• 2012-now post-doc at the Biomaterials Science Center (University Basel)

László Dézsi

PhD, DrHabil.
Senior Research Assoc., in vivo lab.
Semmelweis University, Budapest, Nanomedicine Research and Education Center
Phone +36206663502
E-mail dr.dezsi.laszlo@gmail.com
29th of Dec. 1955

PhD, DrHabil., Senior Research Associate in the in vivo laboratory of Nanomedicine Research and Education Center of Semmelweis University since 2012 in Budapest, Hungary.
He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), and in between he received fellowships at Albert Ludwigs Universität, Freiburg, Germany (Prof. E. Bassenge) working in the field of local regulation of blood flow in skeletal and cardiac muscle; as well as at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center (Prof. M. Reivich, Dr. J. H. Greenberg) working in the field of cerebral blood flow/metabolism and cerebral ischemia/reperfusion in stroke models in animals. He had been head of laboratory, CRO monitor and research project manager in vascular and safety pharmacology at Gedeon Richter Pharmaceutical Plc. (1999-2012). He was manager of Analgesic Research Laboratory (2006-2012), a joint venture of Gedeon Richter and University of Pécs, Department of Pharmacology (Prof. J. Szolcsányi). He participated in curriculum development and had been Secretary of Biomedical Engineering Course Committee at Technical University, Budapest (1994-2000). He made his habilitation at Semmelweis University in 2005 and became Private Professor in 2006. He established his own teaching course (2008) "Cardiorespiratoric and neurophysiological measuring techniques" at the Department of Human Physiology (Prof. Z. Benyó).

Currently he is working in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in in vivo models of complement activation related pseudoallergy (CARPA) under the supervision of Prof. J. Szebeni.



Eleni Efthimiadou

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Phone: +30 6937677557
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www.ims.demokritos.gr/nanotherapy

Date of birth: 28 December 1979

ACADEMIC CAREER

I completed my MSc and PhD at the University of Athens (Greece) in the domain of Chemistry and I deal with the synthesis, characterization and biological evaluation of organic and inorganic compounds and I was founded by the NCSR "Demokritos" during my PhD. The synthetic compounds, organic or inorganic, had been biological evaluated as potential new drugs, MRI contrast agents, antimicrobials and anticancer. After that, I am working for 3 years as a postdoctoral fellow at the Institute of Sol-Gel Laboratory, Institute for Advanced Materials, Physicochemical processes, Nanotechnology & Microsystems, of NCSR "Demokritos" in Athens, Greece, where I am working in the domain of Nanotechnology us potential Drug Delivery Systems (DDS).

In July 2009 I started my postdoc research at the group of Prof. Dr. George Kordas at the. The characterization of the fabricated NCs is on the one hand morphological by electron microscopy (Scanning, SEM and Transmittance TEM) and on the other hand structural by FT-IR, RAMAN. Furthermore, I contributed to the discovery of new multiple stimuli NCs based on biocompatible polymers (Polymethacrylates) and metallic NPs (Fe3O4 and gold nanoparticles) for targeted therapy and diagnosis, working on an IDEAS program founded by Dr. G. Kordas. The multi-stimuli NCs have investigated about their desired behavior in vitro, acidic pH conditions, Glutathione existence and temperature difference, which are prevailing in the tumor area. A second MSc was acquired in the domain of Chemistry, in the field of Catalysis and Enviromental Chemistry, in The Open University of Greece, Patra, Greece.

In July 2009 I started my postdoc research at the group of Prof. Dr. George Kordas at the Sol-Gel Laboratory, Institute for Advanced Materials, Physicochemical processes, Nanotechnology & Microsystems, NCSR "Demokritos" following a competitive process. I started my postdoc research in the field of Nanobiopharmaceutics within the group of Dr. G. Kordas who was one of the pioneers in the field of nanostructures. Dr. G. Kordas is recipient of an Advanced Grand ERC program named: IDEAS –NANOTHERAPY", a novel nano-container drug carrier for targeted treatment of prostate cancer. During this period, I worked in the development of new synthetic methods for Nanocontainers fabrication and their characterization and biological evaluation. In his laboratory, I successfully developed an efficient method to fabricate smart polymeric nanocontainers (NCs) based on their functionalization. The fabricated NCs exhibit different sensitivities such as pH, Thermo and Redox or combination of them. The NCs were modified with Magnetic Nanoparticles (MNPs) with target moieties, peptides and small molecules as well as coated with different biocompatible (polyethylene Glycol, PEG) and biodegradable polymers (Polylactic Acid, PLA). I also work on the polysaccharides as advanced coated material inducing specific pH, Thermo and Redox properties in this domain. I have experience in peptidic synthesis in solid phase and in the solution also. In this laboratory, I advanced my skills to characterize the fabricated NCs both, structural and morphological. For the NCs characterization, I learn to use and to estimate the result of the X-ray diffraction (XRD), Scanning and Transmission electron microscopy (SEM & TEM), thermogravimetric analysis (TGA) and others. The biological evaluation takes place using MTT & Trypan Blue assays in different cancer cell lines. During this period, I have published 9 papers, in the majority of which I am a co-corresponding author. I am a co-supervisor of two master and two Ph.D. candidates. This is a strong indication that I am willing to inspire young scientists to begin successful couriers. I am collaborating with Pr. C. Charitidis of the National Technical University of Athens, School of Chemical Engineering, Director of Material Science and Engineering Section.

ACADEMIC QUALIFICATIONS

- 2005-2010 Master in Catalysis and Environmental Chemistry, Department of Natural Sciences
- 2006-2009 Doctor of Philosophy (Ph.D), Synthesis and characterization of complexes with medical and medicinal applications
- 2004-2006 Master in Chemistry, Department of Natural Sciences, University of Athens, Greece
- 1999-2004 Diploma in Chemistry, Department of Natural Sciences, University of Athens, Greece
- 1998 Higher school certificate (2nd High School of Farsala, Larisa, Greece)

SCHOLARSHIPS

- 2006 Scholarship from the Institute of Physical Chemistry of the NCSR "Demokritos" for postgraduate studies (PhD thesis)

PROGRAM EXPERIENCE -FUNDING

- 1-3-04 to 30-6-04 Research in Paramagnetic Organometallic Compounds of Rare Earth Elements and study of these compound in health, Program Frame: "E-1094"
- 2007-2009 Research in MRI Contrast Agents, Program Frame: «PEP Attikis, 1.2, Action 1.2.1»
- 2007-2009 Research in Drug Delivery Systems, «Nanoscale Functionalities for Targeted Delivery of Biopharmaceutics», 'NMP' INTEGRATED PROJECT, Contract No NMP4-CT-2006-026723. European Union Program Frame
- 2007-2009 Research in Nanobiophrmaceutics, "IDEAS –NANOTHERAPY". A novel nano-container drug carrier for targeted treatment of prostate cancer

TEACHING EXPERIENCE

- 2012- now Seminar in the Master class of the National Technical University of Athens, School of
Chemical Engineering, of Material Science and Engineering Section.
- 2004-2009 University of Athens, Chemistry Department, laboratory assistant
- 2004-2009 Sivitanidios School in the domain of technical in medicals, cosmetics and similar products

THESIS SUPERVISION

- 2010-now Supervisor of the PhD candidate Chris Tapeinos, Institute for Advanced Materials, Physicochemical processes, Nanotechnology & Microsystems incollaboration with Materials Science Department, University of Patras, Greece.
- 2010-now Supervisor of the PhD candidate Katerina Metaxa, Organic Chemistry, University of Athens, Department of Chemistry, in collaboration with NCSR Demokritos
- 2010-now Supervisor of the BSc student NicoletaVlachou, National Technical University of Athens, School of Chemical Engineering, of Material Science and Engineering Section
- 2010-now Supervisor of the PhD candidate Anastasia Galani, Bio-Inorganic Chemistry, University of Athens
- 2010-2012 Supervisor of the MSc student Katerina Metaxa, Organic Chemistry, University of Athens, Department of Chemistry. Diploma Thesis: Polymeric Systems as anticancer drug carriers, in collaboration with NCSR Demokritos

COLLABORATIONS

- Prof. Karaliota, A. Inorganic Chemistry, University of Athens
- Prof. Charitidis, C. National Technical University of Athens, School of Chemical Engineering, of Material Science and Engineering Section
- Dr.Vourloumis, D. NCSR "Demokritos".
- Dr. Stratikos, S. NCSR "Demokritos".

PUBLICATIONS – CONFERENCE PARTICIPATION

- Number of Publications in peer-reviewed journals (March 2013): 27
- Non self-citations (Oct 2012): 550 (ISI, Scopus)
- h-index (Oct 2012): 15
- Number of presentations in national and international conferences: 31
- Number of oral presentations to international conferences: 7

JOURNAL REVIEWER

- Journal of Nanoparticle research (IF=3.287)
- European journal of Medicinal Chemistry (IF=3.346)
- Materials Science and Engineering C (IF=2.686)
- Journal of Surfaces and Interfaces B: Biointerfaces (IF=3.456)

Ramy El-Sayed

Ramy Elsayed, Hanstavgen 51- lgh 1524, 164 53 Kista, Sweden
Mobile: +49 176 3117 3822
Email: ramy.el.sayed@ki.se

Born January 30th, 1989, Cairo, Egypt

EXPERIENCE

- Oct. 2012-now PhD student, Institute of Environmental Medicine-IMM; Carbon Nanotubes: A biocompatible & multifunctional carrier, Karolinska Institutet- KI, Stockholm, Sweden
- Feb 2011-Oct 2012 PhD student, Department of Laboratory Medicine; Carbon Nanotubes: A biocompatible & multifunctional carrier, Karolinska Institutet- KI, Stockholm, Sweden

Nov 2009-March 10 Research assistant, Functional nano materials division,Drug delivery vesicles, Kungliga Tekniska högskolan- KTH, Stockholm, Sweden

EDUCATION

- Aug 2007-Jul 2009 Master degree of chemical science and engineering; Master major: Biomedical materials; Master thesis: Building Polyacrylic acid/chitosan polyelectrolyte complex on silica Substrate, Kungliga Tekniska högskolan- KTH, Stockholm, Sweden

SELECTED PUBLICATIONS

1. "*Thermostable luciferase from Luciola cruciate for imaging of carbon nanotubes and carbon nanotubes carrying doxorubicin using in vivo imaging system*." Nano letters (2013). **Ramy El-sayed** , Mohamed Eita, Åsa Barrefelt, Fei Ye, Himanshu Jain, Mona Fares, Mikael Crona et al.
2. "*Solid formulation of cell-penetrating peptide nanocomplexes with siRNA and their stability in simulated gastric conditions*." Journal of Controlled Release (2012). Ezzat, Kariem, Eman M. Zaghloul, Samir El-Andaloussi, Taavi Lehto, **Ramy El-Sayed**, Tarek Magdy, C. I. Smith, and Ülo Langel. 3. "Optical properties of thin films of zinc oxide quantum dots and polydimethylsiloxane: UV-blocking and the effect of cross-linking." Journal of Colloid Interface (2012). Eita Mohamed, Ramy El Sayed, Muhammed Mamoun.

Falk Andreas

MSc. Bakk.rer.soc.oec. Dipl.-MTA,
BioNanoNet Forschungsgesellschaft mbH
Elisabethstraße 9-11, 8010 Graz
Andreas.falk@bionanonet.at
www.bionanonet.at

EDUCATION

- 02.1996-09.1996 Study of medicine at Karl Franzens Universität Graz
- 10.1996-09.1999 Akademie für den med. techn. Laboratoriumsdienst
- 29.09.1999 Graduation: dipl. med.-techn. Analytiker" (Dipl.-MTA) Title of the Thesis: „The long-QT syndrom – the molecular background
- 2004 Training as "Internal Auditor" and "quality manager", GALP
- 10.2005-07.2009 Study of business administration at the University of Graz
- 24.07.2009 Graduation „Bakkalaureat Betriebswirtschaftslehre" (Bakk.rer.soc.oec.); Title of Bachelor-Thesis: „Aspekte eines strategischen Netzwerkes am Beispiel der BioNanoNet Forschungsgesellschaft mbH"
- 10.2009-09.2011 Master-Study of business administration at the Karl Franzens Universität, Graz (M.Sc.), 26.09.2011: Graduation "Master of Science"; Titel of the Master-Thesis: „The Role of Forgiveness in Relationship Marketing"

EMPLOYMENT

- 11/1999-08/2005 Technical Manager Laboratory Birkmayer, Laßnitzhöhe at the Institute of Nutrition and Metabolism
- 08.2005-now Research Associate at the Institute of Nutrition and Metabolism, Laßnitzhöhe
- 11.2005-now Biomedical analyst at the Institute of Medical Technologies and Health Management, JOANNEUM RESEARCH ForschungsGmbH, Working Group Bioanalysis;
- Since 10.2008: JOANNEUM RESEARCH - HEALTH – Institute for Biomedicine and Health Sciences, Working Group Nanotoxicology and Nanomedicine
- 10.2008-now Coordinator and Network Manager BioNanoNet Forschungsgesellschaft mbH; Responsibilities: organizational, operational network management, quality assurance, project proposals and project coordination
- Since 02.2013: CEO at BioNanoNet Forschungsgesellschaft mbH
- 01.2009-now Coordinator of the European Center for Nanotoxicology (EURO-NanoTox)
- 05.2009-now Coordinator of working group Medical Sensor Solutions (MSS)

MEMBERSHIPS

- Member of High level group of EU Member States and FP7 Associated States on Nanoscience and Nanotechnologies (national expert, nominated by bmwf and bmvit)
- Co-Founder and managing editor of ONLINE-Journal EURO-Nano-Tox-LETTERS, ISSN: 2074-8515; Scope: nanotoxicology, nanomedicine
- Designated Contact Point for Austria in OECD Working Party on Manufactured Nanomaterials
- Member of NANOfutures asbl
- Member of European Technology Platform Nanomedicine ETPN.
- Member of NanoSafetyCluster, co-leader in communication-dissemination working group
- Member of experts group of the Austrian NanoInformationPortal NIP (working group within the Austrian Nano Action Plan)
- Member of Management Committee: COST Action TD1204 "MODENA – Modelling Nanoparticle Toxicity"
- Member of Scientific Advisory Board of CLINAM-Conference
- Member of Scientific Advisory Board of BioNanoMed-Conference
- Member of International Scientific Advisory Board of NANOSOLUTIONS-project

RESEARCH (main areas)

Nanosafety, Nanotoxicology, Nanomedicine, Oxidative Stress

PROJECTS

- 03.2005-02.2012 „Nano-Health - Nano-structured Materials for Drug Targeting, Release and Imaging", Nano Initiative, Austria (FFG),Activities: administrative coordination; dissemination and communication.
- 05.2011-10.2013 „NANOFORCE Nanotechnology for Chemical Enterprises - How to link scientific knowledge to the business in the Central Europe", CE, Grant agreement: 3CE394P1; Activities: Work package leader WP4; Project leader PP7, Communication manager PP7.
- 10.201-09.2013 NanoProdEx - Nano-Produkte – Identifikation und Exposition, NANO-EHS (FFG), Grant agreement: 835354; Activities: project coordinator, project manager.

11.2012-10.2016 SPIDIMAN - Single-Port Insulin Infusion for Improved Diabetes Management, EU-FP7-HEALTH, 2012.2.4.3-1; Grant agreement 305343; Activities: project manager.

• 3.2013-08.2016 "NANoREG - A common European approach to the regulatory testing of nanomaterials", EU-FP7-NMP-2012-LARGE-6; Grant agreement 310584; Activities: Task leader, national coordinator, project partner.

PUBLICATIONS

• **Falk A.**, Hartl S., Sinner F., 2013, „Regulation and Safety Implementation of Nanotechnology for Chemical Enterprises in the Central Europe Space", J. Phys.: Conf. Ser. 429 012068, DOI: 10.1088/1742-6596/429/1/012068.

• Wonisch W., **Falk A.**, Sundl I., Winkelhofer-Roob B.M., Lindschinger M., 2012, "Oxidative Stress increases continuously with BMI and with unfavourable profiles in males", The Aging Male, 1-7. DOI: 10.3109/13685538.2012.669436.

• Roblegg E.,**Falk A.**, Froehlich E., Zimmer A., Sinner F., 2011, European Center for Nanotoxicology EURO-NanoTox – An Austrian Activity, Academy of Science Malaysia Journal, 5 (1).

• Weinhandl H., Pachler C., Mader J. K., Ikeoka D., Mautner A., **Falk A.**, Suppan M., Pieber T. R., Ellmerer M., 2007, "Physiological hyperinsulinemia has no detectable effect on access of macromolecules to insulin-sensitive tissues in healthy humans."

• Pachler C., Ikeoka D., Plank J., Weinhandl H., Mader J., Suppan M., **Falk A.**, Bodenlenz M., Mangge, H., Pieber T. R., Ellmerer M., 2006, „Direkte Messung des Effektes von Insulin auf die Sekretion von proinflammatorischen Zytokinen im subkutanen Fettgewebe beim Menschen".

• Weinhandl H., Koehler H., Pachler C., Mader J., Mautner A., **Falk A.**, Ikeoka D., Bodenlenz M., Pieber, T. R., Ellmerer M, 2006, „Untersuchung des Effektes Physiologischer Hyperinsulinämie auf den Zugang von Makromolekülen zu insulin-sensitiven Geweben".

Sara Fortuna

Sara Fortuna has been awarded her PhD in Chemistry from Warwick University in September 2010, and after spending two years in SISSA in Dr. Fabris group looking at metal-supported organometallic nanostructures, she is now working in Prof.Scoles group in the Department of Medical and Biological Sciences at the University of Udine on a project entitled "Molecular Simulations for Nanomedicine".

Her area of expertise is in the multiscale modelling of noncovalently bound molecular systems. She acquired competences in both soft-matter (lattice and off-lattice models, Monte Carlo and molecular dynamics simulations) and DFT techniques. This skill set is suitable for and transferable to the study of the structure-properties relationship of a large variety of chemical systems. Now her research focuses on the peptide-protein interactions for the optimisation of peptide-based binders for molecular recognition.

She is author of 9 papers published in reputable peer reviewed journals (J.Phys.Chem, Langmuir, J.Am.Chem.Soc.), and she has been invited to present her work at seminars and international workshops. She is member of the Institute of Physics (IOP) and the Royal Society of Chemistry (RSC), and peer review for American Chemical Society (ACS) journals (J.Phys.Chem, J.Phys.Chem.Lett., J.Chem.Theory Comput.).

Vivek Sambhaji Gaware

Faculty of Pharmaceutical Sciences,, University of Iceland, Hagi, Hofsvallagata 53
107 Reykjavik, Iceland
E-mail: vsg3@hi.is; vivekgaware@gmail.com
Tel. No.: +354-8416772

Born On 14.12.1981 in Indian
Languages Known: English, Hindi & Marathi

ACADEMICS

• Ph. D. Student (Sep. 2008-till date), Supervisor- Prof. Már Másson; Faculty of Pharmaceutical Sciences, University of Iceland, IS-107, Reykjavik, Iceland

• M.Sc. (Organic Chemistry) (2002-2004), Fergusson College, University of Pune, Pune-411004, Maharashtra, India

• B.Sc. (1999-2002), R. B. N. B. College, University of Pune, Shrirampur-413709, Maharashtra, India.

INDUSTRIAL EXPERIENCE

1. Nycomed Pharmaceuticals Pvt. Ltd. (June 2006 to Aug. 2008), I worked as a Research Associate in Medicinal Chemistry Division in then, Nycomed Pharmaceuticals Pvt. Ltd. (now, Nycomed: A Takeda Company), Mumbai - 400 093, Maharashtra, India.

2. Glenmark Pharmaceuticals Ltd. (Jan. 2006 to June 2006), I worked as a Research associate in New Drug Discovery Division in Glenmark Pharmaceuticals Ltd, Mahape, NaviMumbai-400701, Maharashtra, India.

3. National Chemical Laboratory, Pune (CSIR INDIA) (Nov. 2004 to Jan. 2006), In National Chemical Laboratory, I worked as a Project Assistant, NCL, Dr. Homi, Bhabha Road, Pune – 411008, Maharashtra, India.

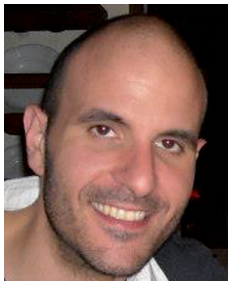
PUBLICATIONS

• **Gaware, V. S.**, Håkerud, M., Leósson, K., Jónsdóttir, S., Høgset, A., Berg, K., & Másson, M. (2013). Tetraphenylporphyrin Tethered Chitosan Based Carriers for Photochemical Transfection. *Journal of Medicinal Chemistry*, 56(3), 807-819.

• Padiya, K. J., Gavade, S., Kardile, B., Tiwari, M., Bajare, S., Mane, V., **Gaware, V. S.**, Varghese, D., Harel, &. Kurhade, S. (2012). Unprecedented "In Water" imidazole carbonylation: paradigm shift for preparation of urea and carbamate. *Org Lett*, 14(11), 2814-2817.

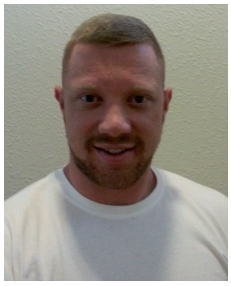
• Benediktsdottir, B. E.,**Gaware, V. S.**, Runarsson, O. V., Jonsdottir, S., Jensen, K. J., & Masson, M. (2011). Synthesis of N,N,N-trimethyl chitosan homopolymer and highly substituted N-alkyl-N,N-dimethyl chitosan derivatives with the aid of di-tert-butyldimethylsilyl chitosan. *Carbohydrate Polymers*, 86(4), 1451-1460.

• Song, W. L.,**Gaware, V. S.**, Runarsson, O. V., Masson, M., & Mano, J. F. (2010). Functionalized superhydrophobic biomimetic chitosan-based films. *Carbohydrate Polymers*, 81(1), 140-144.



Giuseppe Gianfranceschi

Giuseppe Gianfranceschi has been awarded the Master degree in Molecular and Industrial Biotecnologies from University of Perugia in 2011 with 110/110 cum laude. Before graduation he carried out an internship within the course in the Department of Experimental Medicine and Biochemical Sciences, University of Perugia, learning about the primary techniques of recombinant DNA. He is now pursuing his PhD in "Clinical Sciences and Technologies", working in Prof. Scoles group in the Department of Medical and Biological Sciences at the University of Udine on a project entitled "Application of Advanced Nanotechnology in the Development of Innovative Cancer Diagnostic Tools". Specifically, he's involved in a project aimed at dissecting the mechanisms responsible for cardiac stem cell senescence and at developing innovative pharmacological strategies able to interfere with the cell senescence processes. Moreover, he's interested in studying and using miRNAs as circulating biomarkers in heart failure.



Paul Grobler

Grace Haven 3, Potchefstroom, North-West, South Africa
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Born 1986-05-29 in Pretoria, SA

EDUCATION

• High School: Driehoek High School

• University: North-West University, Potchefstroom Campus

• Tertiary education: B.Pharm, 2008 ; Mcs Pharmaceutical Chemistry, 2010
• 3rd year PhD Pharmaceutics student

Bartosz F. Grzeskowiak

Nietazkowo, ul. Dudycza 4/2, 64-030 Smigiel
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mobile: 695837555

date of birth: 14th January 1986

EDUCATION

• X.2010-now International Scholarship, The PhD program in Nanoscience and Nanotechnology, NanoBioMedical Centre, Faculty of Physics, Adam Mickiewicz University in Poznan, Poland, PhD thesis advisor: Prof. dr hab. Ryszard Slomski, Topic: Development of vector nanodelivery systems for transgenesis
• 10.2007- 6.2008 ERASMUS Program, Biotechnology, University of Natural Resources and Applied Sciences in Vienna, Austria
• 2005-2010 MSc. in Biotechnology, Poznan University of Life Sciences, Faculty of Agronomy and Bioengineering, Poland, Excellent final grades. Master's thesis performed in the Human Genetic Institute PAS, Poznan, Poland
• 2002-2005 High School, Leszno, Poland

PROFESSIONALS EXPERIENCE

• 01.1.-30.5.2009 Apprenticeship at the Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Poland
• 22.6.-21.7.2009 Internship at the Institute of Bioorganic Chemistry PAS, Poznan, Poland
• 01.9.-27.10.2009 Internship at the University of Natural Resources and Life Sciences in Vienna, Austria, Department of Biotechnology
• 01.1.-31.12.2012 Internship at the Technical University of Munich, Institute of Experimental Oncology and Therapy Research, Munich, Germany

Olga Ye. Gudkova

e-mail: orbf@mail.ru

Date of birth: 26.08.1985
Speciality – biophysicist. Position – researcher in the Laboratory of biophysics of cells membranes in critical states, V.A. Negovsky Research Institute of General Reanimatology, Russian Academy of Medical Sciences, Moscow.
The author of 8 articles in scientific journals. My scientific interests: atomic force microscopy, red blood cells, modificators, influence of ionizing radiation on cells membranes.

Robert Hennig

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SCIENTIFIC CAREER

• Since 2011 Graduate student at Department of Pharmaceutical Technology, University of Regensburg
• 2010 Diploma in Pharmaceutical Science at Department of Pharmaceutical Technology, University of Leipzig
• 2005-10 Pharmaceutical studies at University of Leipzig

PEER-REVIEWED JOURNAL ARTICLES

• Pollinger, K.; **Hennig, R.**; Ohlmann, A.; Fuchshofer, R.; Wenzel, R.; Breunig, M.; Tessmar, J.; Tamm, E. R.; Goepferich, A.: Ligand-functionalized nanoparticles target endothelial cells in retinal capillaries after systemic application. In: Proc. Natl. Acad. Sci. U.S.A. 2013, 110 (15), 6115–6120.
• Pollinger, K.; **Hennig, R.**; Breunig, M.; Tessmar, J.; Ohlmann, A.; Tamm, E. R.; Witzgall, R.; Goepferich, A.: Kidney podocytes as specific targets for cyclo(RGDfC)-modified nanoparticles. In: Small 2012, 8 (21), 3368–3375.

Simone E. Hieber

CURRENT POSITIONS

• Since 02.2007 Research Assistant (since 2012 Research Associate), Biomaterials Science Center, University of Basel
• Since 02.2010 Academic Coordinator, Biomedical Engineering, University of Basel

EDUCATION

• 02.2007 PhD Computer Science, Computational Science and Engineering Lab, ETH Zurich, Switzerland, Thesis: Particle methods for flow-structure interactions
• 10.2001 Diploma Engineering Cybernetics, University of Stuttgart, Stuttgart, Germany, Thesis: Model-based optimization of the ethanol rate in S. Cerevisiae
• 05.2001 Master of Science Mathematics, Michigan Technological University, Houghton, MI, USA, Thesis: An investigation of the mesh dependence of the stochastic discrete droplet model applied to dense liquid sprays

RESEARCH ACTIVITIES

• Modeling and Simulation (e.g. particle methods, level sets, FEM)
• Image Processing (e.g. segmentation, registration)
• Biomedical Engineering (e.g. virtual surgery, cancer research)

TEACHING ACTIVITIES

• Biomedical Simulations
• Numerical and Symbolic Computing

Ingrid Hilger

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PROFESSIONAL CAREER

• 1983-1990 Studies of Biology at the Christian-Albrecht University of Kiel (WS 86/87: marine biology study visit in Malaysia) (Diploma thesis: summa cum laude)
• 1991-1993 Biologist, Dept. of Clinical Biochemistry, Georg-August University of Göttingen, Topic: Fundamental principles of cellular interactions (Research area: Biochemistry)
• 1993 -1995 PhD student, Institute of Experimental Pathology, Medical School Hannover, Topic: In vitro toxicology: Implementation of in vitro toxicological assays as alternatives to in vivo animal experiments (Research areas: Pharmacology, Toxicology), (PhD Thesis: summa cum laude)
• 1996-1999 PostDoc, Institute of Diagnostic and Interventional Radiology, University Hospital Jena, Germany. Topic: Use of iron oxide nanoparticles for diagnostic and therapeutic purposes (Research areas: Nanotechnology, Biomedicine, Biophysics)
• Since 1999 Leader of the Department „Experimental Radiology", Institute of Diagnostic

and Interventional Radiology, University Hospital Jena, Germany. Topics: Minimal-invasive tumor treatments; molecular imaging in cancer and inflammation

• 2003 WalterFriedrich Award" of the German Radiological Society, awarded in Wiesbaden (Germany), 29th May 2003

• 2000-2003 Habilitation in Experimental Radiology; Thesis: „Magnetic thermoablation of small tumors in the breast" (assistant Professor of Experimental Radiology)

• Since 2008 Full Professor of Experimental Radiology

James Hobson

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Date of Birth: 6th October 1986

EDUCATION

• Oct 2010-Present, University of Liverpool: Ph.D Clinical and Molecular Pharmacology
Development of novel nanoemulsion drug delivery systems for poorly water-soluble drugs, particularly antiretroviral drugs; Jointly supervised by Department of Clinical and Molecular Pharmacology and Department of Chemistry; Founding member and Executive Group member of the British Society of Nanomedicine; Supervisors: Dr Andrew Owen and Prof. Steve Rannard

• Sept 2009-Oct 2010, University of Liverpool: M.Res Pharmacology and Therapeutics; Research Project 1- Cytotoxic evaluation of the components for a novel HAART nanoemulsion drug delivery system; Research Project 2- Oil in water micron and nanoemulsions stabilised by branched copolymer "Pickering Like" stabilisers: Potential for a drug delivery system; Research Project 3- Biological safety assessment of 5 "Pickering Like" stabilisers for a nanoemulsion drug delivery system; Supervisors: Dr Andrew Owen and Prof. Steve Rannard

• Sept 2008-Sept 2009, University of Liverpool: M.Sc Advanced Science (Chemical Biology); Research Project-Characterisation and Functionalisation of Nanoparticles for Applications in Biological Science; Supervisors- Prof. Dave Fernig and Dr Raphael Levy

• Sept 2005-June 2008, University of Liverpool: B.Sc (Hons) Biochemistry; Final year modules: Molecular medicine, Biochemical messengers and signal transduction, Medical glycobiology, Bioinformatics, Gene Expression, Protein structure, function, and organisation; Final Year Honours Project- Purification and analysis of modified esterase for improved stereo isomeric properties; Supervisor: Dr Lesley Iwanejko

Margarethe Hofmann

Dr. Margarethe Hofmann-Amtenbrink (Ing.-grad. in Foundry University of Duisburg, Dipl.Ing., and PhD in Materials Science at Max Planck Institute Stuttgart and Technical University Berlin, Germany) is independent since 1987 with Mat Search Consulting Hofmann in Switzerland. She has managed various Swiss Societies and a Foundation, different research programmes of the Swiss Government and Foundations in advanced materials (Priority Programme Materials), in biomaterials (Gebert Ruef Foundation) and in biotechnology (AO Foundation Davos). For the past ten years M. Hofmann-Amtenbrink has been involved in research projects on nanoparticles for biomedical applications and initiated two EU Research Projects. She is member of several Advisory Boards in Germany and Switzerland (Helmholtz-Zentrum Geesthacht, Europäische Akademie Bad Neuenahr-Ahrweiler GmbH, Competence Center for Applied Biotechnology and Molecular Medicine, University of Zurich), Vice President of the Federation of European Materials Societies, FEMS and member of the Swiss Academy of Technical Sciences.

Lubna Hossain

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PhD student (3rd year), St George Clinical School, University of New South Wales, Sydney, Australia. Involved in a project 'Formulation and evaluation of albendazole loaded albumin nanoparticles for the treatment of ovarian cancer' collaborated with Centre for Advanced Macromolecular Design, Department of Chemical Engineering and Department of Surgery, St George hospital, UNSW, Australia.

EDUCATIONAL QUALIFICATION

• Master of Pharmacy, Jahangirnagar University, Savar, Bangladesh
• Bachelor of Pharmacy, Jahangirnagar University, Savar, Bangladesh

PROFESSIONAL WORK EXPERIENCE

• Worked as a quality Assurance Officer in Lipa Pharmaceuticals Ltd, Sydney, Australia from 2007 to 2011
• Worked as a QA/Regulatory affairs Officer in GMP Pharmaceuticals Ltd, Giraween, Sydney, Australia from 2006 to 2007
• Worked as a QA/AC Officer in healthcare Pharmaceuticals Ltd, Rajendrapur, Bangladesh, (Licensee of Hoffmann-L Roche, Basel, Switzerland) from 2002 to 2005
• Worked as a Production Officer, Novartis Bangladesh Ltd, Tongi, Bangladesh from 1999 to 2002

CONFERENCE ATTENDED

• Drug Delivery Australia, Melbourne, Australia, Nov, 2012
• International Nanomedicine Conference, Sydney, Australia July, 2011 and 2012
• St George Sutherland Symposium, October, 2011 & 2012

PLAN TO ATTEND CONFERENCE IN 2013

• The Lowy Cancer Symposium, UNSW, Sydney, Australia, May 2013
• CLINAM (European foundation for Clinical Nanomedicine), June, 2013
• International Nanomedicine Conference, Sydney, Australia July, 2013

PUBLICATIONS

• First experimental paper has been submitted 'Uptake and intracellular fate of albendazole loaded albumin nanoparticles in ovarian cancer'.
• Another two will be submitted next 6-12 months.

Samir M. Iqbal

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Department of Bioengineering (Courtesy)
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EDUCATION

• 1997 B.E., Electrical Engineering, NED University of Engineering and Technology (Pakistan)
• 2007 Ph.D., Electrical & Computer Engineering, Purdue University, West Lafayette, Indiana (USA)
• 2007 Post-Doc, Bio-Nanotechnology, Purdue University, West Lafayette, Indiana (USA)

APPOINTMENTS

• 5.11-now Assistant Professor, Bioengineering Department (Courtesy), University of Texas at Arlington, Arlington, Texas
• 6.08-now Member Joint Graduate Studies Committee, Bioengineering Program, UT-Arlington and University of Texas Southwestern Medical Center at Dallas, Arlington, Texas
• 7.07-now Assistant Professor, Electrical Engineering Department, University of Texas at Arlington, Arlington, Texas
• 2.07-7.07 Post-Doctoral Research Associate, Birck Nanotechnology Center, Purdue University, West Lafayette, Indiana
• 1.02-2.07 Teaching/Research Assistant, School of Electrical and Computer Engineering, Purdue University, West Lafayette, Indiana
• 1999-2001 Visiting Faculty, Swedish Institute of Technology, Gujranwala, Pakistan
• 1998-2001 Senior Programmer, Eastern Digital Technologies, Karachi, Pakistan
• 1996-1998 Electrical Engineering Officer, Pakistan Navy, Karachi, Pakistan
• 1995-1996 Consultant, International Management Group, Karachi, Pakistan

SOME PUBLICATIONS (out of 30+)

1- Y. Wan, M. A. I. Mahmood, N. Li, P. B. Allen, Y.-t. Kim, R. Bachoo, A. D. Ellington and **S. M. Iqbal**, "Nanotextured Substrates with Immobilized Aptamers for Cancer Cell Isolation and Cytology," Cancer, 118(4), (2012).
2- W. Asghar, M. Islam, A. S. Wadajkar, Y. Wan, A. Ilyas, K. T. Nguyen and **S. M. Iqbal**, "PLGA Micro and Nanoparticles Loaded into Gelatin Scaffold for Controlled Drug Release," IEEE Transactions on Nanotechnology, Appeared Online: 05 Jan 2012, (2012). DOI: 10.1109/TNANO.2012.2183004.
3- Y. Wan, Y. Liu, P. B. Allen, W. Asghar, M. A. I. Mahmood, J. Tan, H. Duhon, Y.-t. Kim, A. D. Ellington and **S. M. Iqbal**, "Capture, Isolation and Release of Cancer Cells with Aptamer-functionalized Glass Beads," Lab on a Chip, vol. 12, no. 22, pp. 4693-4701 (2012).
4- S. D. Vidyala, W. Asghar and **S. M. Iqbal**, "Porous Organic Nano-Films for Coating of Solid-State Devices," Journal of Nanobiotechnology, vol. 9, no. 18 (2011).
5- S. Goyal, Y.-t. Kim and **S. M. Iqbal**, "Vapor-Phase Facile Coatings of Nanotextured Organic Biocompatible Films on Solid-State Substrates," IEEE Transactions on Nanotechnology, vol. 9, no. 5, 618-624 (2010).
6- **S. M. Iqbal**, D. Akin and R. Bashir, "Solid-state Nanopore Channels with DNA Selectivity," Nature Nanotechnology, 2(4), 243-248 (2007).

HONORS

• 2013 University of Texas at Arlington Best Academic Award Advisor 2013, Honorable Mention
• 2013 College of Engineering Nominee for President's Award for Excellence in Teaching
• 2011 Recognized Professor, Phi Kappa Phi
• 2009 NSF CAREER Award
• 2009 Best Paper Award at IEEE LiSSA'09 Workshop, Bethesda, MD, USA (April 9-10, 2009).
• 2009 Best Poster Award at From DNA-Inspired Physics to Physics-Inspired Biology, The Abdus Salam International Centre for Theoretical Physics, Trieste, Italy (June 1-5, 2009).
• 2006, 2008 NSF Fellowship for "NSF Summer Institutes" at Northwestern University.
• 2003-2004 Magoon Award for Excellence in Teaching. Chosen amongst the teaching assistants from 13 engineering schools, based on superior teaching achievements.

Olivier Jordan

Nationality : swiss, french
Ch sous le bois 15A, 1197 Prangins, Suisse
Tél. prof.: (+41) 22 379 65 86
Olivier.Jordan@unige.ch

EDUCATION

• 1984 Lycée International de Ferney-Voltaire: Scientific Baccalaureate with mention
• 1989 Swiss Federal Institute of Technology (EPFL): Engineer physicist diploma.
• 1996 Ph D degree, EPFL. Thesis "Microencapsulation of Langerhans islets by laser-induced photopolymerization."

EXPERIENCE

• Since 12.2010 Senior lecturer (Maître d'Enseignement et de Recherche) at the School of Pharmacy, University of Geneva.
Research projects in the field of injectable biomaterials and drug delivery:
- Biopolymers for regenerative medicine: Chitosan-based injectable hydrogels for bone and skin replacement
- Drug delivery for arthritis and osteoarthritis: microparticules for intra-articular drug delivery
- Peptide delivery for wound healing applications
- Functional implants: heat-releasing implants for hyperthermia treatment in oncology
- Radiopaque polymers for embolization of vascular defects. In vitro testing and evaluation on animal models
- Soft tissue augmentation for the treatment of urinary incontinence
- Biomaterials and physical pharmacy teaching
- Technology valorisation: 5 patents , 2 technology transfers to industrial partners
- Thesis co-direction of 8 PhD students (+5 PhD thesis completed)
• 9.2008-12.2010 Research associate (Adjoint scientifique) at the School of Pharmacy, University of Geneva
• 12.2005-9.2008 Collaborateur scientifique at the School of Pharmacy, University of Geneva
• 9.1999-2005 Maître Assistant at the School of Pharmacy, University of Geneva, Prof. E. Doelker
• 4.1997-8.1999 First assistant at the Division for Surgical Research and Gene Therapy Center, CHUV, Lausanne, Prof. Patrick Aebischer:
-Coordination of a "Swiss Priority Program on Materials" on biomimetic materials for artificial cartilage, implying 4 academic and 2 industrial partners
-Research on a bioartificial pancreas: polymer synthesis, cell encapsulation, in vitro demonstration of functionality, in vivo evaluation.
-Development of nerve regeneration implants: promotion of axonal growth within conduits bearing molecular adhesive cues or releasing neurotrophic factors. Bridging of long nerve gap in rodent models.
• 1992 - 1997 EPFL, Department of Microtechnology: thesis work on a bioartificial pancreas project

MISCELLANEOUS

• Research support: De Reuter foundation (2010), Innovation Promotion Agency CTI (2008), Swiss National Fund for Scientific Research, main applicant (2009) and co-applicant (2004, 2007)
• Co-direction of 4 PhD thesis, supervision of 2 lab technicians
• Teaching in biomaterials and pharmacy (yearly), various seminars
• Expert for Ph D thesis (8) and diploma thesis (5/year)
• Paper reviewing for publishers (approx 2 monthly)
• Inventorship on 5 patents
• Co-founder of a startup in the field of functional implants
• Membership: European federation for pharmaceuticals sciences, European society for biomaterials, Swiss society for biomaterials

Aleksandra E. Kedzierska

MSc, Eng.
Osiedlowa 18, 97-200 Kolonia Zawada, Poland
e-mail: olkakedz@unipress.waw.pl,
aleksandra.kedzierska@inmat.pw.edu.pl
date of birth: 12.06.1986

EDUCATION

• 2012-now PhD studies, Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland
• 2005-2010 Master of Science, Engineer in field of material science , Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Cracow, Poland
• 2002-2005 secondary education , High School „Radosna Nowina 2000", Piekary/ Cracow, Poland

WORK EXPIERIENCE

• 2011-now Laboratory of Nanostructures for Photonics and Nanomedicine, Institute of High Pressure Physics, Polish Academy of Sciences, Warsaw, Poland PhD student, work with international project SONOSCA, participating in accreditation process in laboratory

PUBLICATIONS

• Smoleń, D., T. Chudoba, S. Gierlotka, et al. 'Hydroxyapatite Nanopowder Synthesis with a Programmed Resorption Rate', Journal of Nanomaterials, vol. 2012/(2012), .
• Smoleń, D., T. Chudoba, I. Malka, et al. 'Highly Biocompatible, Nanocrystalline Hydroxyapatite Synthesized in a Solvothermal Process Driven by High Energy Density Microwave Radiation', International Journal of Nanomedicine, vol. 8/(2013), pp. 653-668.

CONFERENCES

• 13-15.03.13 6th Annual Meeting 2013 "Materials for Tissue Engineering", Hafjell, Norway poster "Ultrasonic coating technique of a polymer scaffolds for bone implant applications"
• 17-21.09.12 E-MRS 2012 Fall Meeting, Warsaw, Poland poster "Ultrasound Coating Technique for Bioresorbable Polymer Scaffolds"
• 5-8.09.2012 3rd TERMIS World Congress 2012 "Tissue Engineering and Regenerative Medicine", Viena, Austria poster "Microwave Solvothermal Synthesis of highly biocompatible hydroxyapatite nanopowder"
• 7-9.05.2012 5th CLINAM 2012, The European Summit for Clinical Nanomedicine 2012, Basel, Switzerland poster "Poland's Achievements in Nanomedicine"

INTERNATIONAL TRAININGS

• 8-11.04.13 NAMABIO Training School, Ankona, Italy
• 4-5. 09.12 2nd Joint Meeting COST Action MP 1005, Viena, Austria
• 8-15.02.12 project consultations, University Bar Ilan, Ramat Gan, Israel

Hyunjin Kim

Molecular Imaging & Therapy Branch, National Cancer Center,
323 Ilsan-ro, Goyang-si, Gyeonggi-do 410-769, Korea
E-mail: nalvi77@ncc.re.kr
Tel: +82-31-920-2522

EDUCATION

• M.S. Department of Life Science, Gwangju Institute of Science and Technology, Gwangju, Korea
• B.S. Chemistry, Chonnam National University, Gwangju, Korea

PUBLICATION

• A highly sensitive magnetite nanoparticle as a simple and rapid stem cell labelling agent for MRI tracking, Hyunjin Kim, Hyun-Mi Dae, Cheongsoo Park, Eun Ock Kim, Daehong Kim, In-Hoo Kim, Yun-Hee Kim and Yongdoo Choi, J. Mater. Chem., 2011, 21, 7742
• A fluorescent turn-on probe for the detection of alkaline phosphatase activity in living cells, Tae-Il Kim*, Hyunjin Kim*, Yongdoo Choi and Youngmi Kim, Chem. Commun., 2011, 47, 9825–9827

George Kordas

"Demokritos", NCSR D, IMS, 153 10 Ag. Paraskevi Attikis Athens, Greece;

EDUCATION

• 1979 Ph.D. in Engineering (Doctor Ingenieur), WWIII, Germany.
• 1974 M.S. in Physics (Diplom Physiker), University of Erlangen, Germany.

CURRENT PROFESSIONAL EXPERIENCE

• 1990- Research Director, NCSR, "Demokritos," Athens, Greece.

SCHOLASTIC ACHIEVEMENTS & AWARDS (selected)

• 2012 "Award for Scientific Excellence", National Center for Scientific Research, Thursday 23 February 2012
• 2009 ERC-IDEAS Grant - 232959, NANOTHERAPY A Novel Nano-container drug carrier for targeted treatment of prostate cancer (http://erc.europa.eu/index.cfm?fuseaction=page.display&topicID=492)
• 2002 Weston Visiting Professor, Department of Physical Chemistry, Weizmann Institute of Technology, Rehovot, Israel
• 2001 DAAD Invitation, Max Plank Institute for Polymer Science, Mainz, Germany

SELECTED ARTICLES IN JOURNALS

• Novel PEGylated pH-sensitive polymeric hollow microspheres, Bilalis P.; Boukos N.; **Kordas G.**, MATERIALS LETTERS, 67, 1,180-183 DOI: 10.1016/j.matlet.2011.09.062, JAN 15 2012
• New approach in synthesis, characterization and release study of pH-sensitive polymeric micelles, based on PLA-Lys-b-PEGm, conjugated with doxorubicin, Efthimiadou E. K.; Tapeinos C.; Bilalis P.; et al., JOURNAL OF NANOPARTICLE RESEARCH, 13, 12, 6725-6736 DOI: 10.1007/s11051-011-0579-5, DEC 2011

CURRENT RESEARCH

Project full title: "A Novel Nano-container drug carrier for targeted treatment of prostate cancer", Grant agreement no.: 232959 European Research Council, IDEAS Advanced Grant. This project deals with the development of nanocontainers exhibiting triple stimuli response to cancer. The nanocontainers will be grafted with molecules that will first recognize cancer. The nanocontainers will respond to the local values of cancer such as pH, Redox and temperature. Once the cancer has been recognized by the molecule on the nanocontainers, they will release the drugs only when the local values correspond to the properties of cancer. This way, extremely local therapy will be achieved. The nanocontainers will also be excited externally through modest RF signals. This system will be appropriate for extremely local hyperthermia. This program presents a challenge towards cancer therapy.

Julijana Kristl

Julijana Kristl became Doctor of Pharmaceutical Sciences in 1988 at the University of Ljubljana, Slovenia, and did PostDoc at the University in Geneva. In 1989 she was appointed Assistant Professor, in 1994 Associate Professor and in 1999 Professor.
Academic and Professional Posts: She has given lectures in undergraduate and postgraduate courses of Pharmaceutical Technology, Nanotehnology and Cosmetology. Professor Kristl's main

research interests include new drug delivery systems, nanotechnology and nanomedicine. Her research activity has resulted in more than 160 scientific articles published in national and international scientific journals, numerous invited lectures and reports at scientific meetings. Her international scientific work includes membership in the editorial boards (JDDST, Ars Pharmaceutica, JBN) and reviewer in 27 journals. In the last decade she is actively involved in the management of the University of Ljubljana as a Vice-Rector for education, and is responsible for introduction of Bologna process, implementation of study programs, quality and international cooperation. Among other she served as a Vice-Dean in the period 1991-1995 and as a Dean of the Faculty of Pharmacy from 1999-2003 and as a Head of the Chair for Pharmaceutical Technology from 2003-05.

Anne Maria Rita Kümmel

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Birthday: 18/07/1977

WORK EXPERIENCES

• since 10.2012 Modeler at Novartis Pharma AG, Basel, Switzerland pharmacokinetics and -dynamics, (pre-)clinical data analysis
• 05.2011-09.2012 Visiting Scientist in Modeling and Simulation at Novartis Pharma AG, Basel, Switzerland; pharmacokinetics and -dynamics, (pre-)clinical data analysis
• 03.2008-03.2011 Postdoctoral Researcher at Novartis Pharma AG Basel, Switzerland; Research project: Multivariate Data Analysis for High-Content Screening
• 10.2002-12.2002 Internship at BASF AG, Ludwigshafen, Germany: Unit operations in bioseparation

Sylwia Kusnieruk

Sylwia obtained her master's degree in chemistry from the Faculty of Chemistry at the University of Warsaw in Poland. Now she is studying for a PhD at the Laboratory of Nanostructures for Photonic and Nanomedicine at the Institute of High Pressure Physics of the Polish Academy of Sciences. Her research focus is on nanopowders' synthesis, examination of their properties and practical applications in medicine.

Nalia R. Kuznetsova

Laboratory of Lipid Chemistry
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, ul. Miklukho-Maklaya 16/10, Moscow, 117997 Russia
ul. Ostrovityanova, 33A
natalia@lipids.ibch.ru
+7(495)330-6610; +7(962)920-9247

EDUCATION

• 2007–12 Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia
• 2012 Ph.D. in Chemical Sciences, Area of Specialization: Biochemistry, Drug Delivery
• 2002–07 Specialist in Chemistry, with Honors, Chemistry Department, Moscow State University, Russia

RESEARCH EXPERIENCE AND INTERESTS

• Laboratory of Lipid Chemistry, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, 2007–present (research adviser: Dr. Elena L. Vodovozova); Synthesis of lipophilic prodrugs employing various synthetic procedures and methods of purification (adsorption and reversed phase chromatography, gel filtration, and thin-layer chromatography). Design of liposomal formulations; characterization of nanosized drug delivery systems by methods of dynamic light scattering, electron microscopy, HPLC, PAGE, immunoblotting, and other analytical procedures to control liposome size and composition, chemical stability of prodrugs in vitro and in vivo, and liposome interactions with blood components.
• Centre Interfacultaire des Biomatériaux, Université de Liège, March–September, 2010 (traineeship supervisor: Dr. Christian Grandfils); Assessment of hemocompatibility and other characteristics of liposomes and other nanomedicine formulations in frames of the NanoBioPharmaceutics project.

AWARDED GRANTS AND FELLOWSHIPS

• 2012–now Russian Foundation for Basic Research Young Researcher Fellowship
• 2009 FEBS Collaborative Experimental Scholarships for Central& Eastern Europe

SELECTED PUBLICATIONS AND PRESENTATIONS

• **Kuznetsova NR**, Sevrin C, Lespineux D, Bovin NV, Vodovozova EL, Mészáros T, Szebeni J & Grandfils C (2012) Hemocompatibility of liposomes loaded with lipophilic prodrugs of methotrexate and melphalan in the lipid bilayer. J. Control. Release. 160, 394–400.
• **Kuznetsova NR**, Stepanova E, Boldyrev I, Bovin N, Vodovozova E. Targeting of cytotoxic liposomes to tumor vasculature by Sialyl Lewis X ligand. Poster presentation at Liposomes in Jerusalem 2011 International conference, Israel, May 15–19, 2011.
• **Kuznetsova NR**, Bovin N, Sevrin C, Lespineux D, Grandfils C, Vodovozova E. Hemocompatibility of liposomes loaded with diglyceride esters of methotrexate and melphalan. Poster presentation at the 3rd International NanoBio Conference 2010, ETH Zurich, Switzerland August 24–27 2010 (http://www.ecmjournal.org/journal/supplements/vol020supp03/pdf/v020supp03a152.pdf).
• **Kuznetsova NR**, Kandyba A, Vostrov I, Kadykov V, Gaenko G, Molotkovsky J & Vodovozova E (2009) Liposomes Loaded with Lipophilic Prodrugs of Methotrexate and Melphalan as Convenient Drug Delivery Vehicles. J. Drug Del. Sci. Tech. 19, 51–59.

Anne-Laure Lainé

After a degree in chemistry and chemical engineering with a specialization in pharmaceutical technologies obtained in 2008, I got a fixed term contract as research associate, following my internship, in the French pharmaceutical laboratories Servier (Orleans, France). I was in charge of the development and characterization of nanosuspensions for an anticancer drug. Then, I joined Actelion Pharmaceuticals (Basel, Switzerland) as research associate in preformulation and preclinical galenics. The main duties concerned the formulation support for in vitro and in vivo studies including hydrophobic drug solubilisation and powder engineering, as well as an oral formulation screening and taste masking for a phase I trial. In addition, I was responsible for the development of a nanosizing technique via wet-milling. After a year in Actelion, I decided to start a Ph.D. entitled "Application of lipid nanocapsules loaded with ferrocifens in cancer treatment" under the direction of Pr. C. Passirani in the laboratory INSERM 'Biomimetic micro and nanomedicines" headed by Pr. J.P. Benoit (Angers, France). This gave me the opportunity to deepen my knowledge on nanoencapsulation and to gain skills in cell culture and animal experimentations. In parallel to the thesis, I work as assistant teacher at the Pharmacy school of Angers and I regularly supervise trainees. I recently obtained the 2012 Best Paper Award in EJPB for my first manuscript entitled "Brain tumor targeting strategies via coated ferrociphenol lipid nanocapsules".

Roman Lehner

3rd year Ph.D student
University Hospital Basel, Intensive Care Unit
E-mail: Roman.Lehner@unibas.ch

Roman Lehner is a final year Ph.D student in the group of Prof. P. Hunziker, working within the SNF project "Intelligent Materials". Roman studied molecular biology at the University of Basel from where he received his MSc in structural biology in 2009, where he was involved in the crystal structure determination of the membrane protein Kdgm, a porin, which is involved in the pathogenicity of the bacteria. After his master diploma, he worked as a scientific associate at the Institute of Anatomy in Basel and for the spin-off company BioVersys. His work is focused on the design of a polymer based drug delivery system, which can be functionalized to target particular tissue and combined with a stimulus responsiveness to light to explore activation of caged compounds.

Xiaochun Li-Blatter

Xiaochun Li-Blatter has worked as a scientific collaborator at Biozentrum, University of Basel since 1997. Together with Professor Anna Seelig, she has quantitatively evaluated the mechanism of activation and inhibition of ATP binding cassette transports (P-glycoprotein, SAV1866) and contributed to the drug formulation strategies to overcome the multidrug resistance problem. She has published 14 publications. Currently she holds a position at the Biophysics Facility at Biozentrum. She got a diploma of organic chemical engineering at Beijing Institute of Chemical Fibre Technology.

MAIN FOCUSES

- Characterization of biomolecules (e.g. drugs and proteins) and nanoparticles with respect to size, conformation, stability and oligomeric states.
- Determination of binding constants and the stoichiometry of interactions nanoparticles such as liposomes, polymers and proteins, and ligands which are generally drugs substances and small biomolecules. Characterization of the interactions with thermodynamic parameters; understanding the binding process at the molecular level.
- Developing high through-put screening techniques for inhibitors/substrates of proteins and quantifying their potency.

MAIN RESEARCH TOOLS

- Analytical ultracentrifugation and complementary use of a fluorescence detection system; zetasizer, static light scattering; isothermal titration calorimetry , differential scanning calorimetry; microscale thermophoresis; surface plasmon resonance; fluorescence-based thermal shift; fluorescence and circular dichroism spectroscopy.

RECENT PUBLICATIONS

- The brain entry of HIV-1 protease inhibitors is facilitated when used in combination. Marzolini C, Mueller R, **Li-Blatter X**, Battegay M, Seelig A. (Submitted to Molecular Pharmaceutics).
- P-glycoprotein-ATPase modulation: the molecular mechanisms. **Li-Blatter X**, Beck A, Seelig A. Biophys J. 2012 Mar 21; 102(6):1383-93. Exploring the P-glycoprotein binding cavity with polyoxyethylene alkyl ethers. Li-Blatter X, Seelig A. Biophys J. 2010 Dec 1; 99(11):3589-98.
- Detergents as intrinsic P-glycoprotein substrates and inhibitors. **Li-Blatter X**, Nervi P, Seelig A. Biochim Biophys Acta. 2009 Oct; 1788(10):2335-44.

Neill James Liptrott

The University of Liverpool, Department of Molecular & Clinical Pharmacology
70 Pembroke Place, Block H, Liverpool
L69 3GF
Tel: +44 (0) 151 794 5919
e-mail: neill.liptrott@liv.ac.uk

Date of birth: May 30th 1979

PROFILE

My main interest is in immune cell biology and my current research interests are aimed toward understanding the impact of immune signalling factors, e.g. cytokines, on cell physiology. In turn this research extends to how this affects the metabolism of drugs and toxins in a variety of metabolically active cells. I am investigating the interaction of novel nanomedicines with the cellular physiology of immune cells in order to investigate potential impact on immune function. As a natural progression of this work I am currently attempting to target nanoparticles to defined cell types and investigate their mechanism of cellular uptake.

EDUCATION AND RESEARCH

- 2012-now Senior Postdoctoral Research Associate – Department of Molecular and Clinical Pharmacology, University of Liverpool, Towards nanomedicine interventions in HIV/AIDS. Principal Investigator: Prof. Andrew Owen
- 2011-2012 Postdoctoral Research Associate - Department of Molecular and Clinical Pharmacology, University of Liverpool. Determining the interaction between nanoformulated drug delivery systems and the human immune system. Principal Investigator: Prof. Andrew Owen
- 2007-2011 Postdoctoral Research Associate - National Biomedical Research Centre for Microbial Disease, Royal Liverpool and Broadgreen University Hospital Trust, Liverpool. Investigating the mechanisms governing the intracellular pharmacology of HIV antiretrovirals in primary immune cells. Principal Investigator: Prof. Saye Khoo
- 2004-2007 Ph.D. - Department of Molecular and Clinical Pharmacology, University of Liverpool. Pharmacological and Immunological Factors that Influence Antiretroviral Drug Therapy. Supervisors: Prof. Andrew Owen and Prof. David Back.
- 2003-2004 M.Sc. Human Immunity, University of Liverpool
- 1999-2003 B.Sc. (Hons) Molecular Biology (2.2), University of Liverpool, UK

Tamara Lozano Fernández

Immunology Laboratory. Edificio de Ciencias Experimentales. University of Vigo. Campus Lagoas Marcosende, Rúa das Abeleiras s/n. 36310 Vigo. Spain
Phone: +34 986812626
Email: tlozano@uvigo.es

EDUCATION

- 2004-2009 Degree in Biology
- 2009-2010 Master in Methodology and Applications in Life Sciences
- 2010-Present PhD student at the University of Vigo

FELLOWSHIP

- 2008-2009 Collaboration Grant in the Laboratory of Immunology
- 2009-2011 Master Grant in the Laboratory of Immunology
- 2012-now PhD Grant (FPU, Spanish Ministry of Education)

PUBLICATIONS

- **Lozano, T**; Rey, M.; Moya, S.; Donath, E., Gao, C., Antipov, A. and González-Fernández, África. Título: "Cytotoxicity effect of metal

oxide nanoparticles in human tumor cell lines". Proceedings Journal of Physics: Conference Series 304 (2011) 012046
• Simón-Vázquez R, Peleteiro M, **Lozano T**, Casal A and González-Fernández A. Chapter 15: Nanotoxicology. Book: Nanobiotechnology: Inorganic Nanoparticles vs Organic Nanoparticles. Elsevier, 2012. Vol. 4, p. 443-485.
• Simón-Vázquez R, **Lozano-Fernández T**, Peleteiro-Olmedo M and González-Fernández A. Conformational changes in human plasma proteins induced by metal oxide nanoparticles. (submitted).

Pernille Lund Hansen

Eckersbergsvej 38, 5230 Odense M
Phone: 29 72 30 75
Mail: plhansen@health.sdu.dk

Birth date: 18.08 1981

PHD STUDENT (1.5 2009 -)

Project title: Set-Up and Testing of Components for Therapeutic SiRNA Delivery; Supervisor: Jan Mollenhauer; Place: The Molecular Oncology Group, Institute for Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark. Part of the Lundbeck Center of Excellence, NanoCAN; Thesis handed in the 17th of May 2013.

MASTER IN BIOMEDICINE (2002-2009)

Place: The University of Southern Denmark; Master project (April 2007 - April 2009); Title: Osteogenic differentiation in 3D-osteospheres: Novel approach for studying in vitro bone formation. Supervisors: Professor Moustapha Kassem and Associate Professor Susanne Mandrup; Place: The Clinic for Molecular and Endocrinological Treatment (In Danish: Klinik for Molekylær Endokrinologisk Behandling, KMEB) at Medical Biotechnology Centre, University of Southern Denmark

BACHELOR PROJECT (MARCH 2005 - JANUARY 2006)

Title: Osteogenic differentiation of human bone marrow derived mesenchymal stem cells immortalized by telomerase and grown as 3D multicellular spheroids; Supervisors: Professor Moustapha Kassem and Associate Professor Susanne Mandrup; Place: The Clinic for Molecular and Endocrinological Treatment (In Danish: Klinik for Molekylær Endokrinologisk Behandling, KMEB) at Medical Biotechnology Centre, University of Southern Denmark

PUBLICATIONS

• **Hansen PL**, Blaich S, End C, Schmidt S, Moeller JB, Holmskov U, et al. The pattern recognition molecule deleted in malignant brain tumors 1 (DMBT1) and synthetic mimics inhibit liposomal nucleic acid delivery.
• Chemical communications. 2011 Jan 7;47(1):188-90. PubMed PMID: 20830348.

AWARDS

• 2008-2009 Novo Scholarship Programme in Biotechnology and Pharmaceutical Sciences. Kr 72.000

PRESENTATIONS

• Presentation of research project at Oncology Department, Odense University Hospital, Odense, Denmark, May 23rd 2013. "Development of cancer stem cell targeting nanodrugs" -Talk
• 1ST NanoCAN midterm Review, Odense, Denmark, February 21st 2013 "Set-up of Mouse Models for Nanodrug Testing" -Poster
• 1ST NanoCAN meeting, Odense, Denmark April 14th 2011 "Construction of synthetic cancer stem cells" -Poster
• 1ST NanoCAN meeting Odense, Denmark April 14th 2011 "Construction of synthetic cancer stem cells" -Short oral presentation
• 4th European-Conference for Clinical Nanomedicine (CLINAM 2011) Basel, Switzerland 23-25 May 2011
"Construction of new cancer cell models for nanodrug discovery and testing " -Poster

Ingólfur Magnússon

Faculty of Pharmaceutical Sciences, University of Iceland, Hagi, Hofsvallagata 53
107 Reykjavik, Iceland. Contact Number: +354-6935662
E-mail: inm3@hi.is

ACADEMIC PROFILE

• M.S (Pharmaceutical Sciences) Faculty of Pharmaceutical Sciences, University of Iceland (2011)
• B.S. (Pharmaceutical Sciences) Faculty of Pharmaceutical Sciences, University of Iceland (2009)

RESEARCH EXPERIENCE

PhD student: Faculty of Pharmaceutical Sciences, University of Iceland, (September 2011 till date)

Damiano Mangoni

Damiano Mangoni has been awarded the Master degree in Pharmaceutical Biotechnology from University of Perugia in July 2011 with 110/110 cum laude, spending one year at Laboratory of Clinical Immunology and Allergology (Department of Clinical and Experimental Medicine) in Pr. Spinozzi group studying the role of NKT cells and atypical lymphocytes populations in autoimmune and allergic diseases. He is now pursuing her PhD in "Clinical Sciences and Technologies", working in Prof. Scoles group in the Department of Medical and Biological Sciences at the University of Udine on a project entitled "Application of Advanced Nanotechnology in the Development of Innovative Cancer Diagnostic Tools".
He's now interesting in the study of exosomes in tumor progression and in tumor-stroma interaction, in particular in human gliomas. He works on exosome isolation methods, exosome uptake pathways and internalization by recipient cells, exosomal microRNAs extraction and characterization and in vitro studies to understand the biological effects mediated by exosomes in the complexity of tumor microenvironment.

Aparna Mani

D/o Dr.K.R.Mani, (Retd) Director,
Central Research Institute, Kasauli,
Himachal Pradesh.
E-mail: draparnacmc@gmail.com
Phone: 9095009724

Date of Birth: 12.04.1984

EDUCATIONAL QUALIFICATION

• 1998-1999 10th Standard, St.Joseph's Convent, Coonoor, Nilgiris
• 1999-2001 Higher Secondary, Stanes Higher Secondary School, Coonoor, Nilgiris
• 2002-2008 MBBS, Coimbatore Medical College, Coimbatore
• 2011- Till date MD-PhD (Integrated), Sri Ramachandra Medical College, Chennai

PAPERS AND POSTERS PRESENTED

a) Presented a poster on 'Adverse Drug Reactions in Paediatric Patients- a Meta analysis' at PHARMAPRAC – National Conference on Pharmacy Practice, held at Sri Ramachandra Medical College and RI, on 10th and 11th November 2011
b) Presented an oral paper on " Biomimetic synthesis of silver na-

noparticles using herbal extract and evaluation of its anti-oxidant property" at SRIPS 2012, held at Chettinad Medical College, Chennai.

- Awarded the best paper award for the same

c) Presented an oral paper on "Bioenhancing effect of silver nanoparticles synthesised using herbal extract" at 'HERBOTECH' conference held at Sri Ramachndra Medical College, Porur.

d) Presented an poster on "Evaluation Of Cytotoxic Potential Of Synthesised Silver Nanoparticles In Hep G2 Cell Lines" at IPSCON Conference held at Nagpur.

- Awarded certificate of appreciation for getting selected for the Dandiya prize session

PUBLICATION

1."BIO-MIMETIC SYNTHESIS OF SILVER NANOPARTICLES AND EVALUATION OF ITS FREE RADICAL SCAVENGING ACTIVITY" in International Journal of Biological & Pharmaceutical Research 2012; 3(4): 631-633

Sivakumar Manickam

Professor of Chemical and Nanopharmaceutical Process Engineering, Department of Chemical & Environmental Engineering, Head – Manufacturing and Industrial Processes Research Division, Director – Centre for Nanotechnology and Advanced Materials (CENTAM), Faculty of Engineering, University of Nottingham (Malaysia campus)
Jalan Broga, Semenyih 43500, Malaysia
Tel: +6(03) 8924 8156; Fax: +6(03) 8924 8017
Email: Sivakumar.Manickam@nottingham.edu.my

AREAS OF RESEARCH EXPERIENCE AND AREAS OF INTEREST

• Nanoengineering of Advanced Materials (Synthesis, characterization and process development)
• Development of Nanosuspensions and Nanoemulsions for Pharmaceutical Industries
• Process Research, Engineering and Development of Drugs and Drug Intermediates
• Development of Novel Transdermal Drug Delivery System using Ultrasound (Sonophoresis)

MAJOR AWARDS

• JSPS Research Award by the Japan Society for the Promotion of Science, Government of Japan (November 2003 – September 2005).
• DST (Department of Science and Technology) – SERC (Science and Engineering Research Council), Government of India, Fast Track Young Scientist – 2006

RESEARCH STUDENTS

PhD, 22 (Supervisor/Co-supervisor)
Post Graduate, 14 (MPharm-5 and MSc Chemical Engineering -9)

RESEARCH PROJECTS/FUNDS

• FP7 –NMP – 2010 – LARGE - 4 (Large Scale Integrating Project) - "Rational Bioactive Materials Design for Tissue Regeneration" – (Research Collaborator)
• eScienceFund by Ministry of Science, Technology and Innovation (MOSTI), Government of Malaysia for the Project "Novel Strategy of Ultrasonic Cavitation for the generation of Nanoemulsions and Nanosuspensions in Pharmaceutical preparations" – Project No: 03-02-12-SF0043 – US $ 61,000 (Principal Investigator) – COMPLETED
• eScienceFund by Ministry of Science, Technology and Innovation (MOSTI), Government of Malaysia for the Project "Augmenting the removal of active ingredients of Ganoderma lucidum and their encapsulation in the Nanosized Liquid Vehicles (NLVs) to improve the bioavailability using ultrasonic cavitation" – Project No: 06-02-12-SF0141 - RM 221000 (Principal Investigator) - ONGOING
• Tensor Technology Engineering Sdn. Bhd. for the Project on "Process to Enhance coatings for Core Shell structure of Metallic and Non-metallic materials" – RM 40000 (Principal Investigator) – ONGOING
• eScienceFund by Ministry of Science, Technology and Innovation (MOSTI), Government of Malaysia for the Project "Insulin -containing Palm Oil Nanoparticles: Investigating the correlation between Gastrointestinal Uptake and location of Insulin within Nanoparticle" – Project No: 03-02-12-SF0062 – US $ 50,000 (Research Collaborator) - COMPLETED
• eScienceFund by Ministry of Agriculture (MOA), Government of Malaysia for the Project "Development of Chitosan based nanoemulsion biofungicides for the control of Colletotrichum gloeosporioides, causal agent of anthracnose in dragon fruitplants" – RM 255600 (Research Collaborator) - ONGOING
• MedKlinn International Sdn Bhd, Malaysia for the Project on "Cerafusion Technology for physico-chemical responses and decay of selected tropical fruits and vegetables" – RM 253000 (Research Collaborator) - ONGOING
• Ministry of Higher Education (MOHE), Malaysia under Fundamental Research Grant Scheme (FRGS) for the Project on "Designing a Novel Nanoscale Drug Delivery Platform using Graphene, Dye and Pluronic F127 as Nanohybrid vehicle for improved efficacy of Curcumin into cancer cells" – RM 86000 (Research Collaborator) – ONGOING

Carlotta Marianecci

Place and date of birth: Rome, 17.01.1976

EDUCATION

• 2004 PhD in "Pharmaceutical Sciences" at Department of Chemistry and Pharmaceutical Technologies of the Faculty of Pharmacy ("Sapienza"University of Rome). PhD thesis on: "pH-sensitive niosomes as FANS delivery systems in Alzheimer disease therapy: preliminary evaluation of their interactions with macrophagic cells".
• 2006 Research grant in Pharmaceutical Technologies at the Department of Chemistry and Pharmaceutical Technologies at Faculty of Pharmacy ("Sapienza" University of Rome). Research title: "New vesicular systems in drug release".
• 2007 Winner of a permanent position as assistant professor in the field of Pharmaceutical Tecnologies.
• 2007- Assistant professor at Faculty of Pharmacy and Medicine ("Sapienza" University of Rome).

SCIENTIFIC ACTIVITY

• 2001- Research activity in the field of Pharmaceutical Technology: Preparation and characterization of non-phospholipid pH-sensitive vesicular systems;
- application of these non-phospholipid vesicular systems (niosomes) for topical delivery of drugs;
- studies of internalization mechanisms between niosomes an macrophagic cells;
- characterization of liposomes coated by scleroglucan and a derivative of this polysaccharide;
- stability studies on vitamin A loaded in liposomes, niosomes and nanoparticles;
- characterization of niosomes for pulmonary delivery of glucocorticoids used in the therapy of chronic obstructive pulmonary disease;
- bronchial uptake studies by fibroblasts of free glucocorticoids or loaded in vesicular systems;
- characterization of liposomal systems as carriers of L-Dopa prodrugs;
- interaction studies between cationic liposomes and anionic polyelectrolites and between niosomes with negative charge and cationic polyelectrolites (hybrid niosomes);
- selective internalization study of hybrid niosomes by granulocytes, monocytes and linfocytes;
- deep characterization studies on hydrophilic surfactant vesicular bilayer systems in the presence of increasing amounts of cholesterol at different moisture conditions.
• Author of 40 research papers and about 60 oral and poster presentations.
• Referee for the "Journal of Controlled Release", "Pharmaceuticals" and "International Journal of Pharmaceutics".

Már Másson

Professor in Medicinal Chemistry
Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Hagi, Hofsvallagata 53 107 Reykjavik, Iceland. Contact Number: +354-5254483
E-mail: mmaosson@hi.is

My current research is directed towards synthesis of bioactive chitosan derivatives and investigation of the structure activity relationship. As a part of this work developed protection group strategies to allow reproducible synthesis of chitosan based nanomaterials. I am involved in numerical mathematical modeling of drug delivery systems and processes. The investigated application, which we do in collaboration with other groups, include disinfection and antimicrobial treatment, pulmonary drug delivery, transdermal delivery, delivery of photosensitizers, delivery anticancer agent and bone regeneration.

ACADEMIC PROFILE

- Ph.D (Engineering, Biotechnology) Tokyo Institute of Technology, Tokyo, Japan (1995)
- M.S (Organic Chemistry) University of Copenhagen, Denmark (1990)
- B.S (Chemistry) University of Iceland, Reykjavik (1987)

PROFESSINAL PROFILE

- Professor, University Iceland, Faculty of Pharmaceutical Sciences, (2002)
- Assoc. Professor, University Iceland, Faculty of Pharmaceutical Sciences, (2002)
- Researcher, University of Iceland, Faculty of Medicine (1995)

SOME RECENT PUBLICATIONS

- Vivek S. Gaware, Monika Håkerud , Kristján Leósson, Sigríður Jónsdóttir, Anders Høgset, Kristian Berg, **Már Másson**. Tetraphenylporphyrin Tethered Chitosan Based Carriers for Photochemical Transfection. *Journal of Medicinal Chemistry* 2013 56 807–819
- Berglind Eva Benediktsdóttir, Thórarinn Gudjónsson, Ólafur Baldursson (2013)**Már Másson**. Relationship between the structure of N-alkyl-N,N-dimethyl chitosan derivatives and their permeation enhancing effect in bronchial epithelia in vitro 2013. *European Journal of Pharmaceutica and Biopharmaceutics* in Press
- Berglind E. Benediktsdóttir, Ari J. Arason, Skarphédin Halldórsson. Thorarinn Gudjónsson, **Már Másson**, Ólafur Baldursson Drug delivery characteristics of the progenitor bronchial epithelial cell line VA10. *Pharmaceutical Research* 2013 30 (3) 781-791
- Ramona Lieder, Vivek S. Gaware, Finnbogi Thormodsson, Jon M. Einarsson, Chuen-How Ng, Johannes Gislason, **Már Másson**, Petur H. Petersen, Olafur E. Sigurjonsson. Endotoxins affect bioactivity of chitosan derivatives in cultures of bone marrow-derived human mesenchymal stem cells 2013- *Acta Biomaterialia*, 9 4771-4778
- Berglind E. Benediktsdóttir, Kasper K. Sørensen, Mikkel B. Thygesen, Knud J. Jensen, Thórarinn Gudjónsson, Ólafur Baldursson, **Már Másson** (2012) Regioselective fluorescent labeling of N,N,N-trimethyl chitosan via oxime formation. *Carbohydrate Polymers* 90 1273– 1280

Serena Mazzucchelli

PhD
Regione Lombardia - Direzione Generale Sanità, L Sacco Hospital of Milan- Surgical Research Nanotechnology Laboratory-LITA

EDUCATION/TRAINING

- PhD in biological sciences on October 2009 with a work entitled "Studies on the mechanism and physiological role(s) of the interaction of ataxin-3 with tubulin", tutor prof. Paolo Tortora, Department of Biotechnology and Biosciences, Faculty of Sciences (University of Milano-Bicocca).
- Degree in Biology obtained on 20/10/2006 at the University of Milano-Bicocca, Italy with an experimental thesis on "Studies on the physiological roles of human protein CGI-58, a molecule associated to Lipid droplets". Degree mark: 110/110 cum laude.
- Bachelor Degree in Biological Science obtained on 01/10/2004 at the University of Milano-Bicocca, Italy with experimental thesis on "N- and C- terminal sostitution of leucine with acidic residues points out hydrophobic interaction crucial for stability of Carboxipeptidase from S. solfataricus". Degree mark: 110/110 cum laude.

POSITIONS

- Luigi Sacco Hospital of Milan- Surgical Research Nanotechnology Laboratory-LITA, Fixed-term researcher from August 2012 to present.
- University of Milano-L. Sacco Hospital, Department of Biomedical and Clinical Sciences, Post-doc position from January 2011 to May 2012.
- University of Milano-L. Sacco Hospital, Department of Biomedical and Clinical Sciences, Post-doc position from January 2010 to December 2010.
- University of Milan-Bicocca, Department of Biotechnology and Biosciences, PhD student from October 2006 to October 2009.

COMPETENCES

Expression of hetherologous protein in E. coli using commercial expression systems, protein purification, protein assay, enzymatic assay, elettrophoretic techniques, western blot, dot-blot, immunoprecipitation, FPLC separation, GST-pull down, cell culture from human sample or commercial cell lines, cellular fractionation, immunofluorescence, cytofluorimetry, molecular biology techniques and functionalization of hybrid nanoparticles for biomedical applications.

SELECTED PUBLICATIONS

- "Orientation-Controlled Conjugation of Haloalkane Dehalogenase Fused Homing Peptides to Multifunctional Nanoparticles for the Specific Recognition of Cancer Cells", **S. Mazzucchelli***, M. Colombo, P. Verderio, E. Rozek, F. Andreata, E. Galbiati, P. Tortora, F. Corsi, D. Prosperi*. Angew. Chem. Int. Ed. (2013), DOI:10.1002/anie.201209662 IF13.455
- "Protein-assisted one-pot synthesis and biofunctionalization of spherical gold nanoparticles for selective targeting of cancer cells"; M. Colombo,**S. Mazzucchelli**, V. Collico, S. Avvakumova, L. Pandolfi, F. Corsi, F. Porta, D. Prosperi. Angew. Chem. Int. Ed. (2012) 51 (37), 9272-9275. IF13.455
- "Protein Oriented Ligation on Nanoparticles Exploiting O(6) -Alkylguanine-DNA Transferase (SNAP) Genetically Encoded Fusion"; M. Colombo, **S. Mazzucchelli**, J. M. Montenegro, E. Galbiati, F. Corsi, W. J. Parak, D. Prosperi . Small (2012) 8 (10), 1492-1497. IF:8.349
- "Site-specific conjugation of scFvs antibodies to nanoparticles by bioorthogonal-strain promoted alkine-nitrone cycloaddition"; M. Colombo, S. Sommaruga, **S. Mazzucchelli**, L. Polito, P. Verderio, P. Galeffi, F. Corsi, P. Tortora, D. Prosperi. Angew Chem Int Ed Engl. (2012) (51), 496-499. IF13.455
- "Multiple presentation of scFv800E6 on silica nanospheres enhances targeting efficiency toward HER-2 receptor in breast cancer cells"; **S. Mazzucchelli**, P. Verderio, S. Sommaruga, M. Colombo, A. Salvadè, F. Corsi, P. Tortora, D. Prosperi. Bioconjugate Chem. (2011), 16 (22), 2296-2303. IF:4.930
- "Single-domain Protein A-Engineered magnetic nanoparticles: Toward a universal strategy to site-specific labeling of antibodies for targeted detection of tumor cells";**S. Mazzucchelli**, M. Colombo, P. Verderio, C. De Palma, M. D. Coghi, E. Clementi, P. Tortora, F. Corsi, D. Prosperi;. ACSNano (2010), 10, 5693-702. IF:9.855
- "HER2 expression in breast cancer cells is downregulated upon active targeting by antibody- enigineered multifunctional nanoparticles in mice"; F. Corsi, L. Fiandra, C. De Palma, M. Colombo, **S. Mazzucchelli**, P. Verderio, R. Allevi, A. Tosoni, M. Nebuloni, E. Clementi, D. Prosperi. ACSNano (2011) 5 (8), 6383-6393. IF:9.855
- "Towards a universal method for the stable and clean functionalization of inert perfluoropolymer nanoparticles: exploiting photopolymerizable amphiphilic diacetylenes";C. Morasso, M. Colombo, S. Ronchi, L. Polito, **S. Mazzucchelli** D. Monti, M. Buscaglia, T. Bellini, D. Prosperi. Adv. Funct. Mater. (2010), 20(22), 3932-3940. IF: 10.179

• "HER2 targeting as a two sided strategy for breast cancer diagnosis and treatment: outlook and recent implications in nanomedical approaches"; M. Colombo, F. Corsi, D. Foschi, E. Mazzantini, **S. Mazzucchelli**, C. Morasso, E. Occhipinti, L. Polito, D. Prosperi, S. Ronchi, P. Verderio. Pharmacological Research, (2010), 62, 150-65. IF: 3.875
• "Proteomic and biochemical analyses unveil tight interaction of ataxin-3 with tubulin"; **S. Mazzucchelli** A. De Palma, M. Riva, A. D'Urzo, C. Pozzi, V. Pastori, F. Comelli, P. Fusi, M. Vanoni, P. Tortora, P. Mauri, M. E. Regonesi.; Int. J. Biochem. Cell. Biol., (2009), 41(12), 2485-92. IF: 4.759

Tamás Mészáros

EDUCATION

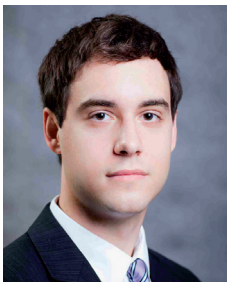
• 2011- Semmelweis University – Ph.D. School, Theme: Liposome, Immunology
• 2002-08 Lóránd Eötvös University – Faculty of Science, Biologist, Immunologist
• 2000-02 Lóránd Eötvös University - Faculty of Primary and Pre-School Education, Biology and Chemistry

WORK EXPERIENCE

• 2009- Nanomedicine Research and Education Center; János Szebeni –György Báthori and Kálmán Benedek
• 2007-09 Semmelweis University, 3rd Department of Medicine (Research Laboratory); Lilian Varga – György Füst
• 2006-07 National Center for Epidemiology (Microbiological Research Group); Ágnes Gyuris – János Minárovits
• 2005-06 Lóránd Eötvös University – Faculty of Science (Department of Immunology); József Prechl – Anna Erdei

PUBLICATION

• 2010 April: „C1-inhibitor autoantibodies in SLE" (**Mészáros T**, Füst G, Farkas H, Jakab L, Temesszentandrási G, Nagy G, Kiss E, Gergely P, Zeher M, Griger Z, Czirják L, Hóbor R, Haris A, Polner K, Varga L.; Lupus. 2010 Apr;19(5):634-8. Epub 2010 Jan 13 -2009 Impact Factor: 2.586)
• 2011 January: „High levels of acute phase proteins and soluble 70 kDa heat shock proteins are independent and additive risk factors for mortality in colorectal cancer." (Judit Kocsis, **Tamás Mészáros**, Balázs Madaras, Éva Katalin Tóth, Peter Gál, Lilian Varga, Zoltán Prohászka, George Füst; Cell Stress Chaperones. 2011 Jan;16(1):49-55. Epub 2010 Aug 22. -2009 Impact Factor: 2.167)
• 2012 June: „Hemocompatibility of liposomes loaded with lipophilic prodrugs of methotrexate and melphalan in the lipid bilayer." (Kuznetsova NR, Sevrin C, Lespineux D, Bovin NV, Vodovozova EL, **Mészáros T**, Szebeni J, Grandfils C. ; J Control Release. 2012 Jun 10;160(2):394-400.)



Gergely Milosevits

166., 2310 Szigetszentmiklos (Hungary)
Mobile: +36308425722
E-mail: ikkuma@gmail.com
Date of birth: 01.01.1988

WORK

• 2012- Resident clinical doctor, II. Department of Pediatrics (Semmelweis University, Budapest)
Tűzoltó u. 7-9., 1094 Budapest (Hungary)
• 2012- Research fellow, Nanomedicine Research and Education Center, Budapest; Institute of Pathophysiology (Semmelweis University, Budapest)
26 Ullői ut, 1085 Budapest (Hungary)
• 2012 Diploma thesis ("Flow cytometric analysis of the physicochemical characteristics and stability of nanopharmaceutical carriers and agents")
Nanomedicine Research and Education Center, Budapest; Institute of Pathophysiology (Semmelweis University, Budapest)
26 Ullői ut, 1085 Budapest (Hungary)
• 2011 Dec 20. Article published in Chemisty and physics of lipids. "Flow cytometric analysis of supravesicular structures in doxorubicin-containing pegylated liposomes." Milosevits G, Rozsnyay Z, Kozma GT, Milosevits J, Tömöry G, Robotka H, Rosivall L, Szebeni J. Nanomedicine Research and Education Center, Semmelweis University and Bay Zoltán Ltd., Budapest, Hungary.
• 10/2009-12 Student researcher, FACS laboratory, Nanomedicine Research and Education Center, Budapest; Institute of Pathophysiology (Semmelweis University, Budapest)
26 Ullői ut, 1085 Budapest (Hungary)

EDUCATION AND TRAINING

• 2006-2012 Semmelweis University (medical university), Budapest (Hungary)

CERTIFICATIONS

• Semmelweis University Diploma (medical doctor)
• ECDL (European Computer Driving Licence)
• State Accredited Language Examination Certificate in german language (intermediate level)
• State Accredited Language Examination Certificate in english language (advanced level)
• President's Award For Educational Excellence (2002, USA)

Mohamed Mohamed

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mohamed.mohamed@unige.ch

EDUCATION

• 2010- Ph.D. in Pharmaceutical Sciences at University of Geneva, Biopharmaceuticals, School of Pharmaceutical Sciences -Switzerland; "Loco-regional treatment of prostate cancer metastase to bone using implant-mediated hyperthermia", Supervisors: Prof G. Borchard & Dr O. Jordan
• 2009 University of Geneva, Geneva, Switzerland, Master's degree in Pharmaceutical Sciences, Master Thesis: "Chitosan based nanoparticles for mucosal vaccination", Honors: Dissertation passed "with Distinction"]
• 2007 University of Geneva, Geneva, Switzerland, Bachelor's degree in Pharmaceutical Sciences

AWARDS

• 2011 1st price at the "Swiss Pharma Sciences Day 2011" (co-author)

TEACHING EXPERIENCE

• 2009- University of Geneva, Geneva, Switzerland, Teaching practical work to Master's and Bachelor's students at the School of Pharmaceutical Sciences

PUBLICATIONS

• **M. Mohamed**, G. Borchard, O. Jordan, In situ forming implants for locally combined chemotherapy and hyperthermia of bone tumors, JDDST, 2012

Sivia L. Montes Fonseca

Calle Chillán 6134 Col. Panorámico
CP. 31140 Chihuahua, México.
E-mail: silvia.montes@cimav.edu.mx

EDUCATION

Graduate School: Bacteriologist and Parasitologist Chemist (2003-2007), Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas
Postgraduate School: Master degree in Biotechnology (2008-2010)

- Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas
- Doctoral degree in Environmental Sciences and Technology (Student)
- Centro de Investigación en Materiales Avanzados, Chihuahua.

POSTERS

• "Toxicity of unpurified and purified carbon nanotubes in macrophages J774 cultures" XXVI Annual meeting and XII State Congress of Clinical Chemistry. October 2009, Chihuahua, México.
• "Citotoxicity of fuctionalized carbon nanotubes with 220 KDa Lectin of E. histolytica in Macrophages J774" XXVIII State Congress of Clinical Chemistry. October 2011, Chihuahua, México.
• "Citotoxicity of fuctionalized carbon nanotubes with 220 KDa Lectin of E. histolytica in Macrophages J774" VII Conference of Research. October 2011, Chihuahua, México.
• "Citotoxicity of Functionalized Carbon Nanotubes in J774A Macrophages" en el 15th European Congress on Biotechnology. September 2012, Turkey.

PAPERS

• **Montes-Fonseca S. L.**, Orrantia-Borunda E., Aguilar-Elguezabal A., González Horta C., Talamás-Rohana P., Sánchez-Ramírez B. Cytotoxicity of functionalized carbón nanotubes in J774 macrophages. Nanomedicine: NBM; 8(6): 853-9.
• **Silvia Lorena Montes-Fonseca**, E. Orrantia Borunda, A. Duarte-Moller, Antonia Luna-Velasco, Manuel Román-Aguirre, Carmen González-Horta and B. Sánchez-Ramírez. CYTOTOXICITY OF CARBON NANOTUBES ON J774 MACROPHAGES IS A PURIFICATION DEPENDENT EFFECT. Journal of Nanomaterials.

Ýrr Mørch

Ph.D., Research scientist at SINTEF Materials and Chemistry/Post doc at Dept. of Physics, Norwegian University of Science and Technology
Sem Sælands vei 2, Kjemihallen 3rd floor, N-7034, Trondheim, Norway.
Phone: +47 40 23 47 44
e-mail: yrr.morch@sintef.no

EDUCATION

• Ph.D. degree in biotechnology at Dept. of Biotechnology, Norwegian University of Science and Technology (NTNU), "Novel Alginate
• Microcapsules for Cell Therapy" (2008).
• Master degree in chemical engineering, NTNU (1999).

PROFESSIONAL EXPERIENCE

• 2011-now Research scientist at SINTEF Materials and Chemistry
• 2011-now Post.doc at Dept. of Physics, NTNU
• 2010 Research Scientist at Dept. of Physics, NTNU
• 2007-2009 Research Scientist at Dept. of Biotechnology, NTNU, funded by The Chicago Diabetes Project, University of Illinois at Chicago, USA
• 2002-2007 Ph.D. student at Dept. of Biotechnology, NTNU
• 2000-2002 Research assistant/teacher, Dept. of Biotechnology, NTNU

PROFESSIONAL SKILLS

Microencapsulation, nanoencapsulation, cell encapsulation (cell therapy), miniemulsion method, polymerization, surface modification and targeting of nanoparticles, biopolymer gelling techniques (internal and external gelling, capsules, beads, films, foams, cylinders), surface modifications and chemical modification of (bio) polymers, enzymatic modification of biopolymers, biocompatibility studies, cell handling and culturing (including staining and imaging and various cell function techniques), rheological measurements (reduced capillary viscosity, uniaxial compression measurements, oscillation measurements), permeability measurements, optical microscopy techniques (including fluorescence microscopy and CLSM), image analysis (including high-speed camera imaging), Circular Dichroism Spectroscopy, lithography, S(T)EM, Zetasizer, NanoSight, Coulter Counter, microfluidics.

AWARDS

• Tekna's Young Scientist of the Year award 2009
• Poster award, International Hydrocolloid Conference, Trondheim July 2006
• Personal Ph.D Student Grant, awarded by the Norwegian Research Council 2001-2006
• Best graduate student award from high school, Reykjavik 1995

PUBLICATIONS

16 peer reviewed publications (times cited: >450 ICI Web of Science, May 2013), 4 public science publications, 9 (first author) presentations at international scientific conferences.

Kouros Motamed

EXPERIENCE

• 2012-now IGDRASOL, Inc., Fountain-Valley, CA (www.igdrasol.com), (A Start-Up Personalized Nanomedicine Therapeutic Company), Chief Scientific Officer, Licensed two Phase 3-ready nanoparticle paclitaxel formulations for US FDA approval, Directed the design & formulation of mAb-targeted paclitaxel nanoparticles
• 2012-now BIOMIGA DIAGNOSTICS, Fountain Valley, CA (www.biomigadiagnostics.com), (A Start-Up Personalized Medicine Diagnostics Company), Chief Technical Officer, Directed assay design, sales and marketing of Point-of-Care, Therapeutic Drug Monitoring (TDM) medical tests. Directed outreach programs to company clients, device manufacturers, key opinion leaders, clinician collaborators and investigator-initiated clinical trials. Formulated long-term visions and strategies for personalized medicine product lines.
• 2007-2012 ABRAXIS BIOSCIENC, LLC (acquired by CELGENE in 2011), Los Angeles, CA (A $10 billion pharmaceutical company), Group Head, MOA and Molecular Group Generation and evaluation of novel in-house and licensed drug delivery systems; Extensive experience in design and execution of MOA studies for FDA filings; Proven experience with modern Cancer Biology models including in vitro & cell-based assays, as well as various in vivo mouse models (xenograft, orthotopic, syngeneic, and spontaneous); Establishment and validation of IHC biomarkers in support of FDA filings.
• 2002-2007 Georgia Health Sciences University, Augusta, GA, Assistant Professor, Dept. of Pathology & Vascular Biology Center; Established novel in vitro co-culture systems of ovarian cancer cells, primary mesothelial cells and macrophages to test the inhibitory function of novel proteins and small molecules. Initiated and managed multi-disciplinary in-house and outside collaborations to evaluate the role of extracellular matrix proteins in mouse models of Hypertension (DOCA salt and Angiotensin II), Diabetes (STZ, ob/ob), Flow-induced Vascular Remodeling, and Stroke (transient middle cerebral arter occlusion, MCAO). Established mouse models of early and late stage ovarian and prostate cancer to study the molecular signaling of tumor growth and metastasis.

PUBLICATIONS

Over 30 peer-reviewed publications in high impact journals
Over 40 published abstracts

PATENTS

5 patents/patent applications

Carolin Müller

University of Southern Denmark
IMM - Institut for Molecular Medicine
JB Winsløws Vej 25
5000 Odense C, Denmark
Email: camueller@health.sdu.dk

EDUCATION, RESEARCH AND WORK EXPERIENCE

• 02.2011-now PhD student at the University of Southern Denmark, Faculty of Health Science-; Institute for Molecular Medicine
• 10.2008-01.2011 Research Assistant (full-time position), University of Applied Sciences, Mannheim, Germany, Institute of Molecular and Cell Biology
• 10.2009-11.2009 Guest researcher; University of Southern Denmark, Odense; Research group Prof. Dr. Jan Mollenhauer
• 10.2003-08.2008 Studies of Biological Chemistry, University of Applied Sciences Mannheim, Germany
• Degree: Diplom-Ingenieur (Dipl. Ing.) Biotechnology
• Diploma Thesis: University of Applied Sciences Mannheim, Germany, in cooperation with German Cancer Research Center (DKFZ) Heidelberg, Germany

Ekaterina Myagkova

e-mail: ball.87@mail.ru

Ekaterina Myagkova, 25 years old, physician, postgraduate student in Scientific Research Institute of General Reanimatology, Russian Academy of Medical Sciences in Moscow, researcher in the laboratory "Pathophysiology of critical states ". Scientific interests are connected with research of the changes of red blood cells membrane in patients with severe trauma.

Scientific publications-thesis: The nanostructure of red blood cell membranes in patients with severe trauma accompanied by massive blood loss. V.V.Moroz, E.A.Myagkova, V.A.Sergunova, D.A.Ostapchenko, V.I.Reshetnyak. General Reanimatology, v. VIII, № 3, P.71.

The surface structure of erythrocyte membranes during of long-term storage of donated blood. V.V.Moroz, A.M.Golubev, V.A.Sergunova, E.A.Myagkova. General Reanimatology, v. VIII, №3, P. 72.

Study of structure of erythrocytes membranes with atomic force microscopy in patients with severe trauma. E.A.Myagkova, V.V.Moroz, D.A.Ostapchenko, V.A.Sergunova, V.I.Reshetnyak. Thesis of reports. X Scientific and Practical Conference "Safety of patients in anesthesiology and reanimatology". 27-28th June 2012.

Liudmila Nikitina

MD, PhD

EXPERIENCE

• February 2012- university assistant, Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria
• February 2009 -February 2012 postdoctoral fellow, Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland
• November 2007- October 2008 postdoctoral fellow, Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria
• December 2006-October 2007 Research assistant, Research center for Obstetrics, Gynecology & Perinatology named after acad. Kulakov, Moscow, Russia
• December 2003-September2006 Research Assistant, Biochemistry Department of Faculty of Medicine of Moscow State University, Moscow, Russia
• 2002-2005 PhD fellow, Biochemistry Department of Faculty of Medicine of Moscow State University, Moscow, Russia
• 1996-2002 Study of Human Medicine, Faculty of Medicine, Moscow State University, Moscow, Russia

RESEARCH INTERESTS

• Reproductive immunology
• Placental transport
• Placental angiogenesis
• Genetic predisposition to pregnancy complications
• Emryology, embryotoxicology
• Nanomedicine, nanotoxicology

Burcin Ozbakir Ulgen

MSc., phD candidate in Biopharmacy Department of Utrecht University, Netherlands
Promoter: Prof. Gert Storm

PREVIOUS EDUCATIONS

• Bachelor Education: Pharmaceutical Sciences, Istanbul University, Turkey
• Master Education: Drug Innovation Master Programme, Utrecht University, Netherlands

PHD PROJECT

HIFU-CHEM: Pharmacokinetic study of doxorubicin encapsulated in thermosensitive liposomes (ThermoDox®). Development of an analytical method for separating liposomal drug from free drug in order to measure release in tumor as well as in blood and healthy tissues.

Anita Palma

Anita Palma has been awarded her PhD in Molecular and Cellular Biology from the University of Rome "Tor Vergata" in December 2011. The main subject of her thesis work was the substrate specificity analysis of Classical Protein Tyrosine Phosphatases, by using the peptide microarray technology. She is currently working in Prof. Scoles group in the Department of Medical and Biological Sciences at the University of Udine on a project entitled "Characterization of Cardiac Stem Cell Biomarkers by nanoproteomic analysis".

Her area of expertise is in the molecular biology. She acquired competences in both cell culture handling and proteomics techniques. She has very good skills in protein purification, western blot analysis and immunofluorescence staining. Now her research focuses on the molecular and morphological characterization of Multipotent Adult Stem Cells by the combination of Atomic Force Microscopy with Fluorescence Microscopy. She is author of 3 papers published in reputable peer reviewed journals and she has 9 communications at congresses and international workshops.

Margriet Park

Margriet van der Zee Park obtained her Master's Degree in Biology from Wageningen University in the Netherlands in 2001. After working as a toxicology research assistant for a year at Michigan State University, she returned to the Netherlands, where she was recruited as the lead toxicologist at Lipid Nutrition BV where she developed its infant toxicology program for food supplement ingredients. She shifted her focus to government in 2004 by work-

ing for the Dutch National Institute of Public Health and the Environment (RIVM) as an Exposure assessor and Toxicologist in the Department of Substances and Integrated Risk Assessment, where she worked on topics such as risk factors in children's toys, consumer and scented products.
In 2007, Margriet was hired to work at RIVM's Center for Health Protection to conduct research aimed to further the risk assessment of nanomaterials. Her research involved an evaluation of the use of in vitro studies for risk assessment of nanomaterials, the possibilities for extrapolating safety data between nanomaterials and she was involved the development of a method to derive appropriate dose metrics for nanomaterials. This work ultimately led to obtaining a PhD in nanotoxicology from Maastricht University in 2012.
Her work at RIVM continues to focus on the risk assessment of nanomaterials. Her current projects involve the development of risk assessment tools and strategies, models to predict nanomaterial toxicity and, more recently, approaches for risk benefit evaluations of nanomedicine.

RECENT PUBLICATIONS

• Colognato R, **Park MVDZ**, Wick P, De Jong WH. 2012. Interactions with the Human Body. In: Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology and Impact on Human Health. Fadeel B, Pietroiusti A, Shvedova AA, editors. Amsterdam: Elsevier.
Park MVDZ, Lankveld DPK, van Loveren H, de Jong W. 2009. The status of in vitro toxicity studies in risk assessment of nanomaterials Nanomedicine 4(6):669-685.
• **Park MVDZ**, Neigh AM, Vermeulen JP, de la Fonteyne LJ, Verharen HW, Briede JJ, van Loveren H, de Jong WH. 2011. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32(36):9810-7.

Zsofia Panna Patko

M.D.
Borsod County Hospital Miskolc, Hungary
3526 Miskolc, Szentpéteri kapu 72-76.
patko.zsofia@gmail.com

EDUCATION

I have graduated at Semmelweis University Budapest at the faculty of Medicine in 2004 as a phisician. The title of my thesis was „The histamine and the polyamine". From 2004 I have studied at Semmelweis University as a PhD student at the Department of Cardiovascular Surgery under the supervision of Prof. Dr. Albert Császár and Prof. Dr.György Acsády. The title of my dissertation is „The role of Mac-1 (CD11b/CD18) in monocyte-platelet aggregate formation".

FOREIGN STUDIES

In 2001 I spent my surgery practice at Otto von Guericke University in Magdeburg, Germany. In 2002 I earned a one-year scholarship of Albert-Ludwigs University Freiburg, where I did research at the laboratory of Internal Medicine, Cardiology, under the supervision of Prof. Karlheinz Peter where I studied the function and interactions of the integrin Mac-1. In the following 3 years I earned more scholarships of this University to keep contact between the hungarian and german laboratories and to continue research (2003, 2005, 2006).

WORKING EXPERIENCES

During my studies as a PhD sutdent I worked as an assistant doctor at the National Medical Center Budapest. Between 2005-2007 I translated medical publications from English and German to Hungarian at the hungarian publisher Medical Tribune. Between 2006-2013 I worked as a clinical research manager at EGIS Pharmaceuticals PLC where I planned and organized bioequivalence studies. Since February 2013 I have been working as a resident doctor at Borsod County University Hospital, Institute of Diagnostic and Interventional Radiology.

Mercedes Peleteiro Olmedo

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Mobile: +34 699137805
Email: mpeleteiro@uvigo.es

EDUCATION

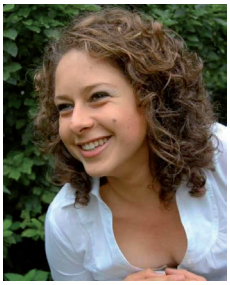
• 2004-2009 Degree in Biology
• 2009-2010 Master in Methodology and Applications in Life Sciences
• 2010-now PhD student at the University of Vigo

FELLOWSHIP

• 2008-2009 Collaboration Grant in the Laboratory of Immunology
• 2009-2011 Master Grant in the Laboratory of Immunology
• 2012-now PhD Grant (FPU, Spanish Ministry of Education)

PUBLICATIONS

• Vicente S, **Peleteiro M**, Diaz-Freitas B, Sánchez A, González-Fernández A, Alonso MJ. Co-delivery of viral proteins and a TLR7 agonist from polysaccharide nanocapsules: a needle-free vaccination strategy (submitted).
• Simón-Vázquez R, Lozano-Fernandez T, **Peleteiro-Olmedo M** and González-Fernández A. Conformational changes in human plasma proteins induced by metal oxide nanoparticles (submitted).
• Vicente S, Diaz-Freitas B, **Peleteiro M**, Sanchez A, Pascual DW, et al. (2013) A Polymer/Oil Based Nanovaccine as a Single-Dose Immunization Approach. PLoS ONE 8(4): e62500. doi:10.1371/journal.pone.0062500
• Simón-Vázquez R, **Peleteiro M**, Lozano T, Casal A and González-Fernández A. Chapter 15: Nanotoxicology. Book: Nanobiotechnology: Inorganic Nanoparticles vs Organic Nanoparticles. Elsevier, 2012. Vol. 4, p. 443-485.



Elizabeth Pietrzykowska

Elizabeth is a materials science engineer. She graduated from the Faculty of Materials Science and Engineering at the Warsaw University of Technology in Poland. Now she is studying for a PhD at the Laboratory of Nanostructures for Photonic and Nanomedicine at the Institute of High Pressure Physics of the Polish Academy of Sciences. Her thesis is about composite materials for regenerative implants. The focus is on forming, under high pressure, nanoceramic powders and the characterisation of the obtained materials (structure, morphology and mechanical properties). The major subject of the Laboratory group is nanopowders' synthesis and their practical applications in medicine.

Nathalie M. Pinkerton

Phone: 1-419-304-3375
E-mail: npinkert@princeton.edu

EDUCATION

• Princeton University Princeton, NJ; Doctor of Philosophy in Chemical and Biological Engineering, expected Fall 2013; Master of Arts in Chemical Engineering, June 2010
• Massachusetts Institute of Technology Cambridge, MA; Bachelor of Science in Chemical Engineering, June 2008 GPA: 5.0/5.0

RESEARCH EXPERIENCE

• Princeton University - Department of Chemical and Biological Engineering Princeton, NJ; Developing a versatile delivery system that specifically targets the lungs via the venous filtration pathway. The platform is an IV injectable, gel microparticle (GMP) loaded with active drug containing nanoparticles (NP). Once trapped in the lungs, the GMPs, composed of PEG based polymers with degradable linkers, release their payload and then degrade away. My research focus is on the polymer synthesis and characterization, NP formulation and functionalization, and GMP characterization. Advised by Dr. Robert K. Prud'homme. Jan 2009 to present

• Novartis Research Center Basel, Switzerland; Formulated nanosuspensions of hydrophobic active pharmaceutical ingredients for oral and parenteral delivery. Investigated process parameters relating to formulation stability and developed a thermodynamic model to predict the stability of nanosuspensions. Advised by Dr. Bernd Riebesehl, Dr. Jörg Brozio and Dr. Andreas Fisch. Summer 2011

• Proctor & Gamble: Brussels Innovation Center Brussels, Belgium; Designed a new process for hydrophobic perfume incorporation in a colloid suspension that increased the stability of finished products. Studied the unstable product and elucidated causes of the instability. Summer 2007

• MIT - Department of Chemical Engineering Cambridge, MA; Modeled and investigated the catalytic synthesis of a copolymer with potential use as a drug delivery agent. Optimized the control of the synthesis and identified key system variables under the supervision of Dr. Clark Colton and Dr. Kevin Brower. Spring 2007, Spring 2008

• MIT - Department of Chemical Engineering Cambridge, MA; Investigated the coating of high-aspect-ratio porous membranes with functional polymers via chemical vapor deposition for applications such as fouling prevention. Analyzed membrane surfaces via SEM and contact angle measurements to discern how the membrane coatings affected hydrophobicity under the supervision of Dr. Karen Gleason and Dr. Malancha Gupta. Spring 2006

• Nestlé Research Center Lausanne, Switzerland; Optimized the in-vitro production of a functional lipid by bacteria in a human gastrointestinal model with the aim of improving processed food nutrition. Explored the conditions in which two lactobacillus strains synthesized the maximum amount of lipid. Assessed the bioavailability of the lipid. Summer 2006

• University of Toledo - Department of Chemistry Toledo, OH; Conducted an X-ray crystallography experiment for Dr. Anthony Pearson of Case Western Reserve University including data collection, analysis, structure solution and refinement. Winter 2003

PUBLICATIONS AND PATENTS

• "Formation of stable nanocarriers by in situ ion pairing during block-copolymer-directed rapid precipitation" **N. M. Pinkerton**, A. Grandeury, A. Fisch, J. Brozio, B. U. Riebesehl, R. K. Prud'homme, Mol. Pharmaceutics, 2013, 10, 319

• "Lung Targeting Dual Drug Delivery System," Patent Application US 2011/0268803 A1: R. K. Prud'homme, P. J. Sinko, H. A. Stone, **N. M. Pinkerton**, L. Shi, J. Wan, S. Ibrahim, D. Gao, 2011

• "Initiated chemical vapor deposition (iCVD) of conformal polymeric nanocoatings for the surface modification of high aspect ratio pores," M. Gupta, V. Kapur, **N. M. Pinkerton**, and K. K. Gleason, Chem. Mater. 2008, 20, 1646

• "1,5-Asymmetric induction during nucleophile additions to arenechromium tricarbonyl complexes, η6-{4-[[Spiro[(1R,2S)-1,7,7-trimethylbicyclo[2.2.1]heptane-3.2'-[1,3]dioxolan]-2-yl]oxy] toluene}chromium tricarbonyl," H. Paramahamsan, A. J. Pearson, **N. M. Pinkerton** and A. A. Pinkerton, Acta Cryst. C, 2008, 64, m147

HONORS AND AWARDS

• Recipient of the Dean's Fund for Scholarly Travel Grant, 2012

• Selected to attend the 2012 NIH National Graduate Student Research Conference, 2012

• Poster, Honorable Mention at NJACS Polymers in Drug Delivery Symposium, 2010

• Princeton University High Meadows Foundation, Sustainability Grant Winner, 2009

• National Science Foundation Graduate Research Fellowship (NSF GRFP), 2009

• National Defense Science and Engineering Graduate Fellowship (NDSEG), 2009

• Princeton University Francis Upton Fellow, 2008

• MIT Alumni Association Community Catalyst Award, 2007

Simona Pînzaru

Doctor in Physics, Associate professor
Cluj-Napoca, Babes-Bolyai University, Biomedical Physics, Theoretical and Molecular Spectroscopy Department
Phone secretariat +40-264-405300
Phone direct +4(0)745387709
Fax number +40-264-550790

Email address: simona.cinta@phys.ubbcluj.ro, simocris02@yahoo.com

Date of Birth 30.09. 1965

EDUCATION

• 1998: Doctor in Physics of Babes-Bolyai University, under the joint scientific coordination of Professor Onuc Cozar from Babes-Bolyai University and Professor Wolfgang Kiefer from the University of Würzburg, Germany

EXPERTISE

• Optical spectroscopy, lasers, optoelectronics, nanotechnology, experimental physics, particularly in applied laser Raman spectroscopy techniques in biomedical, pharmaceutical and environmental field

• Expert evaluator of national and international projects

EMPLOYMENT

• 2003-now: Associate Professor Department of Biomedical Physics, Theoretical and Molecular Spectroscopy Department, Babes-Bolyai University, Cluj-Napoca, Romania

• 2003, 2004, 2005: -Visiting scientist at Institute of Physical Chemistry, University of Wùrzburg, Germany

• 1998-2003- Lecturer, Optics and Spectroscopy Department, Babes-Bolyai University

• 1995-1998 Assistant professor, Optics and Spectroscopy Department, Babes-Bolyai University

AWARD

• The Prize of Excellence for scientific research, Babes-Bolyai University, 2011

PUBLICATIONS

• 59 papers in ISI ranked journals (Hirsch Index 11)

• 48 publications in national journals

• more than 180 contributions in conferences proceedings

• 44 oral presentations in conferences

• 8 invited lectures

• 2 Books/book chapters

• Complete list of publications: Researcher ID: A-4543-2011

• Last publication: **Simona Cinta Pinzaru**, A. Falamas, C. A. Dehelean, Molecular conformation changes along the malignancy revealed by optical nanosensors Journal of Cellular and Molecular Medicine, 17, 2, 277-286,2013:

Paola Posocco

Paola Posocco (31.07.1978) graduated (magna cum laude) in Chemical Engineering at the University of Trieste (UNITS) in 2005. She then started her collaboration with the Molecular Simulation Engineering (MOSE) Laboratory (UNITS). From 2008 to 2009 she was also associated scientist at SUPSI (Lugano, Switzerland) within the Swiss National Science project "Nanovectors for drug delivery in oncology: a combined modeling/experimental study". In 2010 she

obtained her PhD in Nanotechnology (UNITS) with a thesis entitled "Multiscale Molecular Simulation of Nanostructured Systems". Since then and at present, she has a post-doc position at MOSE.
Her research activity is focused on multiscale modeling of complex bio/non bio nanosystems. With an h-index of 13, she is a co-author of 43 ISI publications on international journals, 4 book chapters, and more than 50 conference proceedings in the field of nanostructured materials, drug delivery and target therapy. She won a National Science Foundation-sponsored fellowship award to attend FOMMS 2012 (Mt. Hood, OR, USA) and was selected for the final contest at Premio Nazionale Innovazione-Working Capital 2011 with a research project entitled "Design of multifunctional materials for thin films by multiscale molecular modeling".

Sabrina Pricl

Born 1.8.1961

EDUCATION

• Major Degree (1986) - University of Trieste (Italy) - Chemistry
• PhD (1989) - University of Padua (Italy) - Chemical Engineering

TEACHING AND RESEARCH EXPERIENCE

• From 01.11.90 to 14.02.02: assistant professor at the Faculty of Engineering of the University of Trieste.
• From 01.01.08-01.06.2009: scientific advisor and visiting professor of life science group of iCIMSI-SUPSI, Switzerland.
• From 15.02.02 to date: associate professor of Chemical Engineering at the Faculty of Engineering of the University of Trieste.
• The main research activities are focused on the study, correlation and prediction of the structure.property relationships for complex molecular (nano.bio)systems in life and biomedical sciences. Major expertise in multiscale molecular modeling of complex (bio)materials. Author of more than 400 scientific publications on international journals and conference proceedings in the field of transport properties, molecular modeling and computer simulation of complex biosystems. (h-index = 26, 2054 citations as May 2013; source: Scopus and ISI WOS).

Mazda Rad-Malekshahi

Tel: +31(0)620570625
Email: M.Radmalekshahi@uu.nl

Mazda Rad-Malekshahi is currently a Ph.D. candidate in the department of pharmaceutics at Utrecht University, Utrecht, The Netherlands. He received his pharmacy degree (Pharm.D) from the Tehran University of Medical Sciences in 2009. His current research, under the guidance of Professor W.E. Hennink and Dr. Enrico Mastrobattista, focuses on characterization and pharmaceutical application of peptide-based nanocarriers.

Farshad Ramazani

Email: F.Ramazani@uu.nl

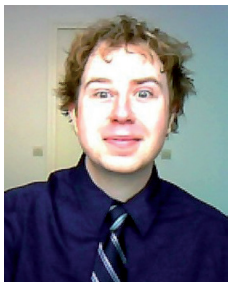
Farshad Ramazani obtained his pharmacy degree (Pharm.D) in 2007 from Tabriz school of pharmacy, Tabriz, Iran. Later on he served as chief pharmacist in different public pharmacies. After been awarded a full scholarship from Iranian health ministry in 2010, Farshad joined the department of pharmaceutics at Utrecht University, Utrecht, The Netherlands. His research is focused on the local delivery of kinase inhibitors for the treatment of cancer under the supervision of prof. dr. ir. W.E. Hennink and Dr. Robbert J. Kok.

Angela Riedel

Diploma in Molecular Biotechnology, Research Assistant, Group of Molecular Oncology, Institute of Molecular Medicine, University of Southern Denmark, J.B. Winsløws Vej 25/1, 5000 Odense-C
Phone: +45-6550-3977
Fax: +45-6550-3950
Email: ariedel@health.sdu.dk

EDUCATION

• Oct. 2003 - Sep. 2008: Student of Molecular Biotechnology at the University of Bielefeld, Germany
• Jan. 2006 - June 2006: Foreign term at the Royal Institute of Technology, Stockholm, Sweden
• Mar. - May 2006: Project thesis in the research group of PD PhD Peter Savolainen, Department of Molecular Biotechnology, KTH, Stockholm, Sweden
• Jan. - Sept. 2008: Diploma thesis in the research group of PD PhD Jan Mollenhauer, Division of Molecular Genome Analysis, German Cancer Research Centre (DKFZ), Heidelberg, Germany
• Dec. 2008: Graduate from the University of Bielefeld with a Diploma in Molecular Biotechnology
• Jan. 2009 – Dec. 2011: Employed as a Ph.D. student at the University of Southern Denmark, Institute for Molecular Medicine, Molecular Oncology, Odense, Denmark (Head: Prof. PhD Jan Mollenhauer)
• Nov. 2010: Winner of the "Best Abstract Award" at the conference "Excellence in Oncology", Athens, Greece
• Jan. 2012: Employed as a research assistant at the University of Southern Denmark, Institute for Molecular Medicine, Molecular Oncology, Odense, Denmark



Douglas K. R. Robinson

Managing Director of TEQNODE SARL, Paris
www.teqnode.com

Douglas K. R. Robinson is an expert in innovation studies, mapping of science and technology fields, ELSA and constructive technology assessment (including scenarios and roadmapping).

Originally trained as a physicist and space scientist, he earned his PhD in Constructive Technology Assessment of Nanotechnology as part of the Dutch TA-NanoNed nanotechnology consortium under the guidance of Professor Arie Rip and in combination with the FRONTIERS and Nano2Life networks of excellence. After postdoctoral research in management of innovation, and a 2 ½ year position as Technical Analyst at the Institute of Nanotechnology (UK) he decided to locate himself permanently in Paris and to apply his expertise through his own small firm.
In May 2011 he created a contract research consultancy with Prof. Rip called TEQNODE Limited located in Paris, France, which provides a platform for applied R&D in national and international projects, with clients including the French Ministry of Research and Higher Education and the OECD.
TEQNODE provides strategic intelligence and future-oriented analysis on emerging science and technology fields through contract research and consultancy. With a focus on early stage and emerging fields, where high uncertainty reigns and expectations about the promising technology are abundant, TEQNODE combines nnovation models to inform analysis and future-oriented assessments of potential innovations to characterise opportunities, challenges and projections of emerging technologies and their embedment in markets and society.
TEQNODE also supports researchers and firms in new and emerging fields of technology and innovation with Societal aspects, Ethics, Governance and Product development aspects, effectively the elements that allow the effective translation of research into applications in society.

At present he has returned to academia as a part-time Post-Doctoral researcher in IFRIS-LATTS (Paris) to gain experience in quantitative analysis of emerging interdisciplinary fields of technology and to contribute his expertise in qualitative analysis of emerging field of science, technology and innovation domains.
Douglas has a number of publications in high-ranked academic peer-reviewed journals including Research Policy and Technology Forecasting and Social Change, and was guest editor of the July 2012 issue of Technology Analysis and Strategic Management journal.

Anna Russo

Anna Russo has been awarded the Master degree in Pharmaceutical Chemistry and Technology from University of Naples "Federico II" in December 2010 with highest grade, then she has spent one year at IBB (Institute of Biostructure and Bioimaging, Naples section) in Dr. Menotti group looking after synthesis and purification of peptides set which modulates the biological activity of growth factors, receptors and enzymes, involved in tumor-associated signaling pathways. She is now pursuing her PhD in "Genetics, Oncology and Clinical Medicine", working in Prof. Scoles group in the Department of Medical and Biological Sciences at the University of Udine on a project entitled "Application of Advanced Nanotechnology in the Development of Innovative Cancer Diagnostic Tools".
She has been visiting PhD student (May-July 2012) at the Centre for Functional Nanostructures of KIT (Karlsruhe Institute of Technology) in Dr. Ljiljana Fruk research group, acquiring competences in synthesis and biofunctionalization of gold nanoparticles, to be used for novel diagnostic tools development. She has very good skills in manual and automatic SPPS by the Fmoc chemistry, Split-mix synthesis, peptide analysis and purification, in biochemical spectrophotometric assays, such as ELISA and FRET by High Throughput Screening.
This skill set is suitable to the study of peptide-protein interactions for the development of peptide-based binders for protein detection. She is co-author of a paper published in Journal of Medicinal Chemistry (2012).

Priyanka Sahariah

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ACADEMIC PROFILE

- M.S., (Organic Chemistry) Department of Chemistry, Gauhati University, Guwahati (2005)
- B.S., (Chemistry) Cotton College, Gauhati University, Guwahati (2002)

RESEARCH EXPERIENCE

- PhD student: Faculty of Pharmaceutical Sciences, University of Iceland, (Dec 2010 till date)
- Research Scientist: Chembiotek Research International (March to Oct 2010)
- Senior Research Chemist: Chembiotek Research International (TCG Lifesciences Ltd.) (Oct 2007 to March 2010)
- Research Chemist: Chembiotek Research International (TCG Lifesciences Ltd.) (April 2006 to Oct 2007)
- Project Fellow: Chembiotek Research International (TCG Lifesciences Ltd.) (June 2005 to April 2006)

Hélder Santos

Born 27 January 1981 in Dalvares–Tarouca, Portugal
Highest Academic degree: D.Sc. Tech. (Chem. Eng.)
Present position: Adjunct Professor (Docent) & Principal Investigator (group leader) in Pharmaceutical Nanotechnology, and Academy of Finland Research Fellow / Associate Researcher

Professional address: Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), FI-00014 University of Helsinki
Professional Phone: +358-9-191-59661; Fax: +358-9-191-59144.
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Dr. Hélder A. Santos is a physical chemist specialized in electrochemistry, biological mimetic models and soft interfaces. In 2003, he obtained his MSc in Chemistry from the Department of Chemistry, University of Porto (Portugal). In 2007, he obtained his DSc in Chem. Eng. from the Laboratory of Physical Chemistry and Electrochemistry, Helsinki University of Technology (Finland). In 2007, Dr. Santos joined the University of Helsinki, Division of Pharmaceutical Technology, as a Postdoctoral Fellow. In 2009, he was appointed Academy of Finland Postdoctoral Research Fellow.
Currently, he is an Adjunct Professor (Docent) in Pharmaceutical Nanotechnology, an Academy of Finland Research Fellow (Associate Researcher) and Principal Investigator/Group Leader at the Division of Pharmaceutical Technology, University of Helsinki. In 2012, Dr. Santos received the ERC starting grant for 2013-2017 worth of 1.5 M€.
Dr. Santos' research is focused on nanobiomaterials, including nanoporous silica/silicon materials, liposomes and solid lipid nanoparticles for biomedical nano/micro-technological applications, controlled drug delivery, nanomedicine, diagnostics and therapy (e.g., diabetes/obesity, cancer and cardiovascular diseases). He is the author/co-author of more than 50 refereed articles, including 9 reviews (> 350 citations, h-index = 11), 6 book chapters and more than 67 conference proceedings/abstracts. He has supervised several undergraduate students and ca. 15-MSc students. He supervised/co-supervised 3-PhD students and is currently the supervisor of 2-MSc students, 8-PhD students and 3 post-doctoral researchers. He has also given more than 37 invited/selected talks in national/international meetings/conferences/lectures.
In 2010, he received the "Talent Prize in Science" awarded by the Portuguese Government (TV-broadcasted for more than 15 million people). Recently, he was awarded with the Young Researcher's Prize 2013 worth of 10 000 € by the Faculty of Pharmacy, University of Helsinki.
Dr. Santos is also an active referee for about 34 international journals and evaluator for 4 international research foundations (The Leverhulme Trust, UK; Research Foundation Flanders, Belgium; The Swiss National Science Foundation, Switzerland; and The Netherlands Organisation for Scientific Research (NWO), The Netherlands). Dr. Santos is also an Editorial Board Member for the journals of Drug Delivery Letters, Biomatter, International Journal of Nanotechnology and Nanoscience, Pharmaceutical Nanotechnology, World Journal of Pharmacology. He is also Associate Editor for Versita in the Chemistry field, the Editor of the recently launched Mesoporous Biomaterials journal and the Editor of a book entitled "Porous Silicon for Biomedical Applications" (in preparation), Woodhead Publishing.
With only 32-years old, Dr. Santos' research career and development portfolio since 2009 totals ~3 M€ awarded by several projects from the European Research Council, the Academy of Finland, and the University of Helsinki Research Funds, among others.

Priya Satalkar

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Institute for Biomedical Ethics.
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Phone: +41-61-267 17 85
Email:priya.satalkar@unibas.ch
Date of Birth: 4th Oct 1979

PROFESSIONAL EDUCATION AND TRAINING

• Sep 2012 on: PhD scholar at Institute for Biomedical Ethics, University of Basel, Switzerland
• 2010 – 2011: Erasmus Mundus Master's in Bioethics (EMMB), Katholieke Universiteit Leuven, Belgium, Radboud, Universiteit Nijmegen, the Netherlands and Universita Degli Studi di Padova, Italy.
• 2009 – 2010 Master's in Medical Anthropology (MSc), University of Amsterdam; the Netherlands
• 2006 – 2007 Master's in Public Health (MPH), BRAC University; Dhaka, Bangladesh
• 1997 – 2003 Bachelor of Medicine and Bachelor of Surgery (MBBS), Mumbai University; India. Medical License no: 2352, Maharashtra Medical Council, India

CONTINUING EDUCATION

• July 2008: Summer Institute on Sexuality, Culture and Society University of Amsterdam, Netherlands
• Feb 2008: Primary HIV Care, Christian Medical College, Vellore, India
• Jan 2008: STI/HIV counseling skills building Y R G Care, Chennai, India
• Jan 2006: Fogarty AITRP-MGIMS International Workshop on Infectious Diseases Epidemiology, Sewagram, India
• Sep 2005: International course in Applied Epidemiology Emory University; Atlanta, GA.USA
• Sep 2003: management and leadership skills building Centers of Disease Control, Atlanta

ACADEMIC AWARDS AND ACHEIVEMENTS

• Suma cum laude in Erasmus Mundus Masters of Bioethics
• Magna cum laude in MSc in Medical Anthropology from University of Amsterdam
• Magna cum Laude in Masters of Public Health from BRAC University, Bangladesh
• Homrasji Kama gold medal in Obstetrics & Gynecology in Mumbai University
• Recipient of Sir Ratan Tata Trust scholarship for Medical Education

Cora-Ann Schoenenberger

Cora-Ann Schoenenberger obtained her PhD in Cell Biology from the University of Basel. During her first postdoc at the Ludwig Institute for Cancer Research in Bern (Switzerland) she studied mammary specific oncogene expression in transgenic mice. She then moved to the Department of Anatomy & Cellular Biology at Harvard Medical School where she investigated the effects of oncogenes on epithelial cell polarity in the laboratory of Prof. Karl S. Matlin. In 1991 she returned to Switzerland and joined Prof. Ueli Aebi at the M.E. Müller Institute for Structural Biology at the Biozentrum in Basel. Here she established her independent research group that focuses on the structural and mechanical plasticity of the actin cytoskeleton. She was amongst the first to probe the nanomechanical properties of cells by atomic force microscopy. The Schoenenberger lab uses an integrated approach that includes different microscopic techniques to study cell and tissue mechanics, as well as structural actin polymorphism at the cellular and molecular level.

Georg Schulz

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• 2008 Diploma in theoretical physics at the University of Freiburg, Germany; Diploma thesis on Exciton dynamics in circular and elliptical aggregates.
• 2008 scientific collaborator at the group of Theoretical Quantum Dynamics (University of Freiburg, Germany)
• 2012 PhD at Biomaterials Science Center on the topic of human brain imaging, in particular magnetic resonance imaging, phase contrast X-Ray computed tomography and small-angle X-ray scattering
• 2012-present PostDoc at Biomaterials Science Center

Nicole Seehaas

Nicole Seehaas (Dipl.-Chem.) studied chemistry at the universities of Goettingen and Hamburg in Germany.
After various positions, including conference management for the pharmaceutical industry and public relations for the "Nanotruck" an initiative of the German Federal Ministry of Education and Research on communication on nanotechnology, Nicole Seehaas joined DECHEMA e.V. in 2007.
Since then, Nicole Seehaas has been active in the Research and Project Coordination group in the coordination and management of FP6 and FP7 European projects, including FP6 Nanomedicine project NanoBioPharmaceutics and FP7 project SYNFLOW. Currently she is responsible for the management and coordination of FP7 large scale project ALEXANDER - Mucus Permeating Nanoparticulate Drug Delivery Systems, coordinated by DECHEMA e.V. She also acts as dissemination manager for the named projects.
DECHEMA (Society for Chemical Engineering and Biotechnology) e.V. is a non-profit scientific and technical society based in Frankfurt am Main, Germany with more than 5,500 members, One aim of DECHEMA is to promote and support research and development in Chemical Technology and Biotechnology.

Victoria A. Sergunova

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Date of birth 23.11.1982

Specialty – biologist. Position – researcher in the Laboratory " Biophysics of cell membranes", Institute of General Reanimatology, Russian Academy of Medical Sciences in Moscow.
The author of 8 scientific articles. The scientific interests: nanostructure of cell membranes, the method of atomic force microscopy.

Laszlo Takacs

CEO and Chief Scientific Officer
Biosystems International

Pofessor, Department of Human Genetics
University of Debrecen Health Science Center

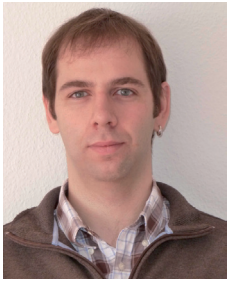
TRAINING

• M.D: Semmelweis University Medical School, Budapest, Hungary

• Ph.D: Hungarian Academy of Sciences
• D.Sc.: Hungarian Academy of Sciences
• Member of the Hungarian Academy of Sciences

SIGNIFICANT ACCOMPLISHMENTS

• Creation of Biosystems International a diagnostics R&D company
• Building BSI, a startup company for biomarker discovery and validation.
• Successful translation of genome and bioinformatics research to biomarker and small molecule drug discovery, leadership of a drug discovery team at Pfizer PGRD Fresnes Laboratories (France). Discovery of many drug target and biomarker candidate genes.
• Leading activities of a gene discovery group, development of a signal exon trap strategy, a screen for secreted protein coding genes. Discovery of various novel genes, with potential biotechnology and pharmaceutical applications at Amgen Inc. (Thousand Oaks CA, US). Discovery of CD26 as a biomarker of cholestatic and inflammatory liver diseases.
• Head of a basic research laboratory at the National Institutes of Health, (Rockville MD. US) discovery of the role of a peptidase in early T cell and bone marrow development. Description of CD25 (IL2 receptor alpha chain) on T cell precursors, investigation of growth requirements of these cells.



Peter Thalmann

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2012: Diploma in experimental physics at the Swiss Federal Institute of Technology Zürich. NFESEM measurements on Si(111): VdI-analysis and single electron detection.
2012-present: PhD student at the Biomaterials Science Center (University Basel)

Helen E Townley

• 2010-2013 Research Fellow, Department of Engineering, Oxford University, 'Nanoparticles for in vivo Near Infra Red Imaging'
• 2007-2010 SEEDA Professorial Fellow
, Department of Engineering, Oxford University, 'Microfluidic separation of biological nanoparticles'
• 2005-2007 Postdoctoral Research, Department of Zoology, Oxford University, 'Optics in Nature'
• 1998-2005 Department of Plant Sciences, Oxford University; 'Production and characterization of a database of defined promoter mis-expression traps'; Cell-cell signalling and the control of programmed cell death (PCD) in Arabidopsis'; 'Improved transcriptional activators and their use in mis-expression traps in Arabidopsis'; 'Calcium signalling networks in Arabidopsis'; 'Characterization of the active site of pyrophosphate: fructose 6- phosphate 1- phosphotransferase (PFP) from plants
• 1998 PhD in Biochemistry, Department of Biochemistry, Bristol University
• 1994 BSc (Hons) in Biochemistry, Department of Biochemistry, Bristol University

Mehmet Hikmet Ucisik

Dipl.-Ing., Department of Nanobiotechnology
University of Natural Resources and Life Sciences (BOKU) Vienna
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Born in Istanbul in April 23, 1981

EDUCATION

• 2009-2013 PhD, Department of NanoBiotechnology, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria
• 2004-2006 MSc, Department of Biotechnology, TU Delft, Delft, The Netherlands
• 2000-2004 BSc , Department of Chemical Engineering, Bogazici University, Istanbul, Turkey
• 1992-2000 Gymnasium, "Deutsche Schule Istanbul", Istanbul, Turkey

SELECTED TRAININGS AND CERTIFICATES

• 2012 European Summit for Clinical Nanomedicine, CLINAM 2012, Basel, Switzerland
• 2011 International Summer School on "Cellular Systems", University of Heidelberg,
Germany
• 2010 Summer School of ICMR on 'Nanoscale Science of Biological Interfaces', University
of California Santa Barbara (UCSB), USA
• 2007 Turkish Patent Attorney Certificate

EXPERIMENTAL AND TECHNICAL SKILLS

Fermentation: Batch and Continuous; Protein Purification: chromatography techniques; Metabolite Analysis including enzymatic analysis and HPLC; Molecular Biology Techniques: Plasmid Extraction, Isolation and Cloning: Polymerase Chain Reaction (PCR), SDS Gel Electrophoresis; Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Fluorescence Microscopy, Surface Plasmon Resonance (SPR), Zeta-Sizer; Cell Culture Experiments incl. Flow Cytometry (FACS). Conceptual Design of Up- and Down-Stream Processing including In-Situ Product Removal Process and Crystallization Unit Operation; Economic Feasibility Studies; Scale-Up.

PUBLICATIONS

• 2013 **Ucisik, M. H.**, Küpcü, S., Debreczeny, M., Schuster, B. and Sleytr, U. B. (2013), S-layer Coated Emulsomes as Potential Nanocarriers. Small. doi: 10.1002/smll.201203116
• 2013 **Ucisik, M. H.**, Küpcü S., Schuster B., Sleytr U.B. CurcuEmulsomes: Nanoformulation for Enhanced Solubility and Delivery of Curcumin, Nanomedicine: NBM (in preparation)
• 2013 **Ucisik, M. H.**, Küpcü S., Breitwieser A., Ilk N., Schuster B., Sleytr U.B. CurcuEmulsomes functionalized with S-layer fusion protein rSbpA-GG for specific binding to human Im Immunoglobulin G (hIgG), Advanced Functional Materials (in preparation)

Mara Cristina Vargas Gonzalez

Investigador Nacional I Sistema Nacional de Investigadores. Monterrey Nuevo Leon, MEXICO; January 1959

ACADEMIC DEGREES

Bachelor in Sciences (Physics); Facultad de Ciencias Fsico-Matematicas, Universidad Autonoma de Nuevo Leon; June 1982. Master in Sciences (Physics); Departamento de Fsica, Instituto de Ciencias, Universidad Autonoma de Puebla; July de 1983. PhD (Physics); Facultad de Ciencias, Universidad Autonoma del Estado de Morelos; Sept. 1997.

PRESENT POSITION

Professor, CINVESTAV del IPN, Unidad Merida, 1998 - to date

PREVIOUS POSITIONS

Visiting Professor Princeton University, Sept 1999 - August 2000. Professor Facultad de Ciencias, Universidad Autonoma del Estado de Morelos, February 1993 - July 1995.

REPRESENTATIVE PUBLISHED PAPERS

• **M. C. Vargas**, P. Giannozzi, A. Selloni and G. Scoles; Coverage-Dependent Adsorption of CH3S and (CH3S)2 on Au(111): a Density Functional Theory Study; J. Phys. Chem.B 105 (2001) 9509-9513.
• X. Wu, **M. C. Vargas**, S. Nayak, V. Lotrich and G. Scoles; Towards extending the applicability of density functional theory to weakly bound systems; J. Chem. Phys. 115 (2001) 8748-8757.
• Alejandro Ramrez-Sols, Jorge Hernandez-Cobos, and **Cristina Vargas**; A New Nonsymetric As(OH)3 Species. Comparison with the Known C3 Species and Thermochemistry at the HF, DFT(B3LYP), MP2, MP4, and CCSD(T) Levels of Theory; J. Phys. Chem. A 110 (2006) 7637-7641.
• J. Hernandez Cobos, **M. C. Vargas**, A. Ramirez Solis, and I. Ortega-Blake; Aqueous solvation of As(OH)3: A Monte Carlo study with exible polarizable classical interaction potentials; J. Chem. Phys. 133 (2010) 114501.
• **M. C. Vargas** and G. Perez- Angel; Crystallization time scales for polydisperse hard-sphere uids; Phys. Rev. E 87 (2013) 042313;
• Antillon Daz A. et al.; New Amphotericin Analogous Compounds and Pharmaceutical Compositions containing them, World Intellectual Property Organization International Bureau; International Publication Number WO 2012/085784 A3.
• More than 500 citations to the rst 2 papers mentioned above.

SUPERVISED THESIS

4 Master Thesis, 1 Bachelor Thesis

Reyhaneh Varshochian

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EDUCATION

• May 2013 PhD student, Pharmaceutics, Tehran University of Medical Sciences, Tehran, Iran; Concentration: Protein delivery by polymeric nanoparticles; Dissertation: Preparation and evaluation of polymeric nanoparticles containing bevacizumab
• 2002-2008 PharmD, Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Dissertation: Reduction of percutaneous absorption of toxic chemicals by dendrimers

AWARDS AND HONORS

• 2010 Number one student in the national Board exam (Pharmaceutics) across Iran and awarded by Iran Ministry of Health and Medical Education
• 2008 Number one student in the national PhD entrance exam (Pharmaceutics) across Iran

PUBLICATIONS AND PERESENTAIONS

• Goodarzi N, Varshochian R, Kamalinia G, Atyabia F, Dinarvand R, A review of polysaccharide cytotoxic drug conjugates for cancer therapy, Carbohydrate Polymers, 92 (2), 1280–1293, 2013
• Kamalinia G, Varshochian R, Goodarzi N, Atyabia F, Dinarvand R, Bioconjugation of cytotoxic drugs to peptides, proteins and aptamers as novel nanostructures for enhanced anticancer drug delivery, Nanotechnology (7): Diagnostics and Therapeutics,PP: 265-290, 2012
• Moghimi HR, Varshochian R, Kobarfard F, Erfan M, Reduction of percutaneous absorption of toxic chemicals by dendrimers, Cutaneous and Ocular Toxicology, 29 (1), 34-40, 2010 [PharmD thesis]
• Varshochian R, Dinarvand R, Jeddi-Tehrani M, Mahmoudi AR, Atyabi F, Sabzevari A, Riazi –Esfahani M, The protective effect of albumin on bevacizumab activity and stability in poly(D,L-lactic-co-glycolic) acid nanoparticles intended for retinal and choroidal neovascularizations treatment, [Submitted in January 2013]
• Abstract in 39th Annual Meeting & Exposition of the Controlled Release Society, Québec, Canada. (CRS 2012)
• Abstract in International Conference on Nanotechnology in Medicine, UK. (NanoMED 2012)
• Abstract acceptance in 8th PBP World Meeting Istanbul, Istanbul, Turkey. (PBP 2012)
• Abstract in the 2nd Symposium on Innovative Polymers for Controlled Delivery, Suzhou, China. (SIPCD 2012)
• Poster in the 13th Iranian Pharmaceutical Sciences Conference, Isfahan, Iran. (IPSC 2012)
• Poster in the 5th Iranian Controlled Release Conference, Mashhad, Iran. (ICRC 2011)
• Oral presentation in the 12th Iranian Pharmaceutical Sciences Conference, Zanjan, Iran. (IPSC 2010)
• Poster in the 11th Iranian Pharmaceutical Sciences Conference, Kerman, Iran. (IPSC 2008)

Suhas V. Vasaikar

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Date of Birth 17-01-1986

CURRENT POSITION AND AFFILIATION

• July 2008-till now Position: PhD Research Fellow, Research field: Systems Biology (Neuroscience), Research area: Systems biology approach to neurodegenerative diseases, Institute: Indian Institute of Technology Delhi

ACADEMIC RECORD

• 2008- PhD (in completion), Indian Institute of Technology Delhi, Thesis Title: "Gene Networks in Neurodegenerative Diseases"
Supervisors: Prof. James Gomes, Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, India
Prof. B. Jayaram, Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, India; Project during PhD work:
Database and web server development for neurodegenerative disease networks analysis.
New method/algorithm developed for biological regulatory network analysis. Qualitative analysis of neuronal apoptosis and proof of concept on HT-22 cell line model.
• 2003-2008 M.Sc. Biotechnology (5yr's Integrated in Bioinformatics and Biotechnology), CGPA : 7.2 (grade 'O'- outstanding), Institute: Institute of Bioinformatics and Biotechnology, University of Pune, India, Thesis: "Molecular Characterization and Identification of Anaerobic Consortium for Microbial Enhanced Oil Recovery growing at 960C"; Supervisor: Dr. Prashant K. Dhakephalkar, Agharkar Research Institute, Pune
• 2008 "Advanced course on Intellectual Property" from World Intellectual Property Organization (WIPO) Geneva, Sept16-Nov25
• 2003 Higher Secondary School Certificate (HSC), GPA: 80.83%; Maharashtra State Board for Secondary and Higher Secondary Education, Pune
• 2001 Secondary School Certificate (SSC), GPA: 79.33%; Maharashtra State Board for Secondary and Higher Secondary Education, Pune

PROFESSIONAL AFFILIATIONS

• Society for Neurochemistry (SNCI), India
• International Brain Research Organization (IBRO), France
• Association of Biotechnology Led Enterprises (ABLE), India

PUBLICATIONS (2008-)

Journal Research articles

1. Vasaikar SV, Padhi AK, Jayaram B, Gomes J (2012) NeuroDNet - an open source platform for constructing and analyzing neurodegenerative disease networks. BMC Neuroscience 14:3.
2. Padhi AK, Kumar H, Vasaikar SV, Jayaram B, Gomes J (2012) Mechanisms of Loss of Functions of Human Angiogenin Variants Implicated in Amyotrophic Lateral Sclerosis. PLoS ONE 7(2): e32479.
3. Vasaikar SV, Gomes J. Rapid Computation of Boolean Attractors in Biological Networks. PLOSComputational Biology (Under consideration).

4. Padhi AK, Vasaikar SV, Jayaram B, Gomes J. Fast prediction of deleterious angiogenin mutations causing amyotrophic lateral sclerosis. FEBS Letters (Accepted).
5. Vasaikar SV, Jayaram B, Gomes J. Analysis of Stable States in Neuronal Apoptotic Pathways for Predicting Drug Targets (Under preparation).
6. Upadhyay M, Samal J, Kandpal M, Vasaikar SV,Biswas B, Gomes J, Perumal V. Depletion of CpG dinucleotides in parvovirus genomes: role of host methylation capabilities (Under preparation).

Proceedings

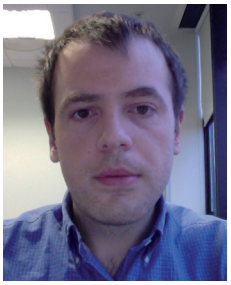
1. Vasaikar SV, Bhattacharya S, Gomes J (2012) A Computational Analysis of Multiple Sclerosis Using Protein-Protein Interaction Network. 3rd Annual International Conference on Advances in Bio technology Biotech 2013, Singapore.

Conference Abstracts

1. Vasaikar SV, Padhi AK, Jayaram B, Gomes J (2011) Network Analysis of Neurodegenerative Diseases: A System based Approach to Uncover Perturbed Networks. Front. Neuroinform. Conference, 4th INCF Congress of Neuroinformatics, Boston USA. doi: 10.3389/conf.fninf.2011.08.00047
2. Vasaikar SV, Gomes J. (2011) Gene Networks in Neurodegenerative Disorders. National Conference Nucleic Acids in Disease & Disorder 2011, New Delhi, India.
3. Vasaikar SV, Padhi AK, Jayaram B, Gomes J (2011) NeuroDNet: a Database for Constructing and Analyzing Neurodegenerative Disease Networks. International Symposium on Systems Biology in Physiology/Medicine, Indian J Physiol Pharmacol, Vol. 55, No. 5, Supp 2011.
4. Vasaikar SV, Gomes J. (2012) Computational Analysis of Protein Interaction Networks in Alzheimer's Disease: A Systems Approach. XIX Annual Meeting of Indian Academy of Neurosciences, New Delhi, India.

SCHOLARSHIPS AND AWARDS

• 2008-2013 National Eligibility Test (NET) qualified. Junior and Senior Research Fellowship obtained from Council of Scientific and Industrial Research (CSIR), Government of India.
• 2008 Selected for Junior and Senior Research Fellowship from Department of Biotechnology, (DBT), Government of India.
• 2007 2nd rank in Technical paper presentation in Thadomal Shahani College of Engineering, Mumbai.
• 2000 Recipient of Maharashtra Talent Search Examination (MTSE) award certificate.



Driton Vllasaliu

I obtained a Master of Pharmacy (MPharm) degree in 2004 at the University of Nottingham and became a UK-registered Pharmacist in 2005, following the completion of pre-registration training in hospital pharmacy. I gained my PhD in 2010 at the University of Nottingham, completing an industry-sponsored project on mucosal delivery of protein therapeutics. This was followed by a 2-year position as a postdoctoral research fellow in the same institution within the Drug Delivery group, School of Pharmacy. I joined the University of Lincoln in November 2012 as a lecturer within the School of Pharmacy.

My research interests include non-invasive (mucosal) delivery of biotherapeutics, nanoparticle-mediated drug delivery and development of in vitro mucosal models for drug delivery studies.
I have authored or co-authored 12 peer-reviewed papers and presented at a number of international scientific meetings.

I am registered with the General Pharmaceutical Council (GPhC) and a member of Royal Pharmaceutical Society, Royal Society of Chemistry, Controlled Release Society (CRS) and Controlled Release Society Young Scientist Committee.

Efstathia Voulgari

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efiv48@hotmail.com

EDUCATION

• 2011-2013 MSc in Pharmaceutical Technology, University of Patras, Greece

• 2005-2011 BSc in Biology, University of Patras, Greece

RESEARCH EXPERIENCE

• 2011 – 2013 Master's thesis, Laboratory of Dr Avgoustakis Konstantinos, Department of Pharmacy, University of : "Magnetic hybrid nanocarriers for targeted delivery of Cisplatin", Patras, Greece
• 2009 – 2011 Barchelor's thesis, Laboratory of Dr Zoumbos Nicholas, Patras University Medical School, Greece

Anne Vroon

Trained as a biomedical scientist, Anne Vroon obtained her PhD in Neuroimmunology and spent several years as a post-doc researcher studying the interaction of the brain and immune system. Her growing interest in applied research and product development made her decide to switch to industry. In the past years, Dr. Vroon gained experience in both research and management of preclinical drug development, working as a scientist on Genmab´s antibody discovery program and as project manager on Prosensa BV´s oligonucleotide development before joining the Cristal Delivery team. She is currently involved in the nonclinical safety and efficacy programme of Cristal Delivery's lead product, CriPec Docetaxel and in addition develops strategies for actively targeted CriPec nanoparticles.

Chang-Fang Wang

E-mail: chang-fang.wang@helsinki.fi
Phone: +358 46 6238205(cell) Address: Viikinkaari 5E, Helsinki

EDUCATION

• Sep. 2012-present, Ph.D at Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, Finland
• Sep. 2007-Jun. 2009, M. Sc. at Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, China
• Sep. 2003-Jun. 2007 B.Sc. at School of Chemical Engineering and Pharmacy, Wuhan Institute of Technology, China

HONORS

• Jun. 2007 First-class of Excellent bachelor thesis in Hubei Province
• Sep. 2004 - Jun. 2007 Student Scholarships in the three years Research Experiences
• Sep. 2012 - present, Polymer/peptide-mediated targeting drug delivery via click chemistry, silicon surface modification, and drug controlled release study.
• Sep.2009 - Aug. 2012 Light-activated molecular linker synthesis, peptide and oligonucleotide solid phase synthsis, conjugation by copper-catalyzed and strain-promoted cycloaddition click reaction
• Sep.2007 –J ue. 2009 Synthesis and modification novel polycarbonates catalyzed by IPPL. Characterization of functional polycarbonates used for gene delivery
• Feb. 2007 - Jun. 2007 Synthesis of chiral molecules and analysis the separation mechanism by 1H-NMR
• Jun. 2005 - Jun. 2006 Measurement of the synthetical iodic compound ratio

Nadine Wilhelm

Dipl. Biol.
Am Hohen Wald 10, 66386 St. Ingbert, Germany

EDUCATION

• Diploma Biologist, Saarland University, Saarbrücken, Germany – October 2004 until July 2009

EXPERIENCE

• Research Assistant, PomBioTech GmbH, Saarbrücken, Germany – September 2009 until May 2011
• Doctoral Student, Fraunhofer Institute for Biomedical Engeneering, St. Ingbert, Germany – since June 2011

Jacek Wojnarowicz

Jacek Wojnarowicz graduated from Tadeusz Kościuszko Cracow University of Technology in the field of Chemical Technolology, with a major in Polymer Technology. In his master's thesis, titled 'Polyurethane nanocomposites containing multiferroic ceramics "Pb0,94Sr0,06 (Zr0,53Ti0,47)0,99 Cr0,01O3", he focussed on synthesis and properties of nanocomposites. Now she is studying for a PhD at the Laboratory of Nanostructures for Photonic and Nanomedicine at the Institute of High Pressure Physics of the Polish Academy of Sciences. He is responsible for the synthesis of cobalt and rare earth-doped zinc oxide, and for the functionalisation of nanoparticle surfaces. He is undertake investigation of agglomerate size and stability of suspensions obtained with the water used in toxicity tests, and also NP characterisation. He is developing a method of zinc oxide storage via a concentrated suspension and dispersion procedurę. His research focus is on nanopowders' synthesis, examination of their properties and practical applications in medicine.

Doudou Xu

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Ms. Doudou Xu received her BSc degree in Bioengineering from Chongqing University of Technology, China in 2007, and her MSc degree in Biomedical Engineering from University of Dundee, UK in 2009. From October 2010, Ms. Doudou Xu became an early stage researcher in an EU FP7 funded project: Nanoporation, and started her PhD degree studying within the same project in Institute for Medical Science and Technology, University of Dundee. Her research work has been focused on establishment of cell culture models of human cancer cell lines in response to encapsulated anti-cancer drugs by novel nano-carriers. Specific achievements include evaluation of γ-cyclodextrin based drug carriers by a range of chemical and biological methods; combination of MR-guided high intensity focused ultrasound and micro-bubbles to control and trigger drug release from the carrier to achieve targeting drug delivery purpose. From May 2013, Ms. Doudou Xu became a fellow of another EU FP7 funded project: Fusimo and continued her PhD study within the project. Fusimo aims at developing a model for focused ultrasound surgery in moving organs, such as the liver. With support in planning of the intervention, monitoring of the treatment progress, as well as by assessing the therapy outcome, focused ultrasound can become a safe and successful non-invasive procedure for tissue ablation in moving abdominal organs. Since then, her research will be focused on small animal study by employing MR-guided focused ultrasound treatment. Specifically, orthotopic tumor models will be implanted into immunodeficient rodents' organ such as liver. Encapsulated cancer drug then will be injected through intravenous, focused ultrasound will be applied to treat moving liver with tumor.

Kewei Yang

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EDUCATION/WORK BACKGROUND

• June.2012-now: Post-Doc in Novartis, Pharmaceutical Development Unit for Parenteral and Topical formulations.
Development of liposomal formulations for delivery of antitumor/anti-inflamation drug substances; continuous manufacture of nanoparticles and microparticles.
• Nov.2011-May 2012: Project scientist in the department of pharmaceutical technology, Friedrich Schiller University of Jena, Germany.
Characterization of liposomal products using HPLC and Asymmetric Flow Field Flow Fractionation.
• July.2007-Nov.2011 Ph.D degree in Pharmaceutical technology in Friedrich Schiller University of Jena (Germany), Department of Pharmaceutical Technology. Bacteria-targeting liposomes for photodynamic antimicrobial chemotherapy; High throughput screening of liposomal formulations
• Mar. 2005—July.2007 Master degree of science in pharmaceutics in Peking University (China), School of Pharmaceutical Sciences. Application of macromolecular micelles as novel drug delivery system
• Sept. 2001—Mar. 2005 Bachelor degree of science in pharmaceutical sciences in Peking University (China), School of Pharmaceutical Sciences.

SELECTED PUBLICATIONS

• **Kewei Yang**, Xin Ru Li, Zhuo Li Yang, Ping Zhu Li, Fei Wang, Yan Liu. Novel polyion complex micelles for liver-targeted delivery of Diammonium glycyrrhizinate: in vitro and in vivo characterization. Journal of Biomedical Materials Research: Part A, 2009, 88A(1): p. 140-148.
• **Kewei Yang**, Burkhard Gitter, Ronny Rüger, Gerhard D. Wieland, Ming Chen, Xiangli Liu, Volker Albrecht, Alfred Fahr. Antimicrobial peptide modified liposomes for bacteria targeted delivery of temoporfin in photodynamic antimicrobial chemotherapy. Photochemical & Photobiological Science, 2011, 10, 1593-1601.
• **Kewei Yang**, Joseph T. Delaney, Ulrich S. Schubert, Alfred Fahr. Fast high throughput screening of temoporfin loaded liposomal formulations prepared by ethanol injection method. (Journal of Liposome Research, 2012, 22(1):31-41.)
•**Kewei Yang**, Burkhard Gitter, Ronny Rüger, Volker Albrecht, Gerhard D. Wieland, Alfred Fahr. Wheat Germ Agglutinin Modified Liposomes for the Photodynamic Inactivation of Bacteria. (Photochemistry and Photobiology, 2012, 88(3):548-556.)

ABSTRACTS OF THE INTERVENTIONS

OCULAR PHARMACOSCINTIGRAPHIC AND AQUEOUS HUMORAL DRUG AVAILABILITY OF GANCICLOVIR-LOADED MUCOADHESIVE NANOPARTICLES IN RABBITS

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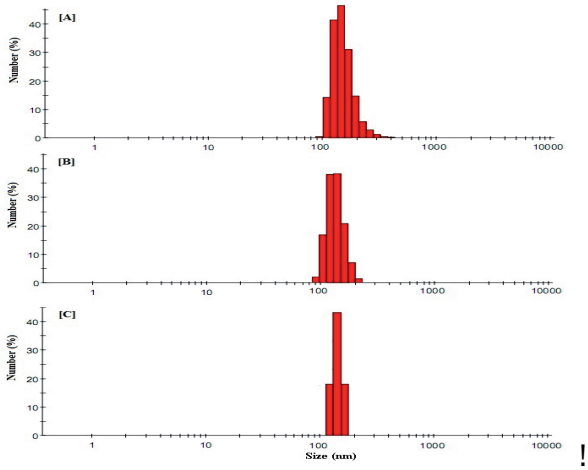
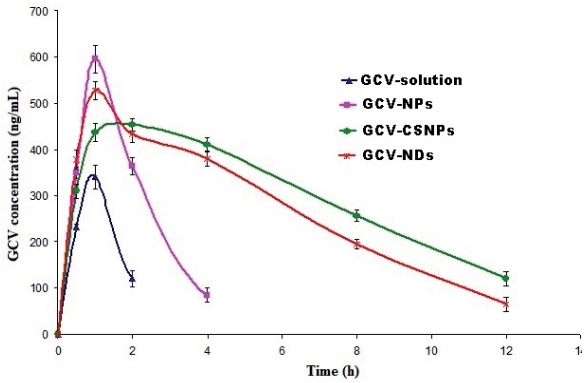
The presentation here describes the improved ocular retention and aqueous humoral drug availability of ganciclovir (GCV) when administered via topical instillation of different kind of nanoparticles onto the rabbit eye. GCV was loaded into PLGA nanoparticles, chitosan-coated nanoparticles and chitosan-coated niosomal nanoparticles. All three formulations contained nanoparticles equally round in shape with a mean particle size in the range of 180-200 nm. The ocular corneal retention property was evaluated by gamma scintigraphy, revealing that the clearance was slowest in case of the chitosan-containing formulations. GCV in chitosan-coated PLGA nanoparticles and chitosan-coated niosomal nanoparticles showed approx. 6-fold higher aqueous humor drug availability as compared to a GCV solution and nearly 2.5 fold higher as compared to the chitosan-lacking GCV-PLGA nanoparticles. The results indicate that the use of a mucoadhesive chitosan coating can improve the ocular residence time and aqueous humoral availability of GCV when administered topically in nanoparticles.



1. Particle size distribution of GCV nanoparticles: (A) reference GCV-NPs, (B) chitosan- coated GCV-CSNPs, and (C) chitosan-coated GCV-NDs.



2. Aqueous humor concentration–time profile of GCV after topical instillation of GCV- solution, reference GCV-NPs, chitosan-coated GCV-CSNPs and chitosan-coated GCV-NDs onto the rabbit eye.

TARGETED CANCER THERAPY VIA MAGNETIC NANOPARTICLES

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INTRODUCTION

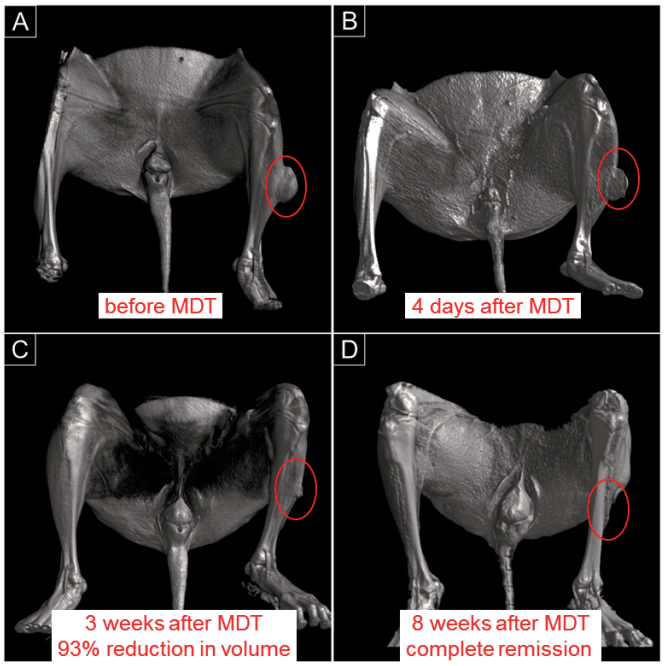
Nano-sized magnetic drug carriers (superparamagnetic iron oxide nanoparticles=SPION) offer a splendid prospect for directed drug application. The goal of this carrier system involved is to achieve active enrichment of effective substances in diseased tissue using magnetic forces. In this study we investigated the biodistribution of chemotherapeutic agent mitoxantrone (MTO) bound to SPION`s with/without external magnetic field in comparison to conventional chemotherapy in tissue and the load of MTO in circulating leukocytes.

MATERIAL AND METHODS

In this study 42 animals, with VX2-squamous cell carcinoma implanted subcutaneously at the left hind limb, were treated with conventional chemotherapy, MTO (Mitoxantron)-SPION intravenously with and without an external magnetic field and MTO-SPION intraarterially with an external magnetic field (Magnetic Drug Targeting=MDT), respectively. The applied external magnetic field was focused to the tumor region. The concentration of the bound MTO was 10% of the regular systemic dose. After treatment the animals were sacrificed for biodistribution analysis of MTO in tissue or blood was taken for detection of MTO-load in leukocytes.

RESULTS

Magnetic Drug Targeting using MTO-SPION given intraarterially with reduced chemotherapeutic concentration revealed a very high concentration in the respective tumor region compared to conventional chemotherapy and led to complete tumor remission with only one application (Fig. 1). Furthermore MDT employing MTO-SPION significantly decreased the chemotherapeutic load in circulating leukocytes compared to systemic administration.



Tumor-size behavior after one course of MDT with MTO-SPION (10% MTO) in tumor bearing rabbit. Red circle indicates the tumor region at the left hind limb during the observation period. Imaging was done using a Flat-panel–CT, Artis zee floor (Siemens Healthcare, Forchheim, Germany).

CONCLUSION

Magnetic Drug Targeting is a promising therapeutic model for cancer therapy. In comparison to conventional chemotherapy complete tumor remission could be achieved with a reduced MTO-SPION application. Furthermore MDT spares the immune system in comparison with conventional chemotherapy.

ACKNOWLEDGEMENTS

The authors would like to thank the Else Kröner-Fresenius-Stiftung, Bad Homburg v.d. H., Germany, the German Research Foundation (AL 552/3-3), the German Federal Ministry for Education and Research (FKZ: 01EX1012B), the Bavarian State Ministry of Environment and Health (U8793-2012) and the Emerging Fields Initiative of the University Erlangen-Nuremberg for financial support.

LIGAND-TARGETED LIPOSOMAL ANTICANCER DRUGS FOR THE TREATMENT OF HAEMATOLOGICAL MALIGNANCIES

THERESA MARY ALLEN, Professor Emeritus, FRSC, Pharmacology and Oncology, University of Alberta, Edmonton; Co-founder and Strategic Advisor, Center for Drug Research and Development, Vancouver (CDN)

Applications of nanotechnology have become widespread. Some of the first products of nanotechnology were drug delivery systems; nanomedicines such as Ambisome® and Doxil®/Caelyx® appeared on the market in the 1990s. Since then, nanomedicines have proved to be versatile delivery systems that enable the in vivo delivery of a wide variety of compounds, including small molecule therapeutics, imaging agents, radiosensitizers, and most recently gene medicines such as siRNA. First generation nanomedicines increased the localization of therapeutic compounds to target sites with increased vascular permeability, such as regions of infection or inflammation, or solid tumors. This is called passive targeting, or the enhanced permeability and retention (EPR) effect.

Considerable research is now going into the development of second generation products that use ligands, such as antibodies or peptides, to mediate the selective intracellular delivery of therapeutics, in particular anticancer small molecule therapeutics. Much experimentation has gone into trying to understand what advantages, if any, ligand-targeted liposomes have over passively targeted (i.e., 'non'-targeted) liposomes, and what might be the appropriate clinical applications. To date, few ligand-targeted formulations are in clinical trials, and none have reached the clinic. Even in animal models, consistent increases in therapeutic outcomes for ligand-targeted vs passively targeted nanoparticles have been difficult to achieve. Both targeted and passively targeted liposomes are distributed to target cells via the same passive distribution mechanism. Hence, when passively targeted and ligand-targeted liposomes have similar circulation half-lives, ligand-mediated targeting does not increase the distribution of liposomes to target tissues compared to passively targeted liposomes. Therefore, improvements in survival, when seen, are not due to increased uptake of targeted liposomes by the diseased tissue, per se, but by increased receptor-mediated uptake of the nanoparticles and their associated/entrapped drug(s), by the target cells. One area where consistent increases in therapeutic outcomes have been seen for ligand-targeted vs passively targeted nanoparticles is in the treatment of animal models of haematological malignancies. This has been attributed to the easy access of the nanoparticles to the malignant cell population relative to the restricted access to the cell in solid tumors. Using combination targeting strategies, including increasing the 'apparent' receptor density on the target cells by targeting to two or more surface receptors, increases therapeutic outcomes even further.

When the application, and the delivery system, is carefully chosen, therapeutic benefit can derive from the used of ligand-targeting strategies. For the delivery of gene medicines such as siRNA, where no therapeutic activity occurs without the intracellular delivery of the therapeutic, ligand-targeted delivery can trigger receptor-mediated internalization, and the intracellular release, of the gene therapeutic. This is enabling the clinical development of these new types of therapeutics, which have proven extremely difficult to delivery by other means. The use of nanomedicines also facilitates the use of combination targeting strategies, which mimic conventional anticancer combination chemotherapy, resulting in improved therapeutic outcomes over monotherapy. We are now seeing the development of third generation multi-component nanoparticles that combine therapeutics with imaging and diagnostic functions (theranostic formulations). In developing these complex multi-component systems, it is important to be aware of the many challenges that impede their clinical development. As nanomedicines become increasingly complex, and incorporate more and more functions, the manufacturing costs, and the issues of reproducibility and quality control, increase considerably compared to those traditionally seen for small molecule therapeutics. Hence, to keep the cost/benefit ratios within reasonable limits, the additional therapeutic benefits that accrue to these systems need to be substantial. Improving our understanding of the relationships between the pharmacokinetics, biodistribution and physical parammeters of the nanocarriers can help in the rational design of improved systems having cost/benefit ratios within acceptable limits.

COMBINATORIAL DEVELOPMENT OF BIOMATERIALS AND SYNTHETIC SIRNA DELIVERY SYSTEMS

DANIEL G. ANDERSON

High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing biomaterials, and in particular siRNA delivery systems. Libraries of nanoparticles, degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to deliver siRNA, both in vitro and in vivo. A number of siRNA delivery formulations have been developed with in vivo efficacy, and show potential therapeutic application for the treatment of genetic disease, viral infection, and cancer.

A NOVEL HEPATITIS B TREATMENT WITH RNAi

CHRISTOPHER ANZALONE

Hepatitis B virus (HBV) is the world's most common serious liver infection, with an estimated 350 million patients worldwide that are chronically infected. HBV can lead to cirrhosis of the liver and is responsible for 80% of primary liver cancers globally. Current therapies for chronic HBV include reverse transcriptase inhibitors and interferon. These therapies either require life-long administration or have significant side effects and limited efficacy, and no current therapy is capable of consistently decreasing viral proteins, which is thought to be a necessary step in achieving a functional cure. We have developed an RNAi-based therapeutic against chronic HBV: ARC-520. This approach is unique because it enables knock down of viral RNAs, including the pre-genomic RNA from which the replicative intermediates are derived, thus reducing both the viral load and the viral proteins that result in disease and negatively impact the immune system's ability to eliminate the virus. ARC-520 has induced multi-log repression of viral RNA, proteins and viral DNA with long duration of effect in transient and transgenic mouse models of chronic HBV infection, without toxicity. Similarly, it has been demonstrated to be safe and effective at decreasing circulating viral DNA and proteins in a chimpanzee with chronic HBV. We expect to treat healthy volunteers in a phase 1 trial in Q3 2013 with ARC-520, followed by a Phase 2a in chronic HBV patients in the first half of 2014.

BIORESPONSIVE MATERIALS AND NANOMEDICINE – TRENDS, CHALLENGES AND BEYOND

LAJOS P. BALOGH
Editor-in-Chief, Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier); AA Nanomedicine and Nanotechnology, Boston, MA 01845

Nanomedicine and nanobiotechnology are two rapidly emerging areas closely related to and depending on modern materials science creating novel bioresponsive materials. Nanomedicine focuses on the patient and nanobiotechnology concentrates on new materials and medical technologies. Nanobiotechnology is developing better and better approaches and solutions to existing problems that have the potential to forever change how patients are diagnosed and treated in the next decade. However, all these changes are happening when both medical science and the pharmaceutical industry are undergoing revolutionary transformations. Apart from the great promises of nanomedicine, there are many general challenges for these paradigm-changing fields, especially in the area of scientific communication and commercialization. In order to produce affordable and not only revolutionary clinical applications, nanomedicine must be fueled by successful business. In this talk, the latest developments in nanomedicine and nanopharmaceutics will be summarized, including popular discussion topics, such as "personalized medicine" and "theranostics". Emerging research and market trends along with the introduction of a few real breakthroughs will also be illustrated.

BUPIGEL™: A SLOW RELEASE BUPIVACAINE LIPOSOME/HYDROGEL COMBINATION ACTING AS AN ULTRA-LONG ACTING LOCAL ANESTHETIC (ULALA) TO TREAT MODERATE-TO-SEVERE PAIN

YECHEZKEL BARENHOLZ[1] ,Rivka Cohen[1], Hiba Kanaan[1] and Gil Grant[2]

[1]Laboratory of Membrane and Liposome Research, Hebrew University – Hadassah Medical School, Jerusalem, Israel,
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There is a compelling need for an ultra-long acting local anesthetic (ULALA) to treat moderate-to-severe pain. Standard local anesthetics have a brief duration of analgesia, and their potential toxicity limits the dose and frequency with which they can be administered. Moderate-to severe pain is currently treated with systemic opioids, but opioids produce many untoward effects including respiratory depression, ileus, urinary retention, pruritus, nausea, vomiting and potential addiction. Thus, ultra-long acting local anesthetics, which are not associated with any of these side effects, are a very attractive option to treat moderate-to-severe pain.
Bupigel™ is an ultra-long acting local anesthetic, composed of a calcium alginate cross-linked hydrogel that entraps large multivesicular vesicles (LMVV) remotely loaded with a large quantity of the local anesthetic bupivacaine. Two Phase I human studies of LMVV remote loaded with the local anesthetic bupivacaine (Bupisome) in healthy volunteers demonstrated prolonged local analgesia, a pharmacokinetic release profile with low plasma levels consistent with slow release of bupivacaine from the LMVV depot, and good safety. These Phase I studies of Bupisome, coupled with Bupigel™ analgesia experiments in mice that showed that Bupigel is superior having longer analgesia in mice than Bupisome, suggest that a single administration of Bupigel™ is expecting to eliminate moderate-to-severe pain in humans for 72 hours or longer.

• Grant G.J., et al. 2004, Anesthesiology; 101: 133-7.
• Grant G.J., et al.2001, Pharmaceutical Research18: 336-343.
• Davidson, E., M., Anesthetic Pharmacology and Preclinical Pharmacology, 110 (4), 1018-1023, 2010.
• Cohen., R., J. Cont. Rel, 2012, 160, 346-352.

DOXIL – THE FIRST FDA-APPROVED NANO-DRUG: LESSONS LEARNED AND MECHANISM OF DRUG RELEASE IN TUMORS

YECHEZKEL BARENHOLZ[1] , Liza Silverman[1] Alexander V. Andriyanov[1], Erez Koren[1], Doron Friedman[1] and S. Nahum Goldberg[2]

[1]Department of Biochemistry, Institute for Medical Research Israel-Canada, Hebrew University-Hadassah Medical School, P.O.B. 12272, Jerusalem 91120, Israel, [2]Radiology Department, Hadassah Hebrew University Medical Center, Ein Karem, Jerusalem, Israel.

PURPOSE
To determine how drug tumor accumulation and therapeutic efficacy (in tumor bearing mice) are affected by the tumor micro-environment and changes there induced by internal (microenvironment composition) and external factors (such as radiofrequency) and by the type of PEGylated liposomal doxorubicin (PLD) used.

MATERIALS AND METHODS
We studied and compared physicochemical properties of two nano-drugs both are long-circulating PEGylated liposomes. One is thermo-sensitive (TS) liposomes with respect to drug release (PLDTS) which show a very fast burst almost complete release at above Tm (420C). The second formulation is Doxil which is not thermo-sensitive (PLD) which demonstrates a very slow drug release in plasma. These were correlated with the PK, the bio- distribution (BD) (of the two liposomal drugs) and their therapeutic efficacies when used with and without exposure to radiofrequency (RF). In order to better understand mechanism of action (MoA) of these nano-drugs we also studied effects of tumor microenvironment composition on doxorubicin release from the liposomes.

RESULTS
Tumor micro-environment plays a major role in drug release, drug accumulation in the tumor, and in the therapeutic efficacy. In addition PLD (non TS) combined with RF had the least tumor growth and the greatest end-point survival, better than PLDTS+RF ($p<0.005$) or all individual therapies ($p<0.001$). Although at 1hr post-treatment greatest intratumoral doxorubicin level was seen with PLDTS+RF ($p<0.05$), at 72hr the greatest doxorubicin fraction was seen in RF+PLD ($p<0.05$), in this group tumor also has the longest exposure to doxorubicin. The later may explain the superior efficacy of PLD + RT combination.

CONCLUSION
Optimizing therapeutic efficacy of nano-drugs based on liposomes requires a better understanding of the role of tumor microenvironment in the drug bio distribution and release profile at the tumor site. If drug release at is too fast as in the case of PLDTS, the benefis of changing the microenvironment on tumor drug localization and therapeutic efficacy may be smaller than for PLDs which lack temperature-dependent fast drug release but rather have slower drug release. Thus the much longer circulation time of drug loaded PLD than of PLDTS and the much higher tumor AUC may be beneficial in many therapeutic instances, especially in extra-hepatic tumors. Most important to explore is the idea that in addition to the EPR effect, the unique microenvironment of the tumor tissue serve as a second and highly important "Achilles' Heel" which can be utilized to improve liposomal nano-drugs anticancer therapeutic efficacy.

FUNCTIONAL RECOVERY OF PARKINSONISM BY CARBON NANOTUBE-MEDIATED SIRNA GENE SILENCING

KATIE BATES
MPhil/PhD student

Neurosurgical procedures are an effective and once again an important component of the therapeutic "toolbox" used to treat Parkinson's disease. However the core surgical techniques that are currently used to treat patients have inherent risks and drawbacks,

hence the enormous demand for new technologies to revolutionise the art of surgery in Parkinson's disease. Impressive advances have been made in the field of nanotechnology and its application in functional neurosurgery; including the invention of novel nanoelectrodes for improved deep brain stimulation (DBS) therapy, improved cell transplantation or regeneration using nanotechnologies as support platforms and more interestingly the genetic manipulation of the striatum or substantia nigra using localised application of nanovectors (both viral and non-viral) or other nanomaterials. This project aims to fuse both nanotechnology and gene therapy for therapeutic use in Parkinson's disease. More specifically, carbon nanotubes (CNT) are used as novel nanocarriers for localised gene transfer in vivo for the treatment of Parkinson's disease. CNT have great potential for biomedical and clinical applications, mainly due to their capacity to pierce and translocate cell membranes. Chemically functionalised CNT complexed with small interfering Ribonucleic acid (f-CNT:siRNA) constructs were stereotactically injected into the subthalamic nucleus (STN) of 6-hydroxydopamine (6-OHDA) induced hemiparkinsonian rats with the aim to silence Bcl-2 expression and suppress pathological neuronal activity in the STN by inducing localised apoptosis. The biological efficacy, specificity and functionality of f-CNT:siRNA constructs were investigated. Gene silencing was observed both at the mRNA and protein levels, in addition to partial functional recovery in hemiparkinsonian rats. Furthermore, f-CNT:siRNA constructs are well tolerated and afford long-term functional rehabilitation in hemiparkinsonian rats up to 15 weeks after administration.

PHARMACEUTICAL DEVELOPMENT - REQUIREMENTS TO GET FROM DRUG CANDIDATE SELECTION TO MARKET

OLAF BEHREND

NANO MILLING CASE STUDY

The bioavailability of many APIs is limited due to its poor solubility and slow dissolution kinetics. Nano milling is one technology amongst others, to potentially increase the in-vivo performance of such poorly soluble drugs. By comminution in a wet milling process, typically mean particle sizes in the range of several 100 nm can be obtained, thus increasing the specific surface area and, thereby, the dissolution rate to a multiple compared to the unmilled API quality. Potential applications can be found in formulation development for clinical use as well as for preclinical studies, e.g. for pharmacokinetic characterization or toxicological evaluation. Case study data will be presented, supportive of the expected performance improvement of nano sized APIs based on both in-vitro methods as well as in-vivo animal models.

CLINATEC: A TECHNOLOGY TRANSLATIONAL RESEARCH CENTER

FRANÇOIS BERGER, C Mestais, D Ratel, A Bouamrani, C Moro, E Labyt, G Charvet, N Aubert, F Lenouvel, F Sauter, T Bosc, A Astier, AL Benabid. Clinatec center- INSERM-UJF-CEA-CHU Grenoble

A major biomedical need is supporting therapeutic innovation in the field of neurology. Brain inaccessibility and functionality probably explain the major delay observed in molecular annotation and therapy for brain diseases. Deciphering the mechanisms of inaccessible brain functional areas and treating them with non-lesional strategies locally addressing neuronal network dysfunction is mandatory. Deep brain stimulation is a perfect example of implanted technology acting locally in a reversible and functional way. It paved the way for more elaborated devices benefiting from the miniaturization and multifunctionality provided by the exponential development of micro-nano-technologies and electronics.

CLINATEC is a technology translational biomedical center implanted inside the technology MINATEC campus. Its objective is to accelerate and make safer translation at the bedside of innovative micro-nano and electronics technologies. 5 connected platforms are available, including a biomedical system integration facility, a biocompatibility and translational biology platform, a preclinical platform, an imaging platform and at the end of the process a clinical unit. The clinical unit is devoted to clinical technology proof of concept trials. It is supported by a unique high technology surgery room and multimodal monitoring associating multimodal intra-operative imaging, electrophysiology, behavior analysis and biomolecular explorations. This unique situation should favor an early synergistic connection between physicians, technologists and biologists from the clean rooms to the bedside.
Several projects have been already implemented. The Brain computer interface program is developing the first integrated ECOG implant for Brain computer-interface. Explorer program develops innovative micro-nano-biomarker-interfaces technologies for the deciphering of inaccessible human brain areas. The NIR program is developing an infra-red optical stimulator for neuroprotection in Parkinson disease. Innovative pump and catheter are also developed for brain delivery of innovative therapies. Clinatec is also an hotel project open to academic and industrial research which could benefit from the platform.
CLINATEC also provides a hotel project open for both academic and industrial neuroscience community.

ANALYSIS IN NANOMEDICINE AND TARGETED DELIVERY – UNDERSTANDING THE IMAGES

GERD BINNIG

Automatically understanding images goes beyond conventional image analysis. Most types of medical or biological images contain very complex contents and conventional image analysis cannot deal with the associated combinatorial problems. Instead of solely applying sequentially formal analysis algorithms a stepwise context driven approach leads to intensive knowledge based processes that finally result in a kind of automated image understanding.
Context driven image analysis opens the door for many different applications, in particular in tissue diagnostics for targeted delivery. Rich quantification of those images, e.g., enable a new discipline one might call Tissue Phenomics. In many cases of histological sample preparations proteins, genes or other molecules are marked representing in a way spatially resolved molecular diagnostics. Nevertheless the main motivation for histology is the investigation of phenotypes . The fingerprints derived from the quantification of phenotypes, the "Phenoms", can be used for many tasks like computer aided diagnosis, developing new biomarkers or creating new insights by correlating them with clinical outcome data or Genomes and Proteomes.

COFM: IN-VIVO MONITORING OF DRUG TRANSPORT ACROSS THE INTACT BLOOD-BRAIN BARRIER

THOMAS BIRNGRUBER
Joanneum Research GmbH, HEALTH - Institute of Biomedicine and Health Sciences, Graz, Austria.

To study the efficiency of nano-carrier systems for drug transport across the blood-brain barrier (BBB), we need to be able to determine the concentration of substances in the brain interstitial fluid (ISF). Such substances can be measured by using the new technique, cerebral open-flow microperfusion (cOFM). cOFM - a continuous catheter based sampling technique that enables direct sampling of brain ISF without limitations due to molecular size, protein binding, charge or lipophilicity. cOFM is therefore well suited to nanoparticle sampling. Like all invasive catheter techniques, cOFM probe implantation causes trauma, destruction of capillaries and therefore disruption of the BBB. A healing period is thus required, and sampling takes place after the BBB has been reestablished.
cOFM features no membrane but brings the perfusate into direct contact with brain tissue. This offers the benefit of constant measurement over a long period with no membrane occlusion due to protein clotting or biofouling. A major problem in long-term im-

planted probe techniques is the formation of a glial scar that hinders substance exchange between the brain tissue and the probe, thereby diminishing measurement sensitivity. The glial scar not only functions as a diffusion barrier but can also alter metabolism and signaling in extracellular brain fluid. Thus, we performed a detailed investigation on histological changes surrounding the cOFM probe implantation site.

PRESENTED DATA WILL SHOW

- The permeability of the BBB during the healing process
- A method of monitoring the BBB permeability during sampling
- Glial scar formation surrounding the probe implantation site and its possible role as diffusion barrier
- A method of absolute substance quantification with cOFM.

DEVELOPMENT OF BIOACTIVE NANOSTRUCTURED 3D-SCAFFOLDS FOR REGENERATIVE MEDICINE

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The development of 3D multifunctional scaffolds for bone tissue engineering and regenerative medicine will be discussed, presenting recent progress and remaining challenges. After presenting a comprehensive summary of current research in the field of scaffolds for tissue engineering, recent results on the development and characterisation of nanostructured bioactive glass based scaffolds will be highlighted. Suitable and emerging technologies for fabrication of foam-like scaffolds will be discussed including a comparison of the simple but versatile foam replica technique and more advanced methods based on rapid prototyping, electrophoretic deposition and electropspinning techniques. Approaches to engineer the bioactive glass scaffold microstructure will be shown. For example, the partial crystallisation of bioactive glass scaffold struts leads to scaffolds with suitable mechanical integrity which are safe for handling without fracture. Moreover coating and infiltration of scaffolds by biodegradable polymers containing functionalised nanoscale inorganic particles of nanofibres increases the fracture resistance of scaffolds and induces the formation of nanostructured hydroxyapatite when immersed in simulated body fluid. The in-vitro characterisation of the scaffolds will be presented, discussing the effects of bioactive glass dissolution products on cell behaviour in relation to osteogenesis and angiogenesis. In-vivo investigations focusing on assessing the vascularisation potential of the new scaffolds will be discussed and the present challenges of this branch of tissue engineering, e.g. the development of vascularised bone, will be highlighted. The effect of nanoscale bioactive glass particles on angiogenesis in comparison with conventional or micrometer scale particles will be discussed. The fabrication of novel silicate scaffolds doped with trace elements (e.g. Sr, Co, Cu) and investigations on the effect of these elements on the scaffold biological performance will be presented to confirm the hypothesis that scaffolds with specific ion release capability ("bioinorganics" or therapeutic metallic ions) are attractive for bone tissue engineering. In addition, 3D nanostructuring strategies for bioactive glass (or glass-ceramic) scaffolds will be discussed, which are intended enhance cell attachment and proliferation. Areas of future research in the field of bioactive materials based on smart composite systems for engineering tissues requiring enhanced vascularisation will be addressed, including the emerging fields of tissue engineering therapeutics and regeneration of complex tissue interfaces where the incorporation of functional nanoparticles is suitable to improve the scaffold performance.

HOW TO OFFER A NANOPRODUCT TO PHARMACEUTICAL DEVELOPMENT

GEORG BOECK

Big pharmaceutical companies have specialized business development and licensing teams to seek external partnerships and collaborations, at all stages of research, development and commercialisation, in order to strengthen global franchises in their key therapeutic areas. It is important for potential partners to understand the evaluation process and the essential information required before starting a license offer.
This presentation will give you insight into the evaluation process and the disciplines involved from a big Pharma perspective. The most important critical quality attributes of nanoproducts expected to start a pharmaceutical development will be addressed.

LANGERHANS ISLETS ENCAPSULATION IN MICROFLUIDIC CARTRIDGE FOR DIABETES CELL THERAPY

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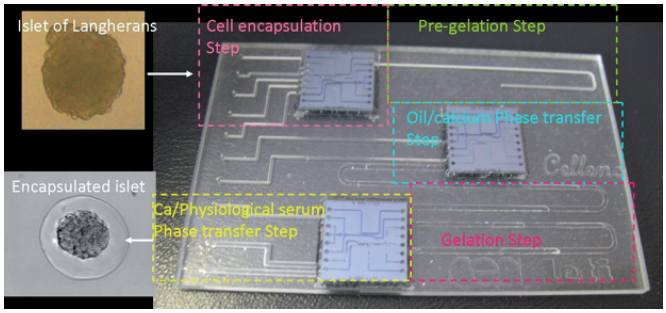
INTRODUCTION

Implantation of Langerhans islets is a promising treatment for diabetes type 1. In order to limit or suppress immunosuppressive treatment one alternative is to encapsulate Langerhans islets in biocompatible alginate shell. Finding an adapted process for cell encapsulation is a challenge. The process must product spherical and monodisperse capsules in a limited time with a good reproducibility. Up to now conventional systems are mainly manual which induces batch disparities and do not provide repeatable clinical results and do not provide as not in continuous flow a fully identical protocol to the islets. Recently, microfluidic devices have been proposed in order to produce spherical and monodisperse droplets [1] [2]. The potential of microfluidic device is not only to produce more reproducible droplet but also to integrate the gelation step in order to provide uniform batch of alginate capsules The novel device described here (Pict 1) is the first step towards a full automated and reproducible process for cell encapsulation with an integration of all required steps of the encapsulation protocol.



Pict 1: *full integrated continuous flow encapsulation prototype*
In this paper, we will focus on one of the main constraints for cell encapsulation application which is the sphericity of the capsule. Critical steps to preserve the droplet sphericity are pointed out. Finally, an optimization of the pre gelation process will be assessed to guarantee the sphericity of the retrieved capsules after phase transfer phase. In order to study these points we used the restricted part of the cartridge illustrated below (fig 1)

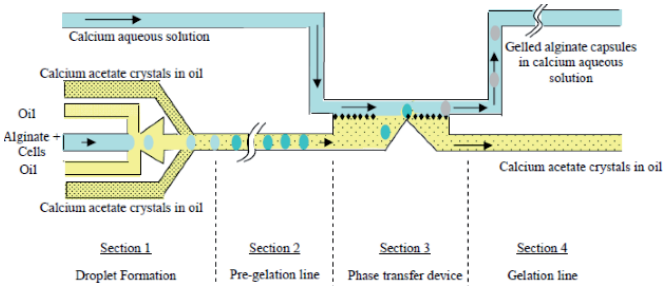


Figure 1: *Schematic presentation of the study cartridge.*

Section 1 *is composed of a typical MFFD device, which produces an alginate monodisperse emulsion. Two channels are added to inject calcium acetate crystals suspended in oil. This Acetate calcium ad on is used to initiate the pregelation of the droplet before crossing the oil/aqueous phase interface(section 3)*

Section 2 *define the full pregelation stage in continuous flow with a duration fixed by length of the channel*
Section 3 *depicts the phase transfer device in which the oil and the aqueous calcium flows formed an interface crossed by pre-gelled alginate droplets [5].*
Section 4 *(as section 2) use a length determined channel to address the complete capsule gelation*

MATERIAL AND METHOD

Microfluidic cartridge fabrication

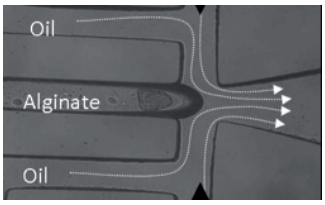
The microfluidic cartridge is an assembly of three silicon chips sealed to a microfluidic plastic cartridge. The three silicon microfluidic chips were fabricated using standard lithography techniques. Substrate and walls were made of silicon and a glass cover seals the flow channels. The channel depth and width were 200µm. Walls were silanized to achieve hydrophobic coating (water contact angle: 108-110°). Production of aqueous alginate droplets without the use of surfactant was then possible. These three silicon chips ensure the three key microfluidic functions which are flow focusing to create alginate droplet, and two phase transfer devices [3]. The plastic microfluidic cartridge is an assembly of two plastic cartridges sealed with conventional technology. Microfluidic channels are milled in one of them, the other one working as cover.

Encapsulation process

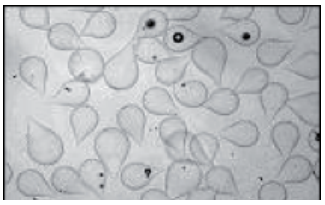
Super refined soybean oil (CRODA EP-NP-LQ-(MH)), was used as continuous phase. The encapsulation polymer was a 3% (w/w) Pronova SLG 100 alginate from Novamatrix (271108/3). Alginate was prepared in an aqueous solution of 150 mM NaCl, 10mM Hepes. The pH of the alginate solution was adjusted to 7.4. Oil and alginate solutions were motioned by regulated pressures delivered by micropumps (Fluigent® micro-pumps MFCS-8C).. Pre gelation time was fixed to 30 seconds and gelation time to 1 minute. Gelation solution was composed of 100mM CaCl2, 80mM NaCl, 10mM Hepes and the physiological serum solution of150mM NaCl, 2mM CaCl2 and 10mM Hepes, pH is adjusted to 7.4.

RESULTS AND DISCUSSION

Capsule production without pregelation step



Pict 3: MFFD device part



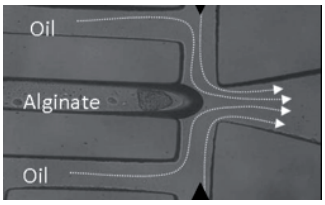
Pict 4: tear shape capsules

The average capsule diameter is 175µm (CV<5%) with a characteristic form of tears (pict 4). This form is characteristic to the external gelation [4] and also in our case with non pregelled capsules going through the phase transfer module.

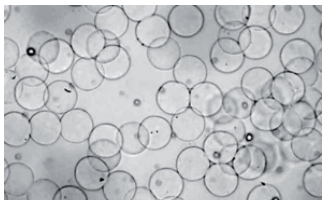
Enhancement of the sphericity shape with pre gelation step

Based on the work of Zhang et al. [5], we mixed in this optimized approach, two additional channel for pregelation on the MFFD device part itself followed by a pregelation channel before going in the phase transfer (pict 4- 5).

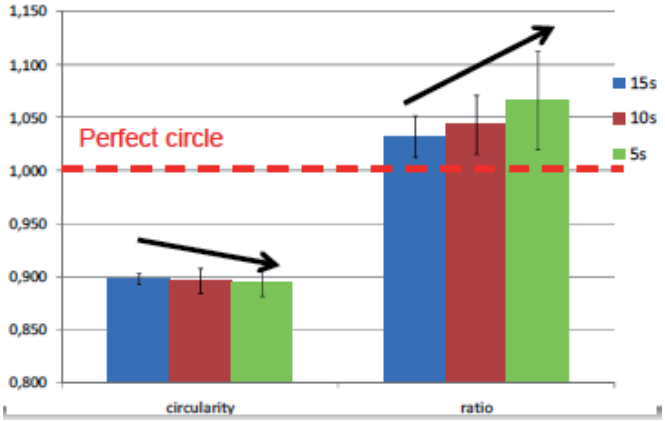
Oil + Calcium



Pict 4: MFFD with 2 additional channels



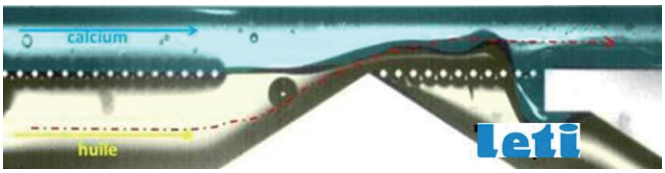
Pict 5: optimized capsules



Pict 6: Capsule Sphericity parameters function of pregelation duration

Phase transfer

This patented module [6] is generic and used to transfer capsule from Oil to Ca+ phase for final gelation and also to transfer gelled capsules from Ca+ to final physiological fluid.
Phase transfer's geometry, pillar size and pitch are related to the channel width, capsule size and used pressure.



Pict 7: Phase transfer device

CONCLUSION

We have developed a new integrated microfluidic device coupling a flow focusing device for generating alginate droplets, to a phase transfer device to extract capsules from the oil phase into the calcium aqueous phase. The phase transfer ratio is equal to 100%. Alginate capsules are spherical and the whole process of encapsulation is performed at physiological pH providing high cell viability. This device works for 2 hours without blockage with a frequency from 60 capsules per minute up to 200 capsules per minute depending on the inlet pressure. The whole fabrication procedure of the device has been performed using industrial process, allowing future large scale-fabrication and highly reproducible devices.
Currently, this system has a lower rate than classical encapsulation macrosystems but it offers new opportunities for procedure automation and production of standardized batches of encapsulated cells.

REFERENCES

[1] S.L. Anna, N. Bontoux and H.A. Stone, Applied physics letters, 2003, 82, 364.
[2]F.E.A. Köster, S.H. Duan, J.J Agresti, A. Wintner, C. Schmitz, A.C. Rowat, C.A. Merten, D. Pisgnano, A.D. Griggiths and D.A. Weitz, Lab on a chip, 2008, 8, 1110.
[3] P. Dalle, S. Morales, J. Berthier, P-Y. Benhamou, A. Bellemin Comte, F. Boizot, G. Costa, F. Rivera, Nanotech 2012, (2),298-301.
[4] L. Capretto, S.Mazzitelli, C. Balestra, A. Tosi and C. Nastruzzi, Lab on a Chip,2008, 8, 617-621.
[5] H. Zhang, E. Tumarkin, R.M.A. Sullan, G.C. Walker and E. Kumacheva, Macromolecular Rapid Communications, 2007, 28,527-537.
[6] J. Berthier, S. Le Vot and F. Rivera, Patent WO2010146261.

IN VITRO MODELS SHOWING LIMITED ORAL UPTAKE OF NANOPARTICLES

HANS BOUWMEESTER, Agata Walczak, Evelien Kramer, Peter Hendriksen, Ad Peijnenburg, Ruud Peters, Hans Marvin, Meike van der Zande.

Nanoparticles (NPs) exist in great diversity, which over the past decade have found numerous applications. Fields of application include incorporation of nanoparticles in consumer products, like food (supplements) and medicinal products. Especially nanomedi-

cine is a rapidly growing area of interest. Current research mainly focuses on intravenous administration, while oral delivery is actually the preferred route of administration. There the challenge is to have maximal uptake of intact NPs that survive the harsh conditions of the human digestive tract and to control the release of the drug at the desired target.
In our recent studies we developed in vitro models for the human digestive tract (in vitro digestion models and in vitro translocation models) and use model nanoparticles to elucidate the relation between physicochemical properties of nanoparticles and their biological effect.

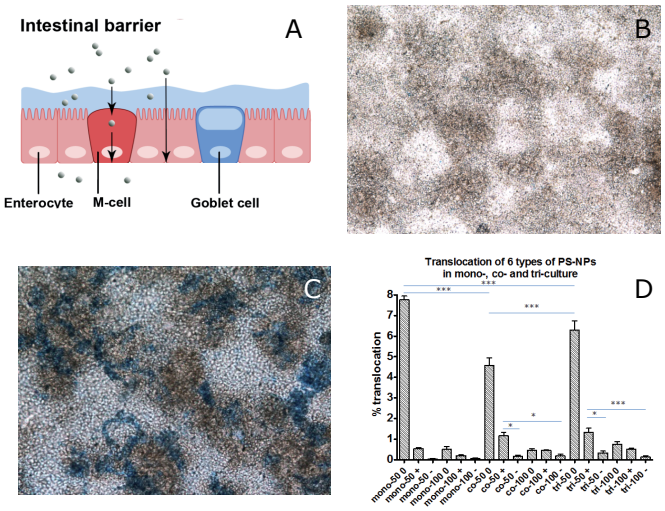


Figure 1. *Schematic illustration of a triple cell culture in vitro model of the gastrointestinal epithelium consisting of enterocytes (Caco-2), Goblet cells (HT29-MTX) and M-cells (A). Alcian blue mucus staining showing the absence of mucus on Caco-2 cells (B) and the presence of mucus on the triple cell culture grown on 3 µm pore sized transwell membranes (C). Translocation of 50 and 100 nm polystyrene nanoparticles with a neutral, positive or negative surface charge through a mono- (Caco-2), co-(Caco-2/HT29-MTX) and tri-culture model (D).*

Here, we present data from studies where we exposed different in vitro models of the gastrointestinal epithelium to 50 and 100 nm polystyrene nanoparticles with either a neutral, positive or negative surface charge. We compared the barrier properties of a simple and routinely used Caco-2 monoculture model with a more complex mucus secreting co-culture model of Caco-2 and HT29-MTX cells and a highly complex triple cell culture model that includes M-cells next to Caco-2 and HT29-MTX cells (Figure 1). Caco-2 cells were seeded in a density of 50.000 cells/cm2 with or without HT29-MTX cells in a ratio of 3:1 on membranes with a 3µm pore size, and were maintained for 21 days. For the triple cell culture model we exposed the Caco-2 cells to Raji-cells for day 16 to day 21, which triggers the differentiation of Caco-2 cells into M-cells.
Commercially available polystyrene nanoparticles were characterized by determination of the hydrodynamic size (DLS) and the zeta potential in water and cell culture medium (DMEM) at the beginning (t=0h) and end of the exposure (t=24h). While the zeta potential in water was different for the differently charged nanoparticles, in DMEM all nanoparticles had a zeta potential around 11 mV.
For translocation studies the integrity of the monolayer is pivotal which was ensured byObserved minimal translocation of marker compounds Lucifer Yellow (<5%) and both low (< 1%) and high molecular weight dextrans (<1%). The functionality of the monolayer was also confirmed, as translocation of the marker compounds increased up to 15-20% for Lucifer Yellow and up to ~5% for both the low and high molecular weight dextrans after opening the tight junctions with EGTA. Next we assessed if the membranes with 3µm pores itself pose a barrier for the used polystyrene nanoparticles. After 24 h of exposure 20 to 40% of the polystyrene nanoparticles were translocated.
In the main experiment we exposed the tree types of in vitro monolayers for 24 h to 250 µg/ml of 50 and 100 nm neutrally, positive or negatively charged polystyrene nanoparticles. During the exposures the monolayer integrity was monitored by measuring TEER values, which were shown to be unaffected. The highest translocation was observed for the neutrally charged 50 nm polystyrene nanoparticles in the Caco-2 monoculture model (8%). The presence of mucus on top of the monolayer in the co-culture model significantly reduced the translocation (4.5%), while the additionally incorporated M-cells in the triple culture model increased the translocation to 6.5%. Particle size had a pronounced effect, since the 100 nm polystyrene nanoparticles had a maximal translocation of 1% or lower. Next to size, surface charge dramatically reduced the translocation in all model variants. In the co and triple culture models translocation of the negatively charged 50 nm polystyrene nanoparticles was significantly lower (>0.5%) than the 50 nm nanoparticles with a positive charge (1- 1.5%).
We compared the barrier properties of a simple and routinely used Caco-2 monoculture model with a more complex mucus secreting co-culture model of Caco-2 and HT29-MTX cells and a highly complex triple cell culture model that includes M-cells next to Caco-2 and HT29-MTX cells. For the gastrointestinal translocation, clearly both size and surface charge are important characteristics of the nanoparticles. Influence of surface charge on translocation was striking, since we observed no differences in the zeta potential (or surface charge) of the nanoparticles in cell culture medium. Further studies to explain these findings are on-going. An integrated in vitro digestion model with the described in vitro translocation model would further increase the physiological relevance of the model system. In our view this model can elegantly be used to elucidate the relation between physicochemical properties of nanoparticles and their biological effect, potentially leading to design of new nanomedicine.

HAPTEN-BINDING BISPECIFIC ANTIBODIES FOR TARGETED PAYLOAD DELIVERY

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Bispecific antibodies (bsAb) that combine binding modules for cell surface targets with modules that bind haptens such as digoxigenin (Dig) can be applied for targeted payload delivery. Cell surface targeting moieties of these bsAbs are IgG-derived entities that bind to tumor antigens such as Her2, IGF1-R, CD22 or LeY. Hapten-binding moieties were generated from an antibody that binds Dig, and subsequently fused to the cell surface binding entities via flexible connector peptides. These molecules can be expressed in mammalian cells and purified in a similar manner as unmodified IgGs. They are stable with low propensity to aggregate, and retain binding specificity and affinity towards cell surface antigens as well as to Dig. Payloads can be generated which retain full functionality, and they can be complexed to bsAb in a defined stoichiometric ratio. Such payloads include small compounds (fluorescent Dig-Cy5 and Dig-Dox), as well as proteins (e.g. Dig-GFP), nucleic acids (siRNA), and nanoparticles. Binding analyses, confocal microscopy, imaging experiments, and functional (mRNA knockdown) assays proved that complexed payloads become specifically targeted to cells that express the recognized antigen in vitro as well as in xenograft models in vivo.

NEGLECTED DISEASES: NEW DRUGS AND DIAGNOSTICS THROUGH PUBLIC-PRIVATE-PARTNERSHIPS

RETO BRUN

Neglected diseases (NDs) represent a health burden to a significant part of our world's population. Diseases caused by viruses, bacteria, protozoans and helminths kill millions of people each year and are responsible for vast morbidity and disability. The existing diagnostic tools and medications are inadequate for most of these diseases, especially drugs which lack efficacy, are toxic or require long and complicated application. For NDs Public-Private-Partner-

ships i.e. the Foundation for Innovative New Diagnostics (FIND), the Medicines for Malaria Venture (MMV) or the Drugs for Neglected Diseases initiative (DNDi) closed the R&D gap and took over the role the pharmaceutical industry had before. The main goal is to get new products that are safe, effective and affordable for patients in resource poor countries. With regard to sustainability affordable drugs are more important than drug donations. Nanotechnology is so far not in use for NDs but has great potential to target drugs to infected cells or organs.

BUSINESS DEVELOPMENT STRATEGY

PETER BURCKHARDT

Like with every strategy it is not sufficient to just fill in a paper. The strategy is rather the endpoint of a intense discussion with all involved persons. With regard of the business development strategy there are a few points to consider carefully. The business development has to directions: one is to ultimately sell or license out parts or all of the company. The other one is scouting for projects to complement the own business line. To develop a business development strategy for the first aspect (business development with an outward focus), one has to agree with the end-point of the start-up company. This can be a trade sale, a licensing deal or a the independent company after an IPO. It is of utmost importance that all shareholders – founders and investors – share the vision where to go. The next decision point is to find out at which milestones reached an exit makes sense. All these steps have to be defined before they occur. However, like everything in the business plan it has to be adapted depending on the progress.

The second part of a business development strategy is to identify gaps in the portfolio and subsequently to decide whether there is a need to complement the product range, the patent portfolio or any other part of the company by licensing in or buying the missing parts. This is clearly the more difficult part as the requested funding for such operations often lack.

Another important part of the business development strategy is the funding of the company. Both strategies, the business development strategy and the financing strategy are strongly interwoven. When to get how much money to fund the company without giving away the shares too cheap is key. Therefore the value increasing steps have to be identified carefully.

Albeit the product development path and bringing together the right team as well as ensuring the financial resources are the most important cornerstones for a success, the business development has to be envisaged from the very first moment to ensure the ght moves in ther optimal time.

MEDICINE IN THE INFORMATION AND KNOWLEDGE SOCIETY

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The intervention analyzes the impact that digital communication and information technology (CIT) can have on medicine both as a science, and as a practice. The technological changes that lead to what I call 'Medicine 2.0' occur on several levels, such as: information overload, the physician-patient relationship, the self-perception of both physicians and patients, as well as the concept of the human body and what is understood as sickness and as health.

Just as industrial society once produced, and still produces, a series of pathological phenomena, the consequences of which we are still experiencing with some lag as social, economic, energy, and climate crisis, so does the so-called information and knowledge society – and this is the thesis that these notes lead to, the so-called information and knowledge society gives rise to a pathology that I believe to be far from analyzed in a critical and systematic fashion on several levels, one of which is medicine.

It is a complex subject not only where it concerns the impact of CIT in medical research, but also in relationship to the changes in interaction between physician and patient. This is also true regarding both the terminology and the different ways in which knowledge, medications, and healing or prevention are exchanged and accessed – by patients and doctors – and their respective public health institutions, as well as by the authorities that shape the conversation on social justice within and amongst different societies in a digitally globalized world. We can expect that the social justice aspect of medicine in the information and knowledge society will become intensified both within affluent and technologically advanced communities, as well as between them and other nations or regions of the world.

It is clear that a systematic study of the pathology(ies) of the information society(ies) cannot be limited to diagnosing the cause of certain phenomena such as the increasingly acute stress brought on by various forms of information overload as well as by phenomena of the digital world both in daily life and in the workplace, which leads to different types of organic and psychological disturbances, as well as to a crisis not only for companies, but in daily social interactions as well, including the education system.

Digital technology has been revolutionizing medical science for several years now both in the fields of diagnosis, and of healing methods. The tendency that we can foresee in relationship to nanomedicine, is towards a personalized medicine in which the physician-patient relationship leans steadily more towards the patient's side. He/she then becomes an agent who is vested with an autonomy that can paradoxically result more in a problem than in an improvement, given that frequently, their capacity to interpret the data related to their case correctly is very limited. Clearly, this development within medicine of the physician-patient relationship can also give rise to control systems based on CIT, which bring with them, paradoxically, the (potential) loss of the patient's autonomy, as well as an information overload when the data are not sufficiently clear, especially when they have possible consequences for the patient/agent.

With the purpose of defining a pathology of the information society, an anthropological framework is proposed based on concepts developed by Swiss physician and psychiatrist, Medard Boss, illustrating with a few examples a systematic analysis of said pathology.

The intervention concludes by proposing guidelines for an ethical outline as a reference for preventive and therapeutic care in the information and knowledge society.

USING NANOPARTICLE TRACKING ANALYSIS (NTA) FOR ACCURATE AND COMPLETE NANOSUSPENSION CHARACTERISATION

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A new sizing and characterization technique, Nanoparticle Tracking and Analysis (NTA) is described which allows nanoparticles in a suspension to be individually but simultaneously detected and analysed in real time using a laser-based microscope system. NTA has been commercially developed over the past six years and now, with over 600 systems installed, is becoming a key characterisation technique in many fields of nanoparticle characterisation particularly in the application of nanomedicines, nanopharmaceuticals and exosomes research and development. Here we discuss the technique, its application to these fields and how recent developments are further enabling advancements in these fields.

In this method a laser beam passes through a suspension at a low angle. The particles scatter light which is collected onto a CCD (or sCMOS) camera by a microscope-type configuration (Fig 1a). Particles between 10-2000nm are tracked individually (Fig 1b) and their diffusion coefficient, and therefore size, calculated directly from their speed (Fig 1c).

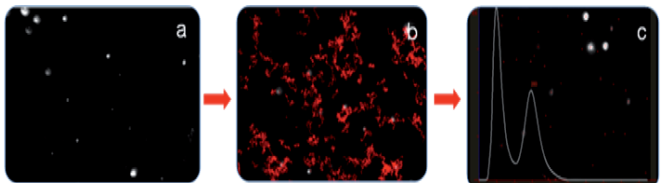

***Figure 1**: a) Scattered light from particles collected on CCD, b) particles tracked and c) size distribution calculated.*

This technique provides significant advantages for sizing over traditional light scatter techniques (e.g. DLS) as the individual tracking of particles results in a greater ability to cope with polydispersed suspensions providing higher resolution.
The technique is also capable of measuring particles in a range of media (not requiring a particular media, or being restricted to pure water). This is relevant across all biological applications, as it is well known that colloidal properties are strongly influenced by their environment.
In addition to its simplistic but powerful sizing approach the technique generates directly a measure of absolute particle concentration, it can be integrated with fluorescence filters to allow fluorescently labeled/loaded particles to be selectively analysed, the application of an electric field across the sample allows the measurement of zeta potential, and the measure of light scatter gives a relative indication of sample material density (or refractive index). Crucially these measurements are not independent but instead give a particle's individual fingerprint with the capability to measure the size, intensity, fluorescence and zeta potential of the same particle. An example of this is shown below (Fig 2) where not only is a tri-modal distribution evident, but it is discernible from the measurement that the 30 and 60nm material (gold, in this case) is of an higher refractive index than the 100nm material (polystyrene here).

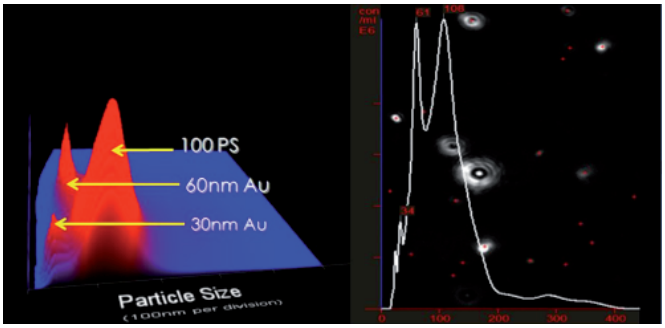

***Figure 2**: Trimodal measurement of a mixture of 30 and 60nm gold and 100nm latex polystyrene particles.*

In the field of pharmaceuticals, a crucial question under inspection is that of protein aggregation and its measurement thereof [1]. In this field NTA has been identified as a technique for characterizing this potentially over previously available techniques [2].
This technique has variously been applied to drug delivery particles such as liposomes, viral vaccines, bacteriophage, VLPs and controlled release drugs. In viral vaccines, for example, where the standard technique for titering a sample (to measure the concentration of virus) takes several days and could not take into account aggregation, the NTA technique is identified as a rapid and accurate alternative [3]. As the technique can also be integrated with fluorescence filters this gives the potential for fluorescently labeled/loaded particles to be selectively analysed. This can be of particular import where a suspension is not purified.
The technique is also being extensively used for characterisation of nanovesicular bodies (also called exosomes) which are shed from cells for amongst other things cell signalling. These bodies are typically in the 50-130nm size range within which NTA is the ideal technique for quantifying these bodies [4]. In this case using NTA in conjunction with fluorescently labelled antibodies, it then becomes possible to speciate the exosomes, which, it is generally believed could lead to significant diagnostic applications.
The technique, several novel developments and their application to the above fields will be described, explaining the importance of obtaining a complete particle characterisation, and in a relevant media. The technique will be compared and contrasted to other techniques. Several examples exploiting both light scattering and fluorescent labelling of samples will be shown.

REFERENCES

[1] Carpenter, J. F., Randolph, T. W., Jiskoot, W., Crommelin, D. J., Middaugh, C. R. and Winter, G. (2010), Journal of Pharmaceutical Sciences, 99: 2200–2208.
[2] Filipe V, Hawe A, Jiskoot W (2010) Pharmaceutical Research, Volume 27, Number 5, 796-810
[3] Anderson, B., et al. (2011), Bacteriophage, Volume 1, Issue 2 March/April 2011
[4] Gardiner, C., Ferreira, Y. J., Dragovic, R. A., Redman, C.W.G. and Sargent, I. L. (2013), Journal of Extracellular Vesicles 2013, 2: 19671

"NANOTECHNOLOGY APPLICATION TO A NOVEL CELL BASED PERSONALIZED IN VITRO MODEL OF NEURODEGENERATIVE DISEASES"

DANIELA CESSELLI

Niemann Pick type C (NPC) disease is an autosomal recessive neurodegenerative lysosomal storage disorder, caused by the abnormal function of NPC1 or NPC2 protein. Both proteins are involved in the intracellular trafficking of cholesterol and other lipids. The deficiency of either of them leads to the accumulation of the endocytosed unesterified cholesterol, gangliosides and other glycosphingolipids (GSLs) within the lysosomes [1].
Clinically, NPC disease presents a highly variable phenotype, with a time of presentation ranging from fetal to adult age. Despite the recent progress in characterizing the biochemical and genetic defects in NPC disease, the underlying patho-physiological mechanisms of this disorder are not clear and the currently available therapeutic interventions are limited. In particular, the study of the molecular pathways linking the lipid accumulation to the neurodegeneration has been hindered by the limited availability of neuronal models of the disease. Indeed, the possibility to isolate stem cells from accessible cell sources and to commit them to a neuronal lineage could be of great utility in dissecting the molecular pathway leading to the neurodegeneration in NPC patients.
In order to test if highly plastic stem cells can be obtained from skin biopsies, we applied a well established method that allowed us to expand, from different adult human tissues (heart, liver and bone marrow), a population of cells named Multipotent Adult Stem Cells, or MASC, characterized by a high differentiation potential that includes neuroectoderm derivatives[2, 3].
Cell cultures were successfully grown from skin biopsies of five NPC patients and two healthy donors. As expected, only cultured cells obtained from patients were positive for Filipin staining of free cholesterol, while all cell lines were immunophenotypically similar to MASC and expressed the pluripotent stem cells markers OCT-4. Therefore, to verify if these cell lines share with MASC the ability to differentiate toward a neuronal fate and in order to develop an in vitro model that could be suitable for dissecting the molecular pathway leading to neurodegeneration in each patient, we applied a differentiation protocol able to drive MASCs towards a neural fate (Beltrami et al., 2007). A multistep method was adopted and differentiation was monitored both by evaluating morphological parameters and revealing the presence of neural markers such as nestin, MAP2, O4 and GFAP. After two weeks of neural induction, a large fraction of cells (≈50%) acquired a neuron like morphology and stained positive for neuronal markers such as nestin and MAP2, suggesting that cells obtained from skin biopsies retained the ability to differentiate toward a neural phenotype. No GFAP, O4 and OCT4 positive cells were detected at the end of differentiation. By using a specific Image analysis software (Neurite outgrowth image analysis, BD pathway 855 system), we measured parameters linked to neurite outgrowth like neurite maximum length and neurite root count. Performing this analysis we found that, once differentiated, cells obtained from patients developed significantly more abundant and longer neurites than the healthy counterparts. Important-

ly, distortion of neuron shape, ectopic dendrites and meganeurite formation are the main features described in NPC brain cells, thus suggesting that the in vitro differentiation system is, at least in part, able the recapitulate the aberrancies present in vivo.
These results indicate that it is possible to isolate cells with stem cell properties from skin biopsies of NPC-patients and that these cultures retain the ability to differentiate toward a neuronal lineage. The in vitro model we developed will be useful to evaluate the impact of different mutations on the pathological phenotype and might represent a powerful tool to perform drug screening on cells obtained from NPC patients presenting different genotypes.

1. Patterson M, Vanier MT, Suzuki K, et al. Niemann Pick disease type C: a lipid trafficking disorder. The metabolic and molecular basis of inherited disease. Mc Graw-Hill. 2001:611-634.
2. Beltrami A.P, Cesselli D., Bergamin N., et al. Multipotent calls can be generated in vitro from several adult human organs (heart, liver and bone marrow). Blood. 2007;110:3438-3446.
3. Cesselli D, Beltrami AP, Rigo S, et al. Multipotent Progenitor Cells are present in human peripheral blood. Circulation Research. 2009;23.

TETRAVALENT DENGUE NANOPARTICLE STIMULATES ANTIBODY PRODUCTION IN VIVO

LUIZ FELIPE LEOMIL COELHO

Dengue is a major public health problem worldwide, especially in the tropical and subtropical regions of the world. Infection with a single Dengue virus (DENV) serotype causes a mild, self-limiting febrile illness called dengue fever. However, a subset of patients experiencing secondary infection with a different serotype progresses to the severe form of the disease, dengue hemorrhagic fever/dengue shock syndrome. Currently, there are no licensed vaccines or antiviral drugs to prevent or treat dengue infections. Biodegradable nanoparticles coated with proteins represent a promising method for in vivo delivery of vaccines. Here, we used a murine model to evaluate the IgG production after administration of inactivated DENV corresponding to all four serotypes adsorbed to bovine serum albumin nanoparticles. This formulation induced a production of anti-DENV IgG antibodies. However, plaque reduction neutralization assays with the four DENV serotypes revealed that these antibodies have no neutralizing activity in the dilutions tested. Our results show that while the nanoparticle system induces humoral responses against DENV, further investigation with different DENV antigens will be required to improve immunogenicity, epitope specificity, and functional activity to make this platform a viable option for DENV vaccines.

SUCCESSFUL PATENT STRATEGIES FOR NANOMEDICAL RESEARCH

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In the highly globalised and competitive environment of nanomedicine research, a successful patent strategy must achieve the nearly impossible: for a highly complex technology or product, to obtain viable protection in major markets worldwide and establish freedom to operate, but of course with limited in-house patent know-how and restricted resources. Without a patent strategy adapted to your specific business needs, it is likely that decisions are made which will later prove disadvantageous, and that necessary actions will be omitted. It is crucial to tailor the patent strategy to support realistic, predefined business objectives, rather than assuming practices that may be suitable for innovators with completely different business models, such as multinational pharmaceutical companies or university hospitals.
An essential component of the corporate patent strategy is a patent portfolio strategy that is reasonably cost-effective, but sufficiently global in scope, and avoids the pitfalls of patent practices based on national law. For example, the common practice in US academia to disclose fresh data from on-going projects in prestigious scientific meetings and subsequently filing a patent application in the grace period provided for inventors by US patent law simply does not work in the rest of the world, and may actually destroy much of the commercial value of an otherwise great innovation. The portfolio strategy must further take into account the requirements of future stakeholders, such as investors and licensees, and anticipate the scenarios of various types of due diligence processes.
Even more underestimated is the second essential module of any sustainable patent strategy, i.e. that which determines how a company handles its freedom to operate. Since so many innovators simply underestimate how many others work on similar concepts and ideas as they do, they also tend to spend too little effort on investigating and establishing freedom to operate for their core technology and product applications. This is a major strategic error, as patent obstacles to freedom to operate are often relative, rather than absolute. Eagerness to learn about the patent landscape and potentially blocking patents held by third parties means early awareness of business risks and allows the initiation of risk mitigation measures, such as the exploration of viable circumvention options. In contrast, to develop technologies or products without continuous awareness of patent risks is like sailing in the fog with the radar switched off.
A common error is to postpone essential activities to a later stage when funds are expected to be more readily available, like after the first sale of an option to a licence or co-development agreement. However, the problem is that your future partners will simply assume risk wherever risk was not properly analysed and quantified, e.g. expect that additional licences from third parties must be obtained in order to commercialise your product for which you failed to determine freedom to operate, and calculate with a high probability that your patents will not hold. In result, the value of your innovation will be substantially discounted. Even worse, you may not even be able to close a deal which would have been possible if the patent homework had been done properly.
It is therefore of vital importance to link up early with a patent counsel who is familiar with your type of business and with the type of technology that it is based on. Allow your counsel to become familiar with your business objectives and ask for a patent strategy that is tailored to your needs. The result may not be perfect - but much better than having no patent strategy.

MARQIBO, A LIPID NANOPARTICLE FORMULATION OF VINCRISTINE FOR TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Marqibo, a liposomal nanoparticle (LNP) formulation of the anticancer drug vincristine, was approved by the US FDA on August 9, 2012 for treatment of acute lymphoblastic leukemia (ALL) at second or greater relapse. This is the ninth LNP drug that has received regulatory approval. Marqibo relies on the extrusion technique for making liposomes (Hope at al., 1985) and the pH gradient (ΔpH) loading technique for loading drugs that are weak bases into liposomes (Mayer at al., 1986). The extrusion technique allows the rapid production of liposomal nanoparticles in the size range of 100 nm diameter and the ΔpH loading technique allows the efficient (effectively 100%) encapsulation of drugs that are weak bases, which corresponds to more than 50% of all drugs known. The ΔpH and extrusion technology was first applied to the anticancer drug doxorubicin, resulting in Myocet, which was approved in Europe and Canada for the treatment of metastatic breast cancer in 2000.
Vincristine was chosen for LNP formulation for three reasons. First, it is one of the most commonly used anticancer drugs (over two million patients receive vincristine every year), second, vincristine is a cell-cycle specific drug and the extended payout of vincristine from liposomal systems could therefore potentially result in enhanced potency, third a dose limiting side effect of vincristine, neurotoxic-

ity, could possibly be mitigated through use of a liposomal formulation. A series of papers published in the early 90's (Mayer et al., 1990; Mayer et al., 1993; Boman et al., 1993) established that liposomal formulations of vincristine could indeed result in enhanced potency in animal models of cancer, however the enhanced potency depended strongly on the rate of release of vincristine from the liposomes. Formulations with slow rates of release (half-times for release of 12 hours or longer) exhibited markedly improved anticancer properties. LNP formulations of vincristine with optimized release properties were developed (Webb et al., 1995 and Phase I clinical trials established safety in humans (Gelmon et al., 1999). Phase II studies indicated increased activity against non-Hodgkin's lymphoma (NHL) and reduced toxicity compared to retrospective data obtained with free vincristine (Sarris et al., 2000).

This was followed by a pivotal Phase II/III trial to evaluate Marqibo as a single agent treatment for NHL at second or greater relapse, resulting in a New Drug Application (NDA) for Marqibo in the spring of 2004. The Marqibo NDA was reviewed by an Oncologic Drugs Advisory Committee (ODAC) in late 2004 and was not approved despite clinical evidence indicating that Marqibo is an active agent in patients with heavily pretreated aggressive NHL, and was tolerated at approximately twice the dose intensity of standard vincristine (Rodriguez et al., 2009). Marqibo was then licensed to Hana Bioscience in San Francisco (now Talon Therapeutics) in 2006. Talon took Marqibo forward as a treatment for acute lymphoblastic leukemia (ALL) at second or greater relapse, again with positive results (20% complete response, 35% overall response). A new NDA was submitted by Talon in July 2011, which finally received a positive vote by an ODAC panel in March 2012 and resulted in approval by the FDA in August 2012.

REFERENCES

K.A. Gelmon, A. Tolcher, A.R. Diab, M.B. Bally, L. Embree, N. Hudon, C. Dedhar, D. Ayers, A. Eisen, B. Melosky, C. Burge, P. Logan, and L.D. Mayer, Phase I Study of Liposomal Vincristine, J Clin Oncol 17:697-705 (1999).

M.J. Hope, M.B. Bally, G. Webb and P.R. Cullis "Production of Large Unilamellar Vesicles by a Rapid Extrusion Procedure: Characterization of Size, Trapped Volume and Ability to Maintain a Membrane Potential", Biochim. Biophys. Acta 812, 55-65 (1985)

L.D. Mayer, M.B. Bally and P.R. Cullis "Uptake of Adriamycin into Large Unilamellar Vesicles in Response to a pH Gradient", Biochim. Biophys. Acta 857, 123-126 (1986)

L.D. Mayer, L.C. Tai, D.S.C. Ko, D. Masin, R.S. Ginsberg, P.R. Cullis and M.B. Bally "Influence of Vesicle Size, Lipid Composition and Drug-to-Lipid Ratio on the Biological Activity of Liposomal Doxorubicin", Cancer Res. 49, 5922-5930 (1989)

L.D. Mayer, M.B. Bally, H. Loughrey, D. Masin and P.R. Cullis "Liposomal Vincristine Preparations Which Exhibit Decreased Drug Toxicity and Enhanced Activity Against Murine L1210 and P388 Tumors", Cancer Research 50, 575-579 (1990)

L.D. Mayer, R. Nayar, R.L. Thies, N.L. Boman, P.R. Cullis and M.B. Bally "Identification of Vesicle Properties Which Enhance the Antitumour Activity of Liposomal Vincristine Against Murine L1210 Leukemia", Chemotherapy and Pharmacology 33, 17-34(1993)

N.L. Boman, L.D. Mayer and P.R. Cullis "Optimization of the Retention Properties of Vincristine in Liposomal Systems", Biochim. Biophys. Acta 1152, 253-258 (1993)

M. S. Webb, T. O. Harasym, D. Masin, M. B. Bally, and L. D. Mayer, "Sphingomyelin-cholesterol liposomes significantly enhance the pharmacokinetic and therapeutic properties of vincristine in murine and human tumour models" Br J Cancer. 1995 October; 72(4): 896–904.

A. H. Sarris, F. Hagemeister, J. Romaguera, M. A. Rodriguez, P. McLaughlin, A. M. Tsimberidou, L. J. Medeiros, B. Samuels, O. Pate, M. Oholendt, H. Kantarjian, C. Burge and F. Cabanillas, "Liposomal vincristine in relapsed non-Hodgkin's lymphomas: Early results of an ongoing phase II trial", Annals of Oncology 11 69-72, 2000.

Rodriguez MA, Pytlik R, Kozak T, Chhanabhai M, Gascoyne R, Lu B, Deitcher SR, Winter JN; Marqibo Investigators., "Vincristine sulfate liposomes injection (Marqibo) in heavily pretreated patients with refractory aggressive non-Hodgkin lymphoma: report of the pivotal phase 2 study", Cancer. 2009 Aug 1;115(15):3475-82).

LIPID NANOPARTICLE FORMULATIONS OF SIRNA FOR SILENCING GENES IN THE LIVER: PRECLINICAL AND CLINICAL RESULTS

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RNAi-based drugs such as siRNA require sophisticated delivery systems in order to achieve therapeutic benefits. These delivery systems must protect encapsulated siRNA from degradation in the circulation, promote accumulation in target tissue and facilitate intracellular delivery into target cells. In order to be suitable for clinical use, these delivery systems must also be relatively non-toxic and must encapsulate siRNA efficiently into well-defined, reproducible nanomedicines using a scalable manufacturing process. Lipid nanoparticles (LNP) are currently the leading delivery systems for satisfying these demands, four LNP siRNA formulations are currently in clinical trials.

Efficient loading into LNP can be achieved using ionizable cationic lipids that can also be optimized to achieve maximum intracellular delivery of siRNA following uptake into target cells. Ionizable cationic lipids, which can be designed to exhibit little positive charge at physiological pH values, are also significantly less toxic than permanently charged cationic lipid. With regard to manufacture of LNP siRNA systems, formulation processes require rapid mixing of an aqueous stream, containing siRNA, with an enthanolic solution containing cationic lipid and PEG-lipid. We have devised scalable microfluidic mixing technology that results in the formation of LNP siRNA systems over the size range 20-100 nm with siRNA encapsulation efficiencies approaching 100%. The LNP siRNA systems formed exhibit a novel nanostructured core consisting of inverted lipid micelles, some of which contain siRNA. It is shown that LNP siRNA systems containing optimized ionizable cationic lipids are highly potent and relatively non-toxic agents for silencing hepatocyte target genes following i.v. injection, achieving 50% or greater target gene silencing of 10 µg siRNA/kg body weight with therapeutic indices of 100 or higher. This is currently the world-leading "gold standard" for the potency of siRNA-based therapeutics in vivo.

The therapeutic potential of LNP siRNA systems containing optimized cationic lipids has been demonstrated for an LNP formulation containing siRNA to silence the transthyretin (TTR) gene, to treat TTR-mediated amyloidosis (ATTR). ATTR is caused by mutations in the TTR gene, which is expressed predominantly in the liver. Mutations in TTR cause abnormal amyloid proteins to accumulate and damage body organs and tissue, such as the peripheral nerves and heart, resulting in intractable peripheral sensory neuropathy, autonomic neuropathy, and/or cardiomyopathy. ATTR represents a major unmet medical need with significant morbidity and mortality; familial amyloidotic polyneuropathy (FAP) affects approximately 10,000 people worldwide and familial amyloidotic cardiomyopathy (FAC) affects at least 40,000 people worldwide. FAP patients have a life expectancy of five to 15 years from symptom onset, and the only treatment options for early stage disease are liver transplantation and tafamidis (approved in Europe). The mean survival for FAC patients is approximately 2.5 years, and there are no approved therapies. As a result, there is a significant need for novel therapeutics to treat patients who have inherited mutations in the TTR gene. Phase I studies were conducted in 2012 to evaluate the safety and tolerability of LNP TTR siRNA (ALN-TTR02) in healthy volunteers as well as the clinical activity of ALN-TTR02 based on measurements of serum levels of TTR, the disease-causing protein in patients with ATTR. In July 2012, positive clinical results were announced. These results showed that administration of ALN-TTR02 leads to robust knockdown of serum TTR protein levels of up to 94% ($p<0.00001$ by ANOVA). Reduction of TTR was found to be rapid, dose dependent, durable, and specific after just a single dose of ALN-TTR02, and a nearly 80% level of suppression was sustained at one month with just a single dose. ALN-TTR02 was found to be generally safe and well tolerated. Knockdown of circulating TTR is a validated endpoint in ATTR based on data from patients receiving liver trans-

plants. Evidence from other systemic amyloidotic diseases shows that as little as a 50% reduction of the disease-causing protein can result in disease improvement or stabilization. These new data with ALN-TTR02 provide key human proof of concept with associated clinical relevance for the treatment of ATTR, a debilitating orphan genetic disease.
A Phase II trial with ALN-TTR02 is underway in Europe aimed at evaluating safety, tolerability, and potential clinical activity of multiple once-monthly doses of ALN-TTR02 in ATTR patients.

REFERENCES

S.C. Semple, S.K. Klimuk, T.O. Harasym, N. Dos Santos, S.M. Ansell, K.F. Wong, N. Maurer, H. Stark, P.R. Cullis, M.J. Hope, and P. Scherrer "Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionizable aminolipids: formation of novel small multilamellar vesicle structures", Biochim. Biophys. Acta 1510, 152-166 (2001)
N. Maurer, K.F. Wong, H. Stark, L. Louie, D. McIntosh, T. Wong, P. Scherrer, S. Semple and P.R. Cullis "Spontaneous Entrapment of Polynucleotides Upon Electrostatic Interaction With Ethanol Destabilized Cationic Liposomes: Formation of Small Multilamellar Liposomes", Biophys. J. 80, 2310-2326 (2001)
Hafez, N. Maurer and P.R. Cullis "On the Mechanism Whereby Cationic Lipids Promote Intracellular Delivery of Polynucleic Acids", Gene Therapy 8, 1188-1196 (2001)
S.C. Semple, A. Akinc, J. Chen, A. Sandhu, B. Mui, C. Chow, D. Sah, D. Stebbing, E. Crosley, I. Hafez, J.R. Dorkin, J. Qin, K. Lam, K. Wong, L. Nechev, M.L. Eisenhardt, M. Jayaraman, M. Kazem, M. Maier, M. Srinivasulu, M. Weinstein, Q. Chen, R. Alvarez, S. Barros, S.K. Klimuk, T. Borland, V. Kosovrasti, Y. Tam, I. MacLachlan, M. Manoharan, M. Ciufolini, M. Tracy, A. de Fougerolles, P.R. Cullis, T.D. Madden and M.J. Hope; "Discovery of Next-Generation siRNA Delivery Systems Through Rational Design of Novel Cationic Lipids", Nature Biotech. 28, 172-176 (2010).
G. Basha, T. I. Novobrantseva, N. Rosin, Y.Y.C. Tam, I. M. Hafez, M. K. Wong, T. Sugo, V. M. Ruda, J. Qin, B. Klebanov, M. Ciufolini, A. Akinc, Y. K. Tam, M. J. Hope and P. R. Cullis, "Influence Of Cationic Lipid Composition On Gene Silencing Properties Of Lipid Nanoparticle Formulations Of siRNA In Antigen Presenting Cells", Molecular Therapy, 19, 2186 (2011)

OFF-TARGET NANOPARTICLE INTERACTIONS

KENNETH DAWSON

Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, important aspects of biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer, rather than the molecular, scale. Harnessing these endogeneous nanoscale biological processes (for example in creation of new nanomedicines or nanodiagnostics) may well provide the best hope of tacking currently intractable diseases, and the effort to resolve outstanding issues in the field is of the highest significance. However, there could be limitations on current approaches to fabricating biologically active nanoparticles. In particular, the design and fabrication of nanostructures that act in a predictable, verifiable, and reproducible manner in situ in biological environments has been of interest. In this talk we present some recent results on the consequences of 'biomolecular coronas' on pegylated particles with targeting ligands, and show that without special actions the fidelity of the targeting mechanism of the nanparticles (present in simple serum and plasma free environments) is compromised in the more realistic situations of a biological environment.

Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface, A Salvati, AS Pitek, MP Monopoli, K Prapainop, FB Bombelli, DR Hristov, PM, Nature Nanotechnology 8 (2), 137-143 (2013)

Monopoli, M. P.; Aberg, C.; Salvati, A.; Dawson, K. A. Biomolecular Coronas Provide the Biological Identity of Nanosized Materials. Nature Nanotechnology 2012, 7, 779-786.
Kim, J. A.; Aberg, C.; Salvati, A.; Dawson, K. A. Role of Cell Cycle on the Cellular Uptake and Dilution of Nanoparticles in a Cell Population. Nature Nanotechnology 2012, 7, 62-68.
Monopoli, M. P.; Walczyk, D.; Campbell, A.; Elia, G.; Lynch, I.; Baldelli Bombelli, F.; Dawson, K. A. Physical-Chemical Aspects of Protein Corona: Relevance to in Vitro and in Vivo Biological Impacts of Nanoparticles. Journal of the American Chemical Society 2011, 133, 2525-2534.
Cedervall, T.; Lynch, I.; Lindman, S.; Berggard, T.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. Understanding the Nanoparticle-Protein Corona Using Methods to Quantify Exchange Rates and Affinities of Proteins for Nanoparticles. Proceedings of the National Academy of Sciences 2007, 104, 2050-2055.

GLUTATHIONE PEGYLATED LIPOSOMAL DOXORUBICIN (2B3-101) TARGETING THE CNS: PRECLINICAL RATIONALE AND PHASE I/IIA STUDY DESIGN

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BACKGROUND

The overall prognosis in patients with solid tumors with brain metastases or malignant gliomas is generally poor and treatment options are limited. This is partly due to poor blood-brain barrier drug penetration. To safely enhance drug delivery to the brain, glutathione PEGylated liposomal doxorubicin (2B3-101) was developed.

2B3-101: GLUTATHIONE PEGYLATED LIPOSOMAL DOXORUBICIN

2B3-101 is a CNS-targeted product based on the already existing PEGylated liposomal doxorubicin (Doxil®/Caelyx®). The active substance, doxorubicin, is an anthracycline antibiotic that is among the most widely used anticancer agents. It inhibits the growth of many cancerous cell lines, including glioblastoma and breast cancer cell lines [1, 2]. Yet, without active delivery to the brain, the efficacy of doxorubicin against brain tumors is limited.
Glutathione (GSH) was chosen as targeting moiety, since this endogenous tripeptide is found at high levels in the brain and is actively transported across the blood-brain barrier [3]. Previously, we have demonstrated that glutathione PEGylated liposomes (G-Technology®) are able to mediate safe targeting and enhanced delivery of encapsulated drugs to the brain [4, 5].

PRECLINICAL STUDIES: PHARMACOKINETICS, BRAIN DELIVERY, AND EFFICACY

In vivo, 2B3-101 and Doxil/Caelyx had a comparable plasma exposure in mice, while brain uptake as measured by cerebral open flow microperfusion was 4.8 fold higher for 2B3-101 compared to Doxil/Caelyx. This was confirmed in in vitro studies, where uptake of 2B3-101 by human brain capillary endothelial cells was temperature-, time-, and concentration-dependent, while Doxil/Caelyx mainly remained bound to the cells without being extensively internalized.

In two independent experiments performed by Dr. Olaf van Tellingen at the Netherlands Cancer Institute (NKI, Amsterdam, the Netherlands), therapies at a weekly or at a twice-weekly dose of 5 mg/kg resulted in a strong inhibition of brain tumor growth with 2B3-101. The effect of weekly administration of 2B3-101 was more pronounced compared to saline ($p<0.01$) and Doxil/Caelyx ($p<0.05$). Two out of 9 animals receiving 2B3-101 showed a complete tumor regression, which was not observed in the other treatment groups. Twice weekly injections of 5 mg/kg 2B3-101 had a significant effect in limiting brain tumor growth ($p<0.001$) compared to Doxil/Caelyx and saline and a complete regression was observed in 1 animal treated with 2B3-101 (Figure 1). In addition, twice weekly dosing of 2B3-101 increased the median survival time by 38.5% ($p<0.001$) and 16.1% ($p<0.05$) compared to saline and Doxil/Caelyx, respectively.

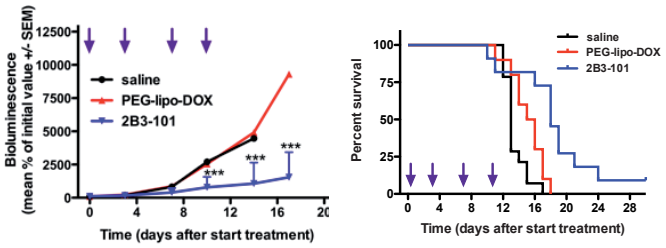

Figure 1**: Treatment with 5 mg/kg 2B3-101 twice weekly results in an inhibition of brain tumor growth (left) and increase in median survival time (right). The inhibition of brain tumor growth as measured by bioluminescence (BLI) was strongest for 2B3-101 (p<0.001 vs saline and Doxil/Caelyx; two-way ANOVA on Log%BLI). Median survival time of mice was 13 days with saline (n=14), 15.5 days with Doxil/Caelyx (PEG-lipo-DOX, n=10, p<0.05 compared to saline) and 18 days with 2B3-101 (n=9, p<0.05 and p<0.001 compared to Doxil/Caelyx and saline, respectively). Experiment performed by Dr. Olaf van Tellingen at the Netherlands Cancer Institute (NKI, Amsterdam, the Netherlands).*

TRANSLATIONAL STUDIES: GMP PRODUCTION AND GLP TOXICITY STUDIES

2B3-101 was prepared using the pre-insertion method, in which GSH-PEG micelles, produced by conjugation of GSH to DSPE-PEG-maleimide, were added to the lipids (HSPC and cholesterol) before extrusion and active loading of doxorubicin [6]. Three batches (each 2L) have been prepared for nonclinical studies and tested for their stability at 5, 25 and 40°C. 2B3-101 was stable up to 24 months when stored at 5°C. Another six batches of 8L each have been produced for the clinical studies; these have comparable characteristics.

No major differences in toxicity and biodistribution were observed between 2B3-101 and Doxil/Caelyx in a repeat-dose intravenous study in rats of both sexes (1.75; 3.5 and 7 mg/kg 2B3-101 or 7 mg/kg Doxil/Caelyx on days 1, 15 and 29).

- The toxicity was similar with regard to effects on body weight, inflammatory lesions of the skin and mucosa, and myelosuppression.
- Histopathology revealed neither cardiotoxicity, nor neurotoxicity.

No acute CNS side effects were seen on EEGs and in a modified Irwin test (a functional neurobehavior battery test).

- No relevant toxicity findings were observed with empty GSH-PEG liposomes.

To confirm the efficacy of the GMP grade 2B3-101, produced by TTY Biopharm, two regimens at maximum tolerated doses were tested in the mouse glioblastoma model (n=10 per group; Dr. Olaf van Tellingen, NKI); 10 mg/kg q4dx4 and 18 mg/kg q8dx2 showed a survival benefit of 57% and 60% respectively when compared to saline, where 18 mg/kg q8dx2 seemed superior (Figure 2).

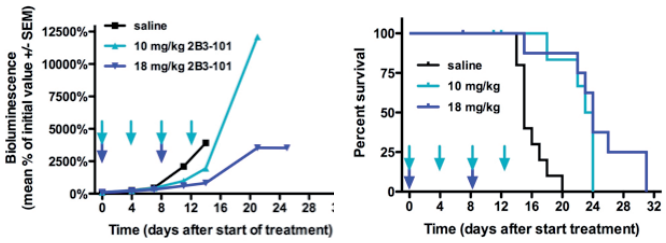

***Figure 2**: Significant glioma growth inhibition (left) and significant survival benefit (right) for 2B3-101 at 10 mg/kg q4dx4 and 18 mg/kg q8dx2 compared to saline (n=10 per group). Experiment performed by Dr. Olaf van Tellingen at the Netherlands Cancer Institute (NKI, Amsterdam, the Netherlands).*

PHASE I/IIA CLINICAL TRIAL – STUDY DESIGN

A phase I/IIa clinical trial in patients with solid tumors and brain metastases or recurrent malignant glioma (clinicaltrials.gov NCT01386580) was designed to (1) determine the safety, tolerability and MTD of 2B3-101 as single agent or in combination with trastuzumab (Herceptin®) and (2) to assess the pharmacokinetics and preliminary anti-tumor activity of 2B3-101. The phase I dose escalation study was initiated with 5 mg/m2 as starting dose, administered as a 60-minute infusion every three weeks. A "3+3" dose-escalation design according to the modified Fibonacci scheme has been applied. Next to general safety measurements, potential neurotoxicity and cardiotoxicity are assessed. Plasma concentrations of 2B3-101 are also measured. Preliminary tumor activity is assessed by CT of the thorax and abdomen and MRI of the brain. Currently, the dose-escalation part is almost completed, and the phase IIa part of the study to determine preliminary antitumor efficacy at the maximum tolerated dose will start soon after.

CONCLUSION

We have created a technology that uses safe, endogenous uptake mechanisms to enhance the blood-to-brain delivery of drugs. Our lead product, 2B3-101, has shown promising preclinical and translational results with regard to safety and efficacy and is currently being evaluated in a phase I/IIa clinical trial in patients with solid tumors and brain metastases or recurrent malignant glioma.

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REFERENCES

1. Wolff J.E. et al. J Cancer Res Clin Oncol 1999, 125: 481-486; 2. Liedtke C. et al. Breast Cancer Res Treat 2009, 121:301-309; 3. Gaillard, P.J. et al. Drug Discov. Today: Technol. 2012, 9:155-160; 4. Gaillard P.J. et al. J Control Release 2012, 164: 364-369; 5. Lindqvist A. et al. Mol Pharm 2012, doi: 10.1021/mp300272a; 6. Barenholz Y. J Control Release 2012, 160: 117-134.

PHASE III STUDIES OF ABRAXANE IN THE TREATMENT OF METASTATIC MELANOMA AND ADVANCED PANCREATIC CANCER

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ABRAXANE® (nab-paclitaxel) is a solvent-free 130 nm albumin-bound nanoparticle form of paclitaxel, which utilizes albumin transport pathways, including caveolae-mediated albumin transcytosis across tumor blood vessel endothelium and potential association with albumin-binding proteins such as SPARC to achieve enhanced drug penetration. ABRAXANE was approved for the treatment of metastatic breast cancer in the United States, Europe, and other countries around the world. In October 2012, ABRAXANE was approved in US for the first-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC), in combination with carboplatin, in patients who are not candidates for curative surgery or radiation therapy. Recently completed phase III trials in melanoma and pancreatic cancer have further demonstrated efficacy and safety of ABRAXANE.

In the randomized, open-label, international study in chemotherapy-naïve patients with metastatic melanoma (CA033), ABRAXANE met the primary endpoint and showed a statistically significant improvement in progression-free survival (PFS) compared to patients receiving dacarbazine chemotherapy (4.8 vs. 2.5 months, respectively [HR: 0.792; 95.1% CI: 0.631, 0.992; P = 0.044]). An interim analysis of overall survival also showed a trend in favor of the ABRAXANE arm compared to treatment with dacarbazine (12.8 and 10.7 months, respectively (HR: 0.831; 99.9% CI: 0.578, 1.196; P = 0.094)). The final OS analysis is pending. The safety profile of ABRAXANE observed in the CA033 study was comparable with other

ABRAXANE pivotal clinical trials.
In the randomized, open-label, international study in treatment-naïve patients with metastatic pancreatic cancer (CA046, MPACT study), ABRAXANE in combination with gemcitabine demonstrated a statistically significant improvement in OS compared to patients receiving gemcitabine alone (median: 8.5 vs. 6.7 months [HR: 0.72, P = 0.000015]). The survival rate also favored ABRAXANE plus gemcitabine arm at one-year (35% vs. 22%, P = 0.0002) and two-year (9% vs. 4%, P = 0.02) as compared to gemcitabine alone. ABRAXANE plus gemcitabine showed statistically significant improvement in secondary endpoints including PFS (median: 5.5 vs. 3.7 months [HR: 0.69, P = 0.000024]) and overall response rate (ORR, 23% vs. 7%, P = 1.1 x 10-10). The safety profile of ABRAXANE observed in the MPACT study was comparable with other ABRAXANE pivotal clinical trials.
In summary, recent positive phase III trial results in metastatic melanoma and metastatic pancreatic cancer have demonstrated the clinical benefits of ABRAXANE supporting and potentially expanding the use of this drug outside of breast cancer and NSCLC.

TARGETED AND PERSONALIZED CANCER THERAPY VIA TUMOR STROMA-ATTACKING NANODRUGS

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Numerous types of solid tumors are characterized by desmoplasia, which is prominent presence of tumor stroma with proliferation of fibrotic tissue and an altered extracellular matrix (ECM). Tumor stroma promotes tumor growth and metastasis. Dense tumor stroma, particularly collagen in tumor extracellular matrix, limits drug penetration in the tumor interstitium and presents a significant barrier for drug delivery, contributing to tumor resistance to chemotherapy. The important roles of tumor stroma in tumor proliferation and chemoresistance make it a highly desirable therapeutic target in desmoplastic cancers, with pancreatic cancer being a primary example.
Albumin is a key plasmic carrier of hydrophobic molecules and is highly accumulated in tumors. ABRAXANE® (nab-paclitaxel) is a solvent-free 130 nm albumin-bound nanoparticle form of paclitaxel, which utilizes albumin transport pathways, including caveolae-mediated albumin transcytosis across tumor blood vessel endothelium and potential association with albumin-binding proteins such as SPARC to achieve enhanced drug penetration. In addition, continued expression of albumin by pancreatic stellate cells (PSC), the major cell type in pancreatic cancer stroma, is associated with maintenance of a quiescent phenotype of PSC.
Recent preclinical and clinical results suggest that ABRAXANE may be an effective therapeutic option for pancreatic cancer by targeting both neoplastic cells and tumor activated stroma. In patient-derived pancreatic cancer xenografts, combination of ABRAXANE with gemcitabine significantly improved antitumor efficacy compared with either agent alone. Histological analysis showed that ABRAXANE alone and in combination with gemcitabine resulted in depletion of the desmoplastic stroma as observed by the less dense, disorganized, wisps of collagen type 1 fibers after 4 weeks of treatment. Consistently, the intratumoral concentration of gemcitabine was increased by 2.8-fold after 5 days of ABRAXANE treatment. It was therefore hypothesized that ABRAXANE may have contributed to the increased intratumoral gemcitabine uptake by targeting pancreatic tumor stroma. A recent clinical study in patients with resectable pancreatic cancer treated with neoadjuvant ABRAXANE plus gemcitabine showed reduction in fibrotic collagenous stroma as well as elimination of SPARC positive cells, further supporting a stroma active mechanism for ABRAXANE. The efficacy of ABRAXANE and gemcitabine combination has been demonstrated in recent phase II and phase III trials in patients with advanced pancreatic cancer. ABRAXANE and albumin-based nanomedicines may provide a novel strategy to improve the delivery and efficacy of therapeutics in patients with pancreatic cancer by targeting tumor stroma as well as cancer cells.

NANOIMMUNOLOGY AND STERILITY

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Nanoparticles are being developed for many industrial and biomedical applications. The clear benefits of using nanosized products in these applications are often challenged by questions regarding safety of these materials. One area of interest involves interactions between nanoparticles and components of the immune system. Nanoparticles can be engineered to either avoid immune system recognition or to specifically interact with the immune system. This presentation will review data regarding nanoparticle-mediated immunostimulation and immunosuppression, which I will use to highlight common challenges in preclinical characterization of nanoparticles. Case studies demonstrating how manipulation of nanoparticle physicochemical properties can influence their interaction with components of the immune system will be discussed. The presentation will focus on areas such as interaction with erythrocytes, effects on blood coagulation system, activation of complement and effects on immune cell function. I will discuss nuances and challenges associated with preclinical immunological characterization of engineered nanomaterials Specifically, I will focus on endotoxin detection and quantification, pyrogenicity testing nanoparticle depyrogenation, sterility and sterilization, nanoparticle interference with traditional immunological tests, and applicability of traditional in vivo immune function tests to engineered nanomaterials. I will present case studies demonstrating the significance of comprehensive physicochemical characterization of engineered nanomaterials prior to their toxicological evaluation as well as correlation between toxicological in vitro assays and relevant in vivo tests.

LIPOSOMES AS LUBRICANTS, A NEW APPROACH FOR OSTEOARTHRITIS

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Moebius Medical is developing a lubricating, intra-articular injectable solution for diarthrosis (synovial joints).
The healthy articular cartilage is smooth and has low friction, allowing the bones in a joint to glide smoothly over one another upon movement. The osteoarthritis cartilage is thinned, eventually completely worn out, resulting in a «bone against bone» joint, reduced motion, and pain.
We developed a medical device based on liposomes. Those liposomes serve as a bio-lubricant and anti-wear protector. The mechanism of action of these liposomes can be described as as “biological ball-bearing” or as biological boundary lubrication.
A clinical trial confirmed the safety and superiority of our treatment over the standard of care, hyaluronic acid. The clinical trial was conducted at Hadassah Medical Center / Orthopedic Department. We will publish the preliminary results of the clinical trial at the conference.

COMPUTER MODELING OF NANOPARTICLE TARGETING TO ENHANCE SELECTIVITY

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One of the major challenges in biomedical applications of polymer nanoparticles is the engineering of nanoparticles to achieve selective targeting of diseased cells with high density of receptors while sparing benign cells. Using computer modeling, we analyze the influence of nanoparticle design on targeting selectivity and discuss strategies for enhancing nanoparticle selectivity (without compromising affinity) for therapeutic and imaging applications.

INTRODUCTION

Nanoparticles have gained popularity as modern multifunctional nanodevices which provide a cargo space for hydrophobic drugs (or RNA), reduce or eliminate toxicity in drug administration, deliver the payload to a desired location, stimulate endocytosis, ensure control in drug release and often serve as imaging and therapeutic agents by themselves.1-5 The fate of nanoparticle delivery either relies on the enhanced permeability and retention (EPR) effect or depends on specific interactions between ligands, antibodies, aptamers, etc. attached to a nanoparticle and receptors expressed on cell surfaces.4,5 In the latter case of active targeting, nanoparticle adsorption and further internalization by cells strongly depend on nanoparticle design, the strength of ligand-receptor recognition interactions and properties of the cell surface, such as density of targeted receptors, their propensity for clustering, etc. While high affinity of a nanoparticle to a targeted cell surface is well-accepted as an important factor in nanoparticle performance, the selectivity of nanoparticle targeting of diseased cells while avoiding interactions with healthy cells (which may also express targeted receptors albeit at a lower concentration) has been only recently identified as an essential function. So far the search for improved nanoparticle selectivity has been directed toward better matching of specific receptors. Considerably less attention has been paid to the design of the nanoparticle itself to optimize its targeting selectivity. At the same time nanoparticle design is much easier to control and understanding how design can influence the selectivity of targeting becomes increasingly important as development of nanoparticulate formulations progresses from the laboratory stage to clinical testing.

METHODS

We use Monte Carlo simulations to investigate the interactions between (solid-core) spherical nanoparticles grafted with flexible tethers carrying targeting groups at their distal end and receptor surfaces containing different densities of mobile receptors The cell surface was modeled as a plane containing mobile receptors each occupying a 2-dimensional circular area with an overall diameter of 6 nm, by analogy with avb3 integrins.[6] The functional center of a receptor was capable of a reversible interaction with binding energy Ebind (which refers to the absolute value of the binding energy) with a ligand tethered to the nanoparticle. The equilibrium chain conformation and equilibrium number of bound ligand-receptor pairs was obtained by moving and binding updates[7,8] performed over more than 8 million Monte Carlo time steps. Our simulation methodology[7,8] accounts for both the enthalpic gains from ligand-receptor interactions and the entropic loss for the nanoparticle (including conformational changes for ligand-bearing and free tethers) in the vicinity of a cell surface and the translational entropy loss for receptors gathering on the cell surface in the vicinity of a nanoparticle. In our simulations we determine the equilibrium number of ligand-receptor binding and calculate of the equilibrium free energy $\Delta F_{\min}$ and the corresponding nanoparticle binding constant $K_{bind} = \exp(-\Delta F_{\min}/kT)$, (with k being the Boltzmann constant and T the temperature), which can be taken as a measure of the nanoparticle affinity to a cell surface. By comparing nanoparticle affinities for cell surfaces with different receptor densities, we can investigate the influence of nanoparticle design (e.g. size, tether length, density, ligand type, etc.) on its targeting selectivity. Such in silica experiments provide a convenient basis for comparison, allow systematic analysis of most experimentally-relevant nanoparticle design parameters and generate predictions which are easily transferable to laboratory conditions.

RESULTS

In physological conditions when a nanoparticle passes through different tissues it can interact via specific ligand-receptor interacons with various cells expressing different concentrations (and possibly arrangements) of targeted receptors. In general the larger the receptor density, the stronger is nanoparticle affinity toward the cell, as shown in Figure 1a, Such dependence is usually non-linear and is strongly influenced by nanoparticle design, as shown in Figure 1b,d. Ideally, for the best therapeutic/imaging results one wants to achieve the situation when there is no (or very small) nanoparticle adsorption to benign cells (with low receptor densities) and there is a significant nanoparticle accumulation at targeted cells with high density of receptors, which corresponds to an abrupt increase in affinity in excess of some critical onset value, ronset (Figure 1a). To achieve this situation, the nanoparticle has to be appropriately designed to exhibit the onset of binding at some intermediate receptor density, which is higher than that of benign cells, but lower than targeted cells.

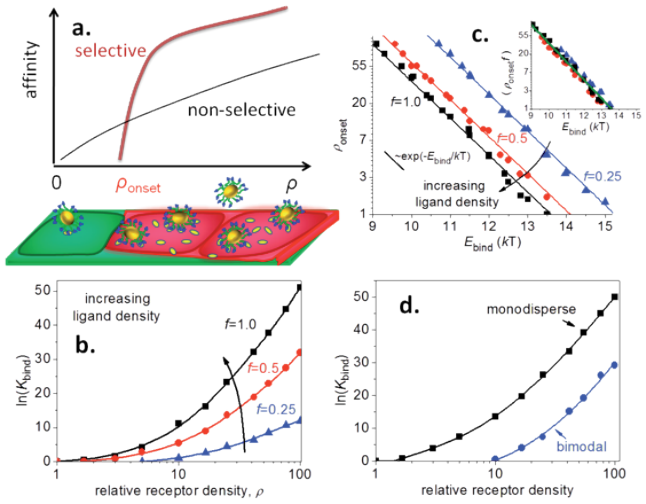

Figure 1. *(a) Schematic presentation of selective and non-selective curves of nanoparticle binding to receptor surfaces as a function of receptor density. The onset density is indicated. (b-d) Results of computer simulations for spherical nanoparticles with core radius of 10nm with 128 flexible tethers. (b) The equilibrium nanoparticle binding constant, ln(K_{bind}) vs. relative density of cell receptors ρ (normalized by the smallest receptor density considered) for a monodisperse protective layer (free tether end-to-end distance Rend=10nm) with E_{bind}=15kT and different fractions of ligated tethers, f: f=1.0 (squares), f=0.5 (circles) and f=0.25 (up triangles). (c) The relative receptor density at the onset of nanoparticle binding ρ_{onset}, as a function of ligand-receptor binding energy E_{bind} for the same system as in (b). The inset shows the product, ρ_{onset} f, as a function of E_{bind} for the same set of data as the main plot. (d) The equilibrium nanoparticle binding constant, ln(K_{bind}) vs. relative density of cell receptors ρ for a protective layer containing 64 ligated tethers with end-to-end distance R_{end}=10nm and 64 non-functional tethers (f=0.5) with end-to-end distance R_{end}=16nm, (circles, bimodal case), or Rend=10nm (squares, monodisperse case) with E_{bind}=15kT.*

Using the results of our computer modeling we determined the onset of binding ρ_{onset}, i.e. the relative receptor density when $\Delta F_{\min}$ =0 (i.e. K_{ind}=1), for nanoparticles with different lig-and densities as a function of the ligand-receptor binding energy Ebind (Figure 1c). In all cases the receptor density at the onset of nanoparticle binding ρ_{onset} exhibits an exponential depend-ence on the ligand-receptor binding energy: ρ_{onset} ~exp(-E_{ind}/kT). This is to be expected, as at the onset of nanoparticle adsorption there will be only one or (a few) ligand-receptor bind-ing(s), which is influenced by the ligand-receptor binding energy Ebind. Furthermore, the on-set of nanoparticle binding is inversely proportional to the ligand density (or the fraction of functionalized tethers f for a given nanoparticle) and all data presented in Figure 1c can be collapsed into a single curve as shown in the inset.8 This implies that for a given nanoparticle one can predict the nanoparticle binding onset ρ_{onset}~(n_{lig})-1exp(-E_{ind}/kT) for different ligand densities (n_{lig}) or different ligand-receptor binding energies (Ebind) if there are experimental binding data for at least one system configuration. The increase in the entropic penalty near a cell surface for longer non-functional tethers leads to significant increase in binding onset for nanoparticles with a "bimodal" protective layer containing short ligated tethers and longer non-functional tethers, as shown in Figure 1d. Thus, hiding ligands inside the polymer protec-tive layer can be used as a successful strategy not only for nanoparticle to escape undesirable interactions upon delivery, but also to enhance targeting selectivity. It is

worthwhile to note that kinetics of nanoparticle targeting can also be an important factor to consider in achieving targeting selectivity.9 Unfortunately an increase of the receptor density at the onset of nano-particle binding, which is desirable for targeting selectivity, often comes at the price of lower-ing the nanoparticle affinity to the target cells. One strategy to overcome this dilemma is to use multivalent ligands while keeping the overall density of targeting groups constant. In such a case, the onset remains practically the same, while the nanoparticle affinity towards a receptor surface with a large receptor density will increase as each stretched tether will bring several targeting groups into the vicinity of cells surface compared to the monovalent ligand case.

To summarize our findings, a higher receptor density at the onset of nanoparticle binding (p_{onset}) can be achieved by lowering the ligand-receptor binding energy (Eb_{ind}), decreasing the ligand density or by increasing tether length, especially the non-functional tether length in bimodal protective layers with ligands hidden inside. Often an increase in binding onset comes at price of decreased binding affinity for the targeted cells. To combat this problem one can employ one of the several strategies, including the use of multivalent ligands. Thus, understanding the effect of different factors on nanoparticle affinity and selectivity allows one to intelligently choose design strategies to engineer nanoparticles with the optimal target-ing performance to achieve the desired therapeutic or imaging goals.

REFERENCES

1. Couvreu Vauthier C, Pharmaceutical Research 2006, 23(7):1417-1450
2. Davis ME, Chen Z, Shin DM. Nat. Rev. Drug Discov. 2008;7(9):771-782
3. Petros RA, DeSimone JM. Nat. Rev. Drug Discov. 2010;9(8):615-627.
4. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nat. Nanotech. 2007;2(12):751-760
5. Torchilin VP. In:Drug Delivery. Springer Berlin Heidelberg; 2010. 3-53
6. Stewart PL, Chiu CY, Huang S, Muir T, Zhao Y, Chait B, et al. EMBO J. 1997, 16(6):1189-1198
7. Wang S, Dormidontova EE. Biomacromolecules 2010, 11(7):1785-179
8. Wang S, Dormidontova, EE Phys. Rev. Lett. 2012, 109:238102-4,
9. Djohari H, Dormidontova EE, Biomacromolecules 2009, 10(11):3089-3097

THE SPECIAL ROLE OF THE IMMUNE SYSTEM IN NANOSAFETY ASSESSMENT

ALBERT DUSCHL

Nanoparticles (NPs) overlap in size with viruses and can thus in principle be recognized by the immune system. Even very small NPs that are in the size range of individual proteins are often eliciting responses of the immune system when they form aggregates after entering biological compartments. Toxicity of NPs can be relevant for all cell types, but immune cells are of special interest for a number of reasons:

• Immune cells can distinguish self from non-self, so they should be able to recognize NPs as non-self. This process is facilitated by localization: Dendritic cells, usually the first responders, are concentrated close to body surfaces where pathogens may enter. The potential uptake routes for pathogens and NPs are identical and NPs are clearly non-self, so interactions can be expected.

• The immune system responds to non-self entities in most cases with tolerance, which is not the same as failure to recognize. Non-self is usually correctly identified as non-self, but is also mostly identified as harmless. Active mechanisms are now initiated to ensure tolerance, for example by inducing the differentiation of antigen-specific immunosuppressive T-cell subsets (Treg). This is a safety assessment – the same analysis that we want to perform for NPs. The reaction of the professional immune cells should be instructive to us.

• Irritation of any sort usually leads to cell stress and inflammation via mechanisms of innate immunity. Importantly, the same types of reactions can be observed following a wide variety of external stimuli that are perceived as dangerous, since their pathways are coupled to many different receptors. We can thus measure endpoints relating to danger without knowing the responsible mechanism. This is valuable for NPs where possible modes of action are unknown.

• A number of NPs have been described to have pro-inflammatory properties that need to be considered for safety evaluation. This can be expected from agents that may be irritating or toxic, but cannot be resolved by the immune system. However, numerous such reports may rest on contamination of NP preparations with bacterial compounds like LPS or with toxic chemicals deriving from the synthesis procedure. Care should be taken to exclude such false positives.

• In many cases NPs will arrive in tissues that are not healthy, like inhalation by persons suffering from diseases like allergic asthma, COPD or bronchitis. The context can modulate the effects of NPs and data from healthy cells, tissues and organisms may not be sufficient to predict effects for risk persons or patients. For medical applications, NPs would - except for vaccines - be applied specifically to health-impaired persons, a context that has to be considered in the pre-clinical testing.

• Nanosafety aspects should be determined as early as possible, ideally in parallel to the development of a product. In this way problematic agents can be identified early on and be either abandoned or modified accordingly. This procedure reduces risks and speeds up development. Immunological assays can play a role here since they will respond to many different types of detrimental interference and since many immune tests can be set up to mimic an already health-compromised status, for example by adding pro-inflammatory agents.

The immune system has evolved to defend the body against pathogens and parasites, not against NPs. If NPs should be toxic, detoxification mechanisms would be called for, while for non-medical purposes it is best if NPs are ignored by the immune system, or are labeled as harmless and are thus tolerated. On the other hand, if specific NPs show reproducible effects on the immune system – activation, inhibition or deviation – such agents may be useful for medical treatments. Nanosafety and Nanomedicine should thus interact as closely as possible for mutual benefit.

NANODEVICES FOR ACTUATING AND MONITORING ENGINEERED CARDIAC TISSUES

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The heart is a non-regenerating organ. Consequently, the loss of cardiac cells and formation of scar tissue after extensive myocardial infarction frequently leads to congestive heart failure. Given the scarcity of cardiac donors, a potential approach to treat the infarcted heart is to repopulate the 'dead zone' with cells capable of spontaneous contraction. Cellular therapy evolved to introduce cells into diseased areas and regain function. However, two main drawbacks of this approach are the lack of control of cell accumulation site after injection, and cell death before forming cell-cell or cell-matrix interactions. These shortfalls motivated the development of the tissue engineering concept, where 3-dimensional (3D) biomaterials serve as extracellular matrix-like scaffolds to the cells, enabling the cells to assemble into effective tissue substitutes, that may restore tissue or organ function. After transplantation the scaffolds either degrade or metabolize, eventually leaving a vital tissue instead of the defected tissue. In this talk I will describe cutting-edge technologies for engineering functional cardiac tissues, focusing on the use of inorganic nanowires and devices for actuating and monitoring tissue performances. In the first part I will describe the engineering of nanowired cardiac patches. Cardiomyocytes are electrically responsive cells, in which signal transduction is mediated by action potential. Therefore, it is important that the scaffolding biomaterial used for engineering of such excitable tissues will not prevent the patch from contracting strongly as a unit. In this study, gold nanowires, ca. 1 µm long with a diameter of 30 nm, were incorporated into 3D scaffold to bridge the electrically

resistant pore walls of the biomaterial, and improve electrical communication between adjacent cardiac cells. Cells grown on these nanocomposite matrices assembled into thicker and better aligned tissues than those grown on pristine scaffolds, and without electrical stimulation the nanowired tissues contracted synchronously. In the second part of the talk I will present the fabrication of cyborg tissues integrating flexible nanoelectronics with engineered cardiac tissues. These nanoelectronic field effect transistor (FET) devices were incorporated into 3D biomaterials and used to monitor the contraction of engineered cardiac tissues and report the pH level of fluid flowing in engineered blood vessels.

THE SPIDER EFFECT: A NOVEL FRAMEWORK TO CATEGORIZE MICROGLIAL ACTIVATION STATUS AND MODULATION

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Microglia cells resemble spiders: at rest, sitting on their webs, waiting for prey; when alerted (activated), moving toward, capturing and eating prey; afterward, returning to their resting place. The spider effect is a novel framework that categorizes the activation status of microglia using morphological attributes. We transected the olfactory bulb of rats and examined the activation of the microglial system histologically. Six stages of bidirectional microglial activation (A) and deactivation (R) were observed: from stage 1A to 6A, the cell body size increased, the cell process number decreased, and the cell processes retracted and thickened, orienting toward the direction of the injury site; until stage 6A, when all processes disappeared. In contrast, in deactivation stages 6R to 1R, the microglia returned to the original site exhibiting a stepwise retransformation to the original morphology. Thin highly branched processes re-formed in stage 1R, similar to those in stage 1A. This reverse transformation mirrored the forward transformation except in stages 6R to 1R: cells showed multiple nuclei which were slowly absorbed. Our findings support a morphologically defined stepwise activation and deactivation of microglia cells. This quantitative system reduces the level of inflammation to a number, providing an objective measure of inflammation status in the CNS.

BONYPID™ - A NEW DELIVERY SYSTEM USING POLYPID TECHNOLOGY FOR THE TREATMENT OF CONTAMINATED OPEN BONE FRACTURES

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PolyPid Ltd developed a novel local drug delivery system based on self-assembly of pharmaceutically approved lipids and polymers that encapsulate drugs .The formulation is self-assembled lipid matrix via the interaction of the lipids (cholesterol and synthetic phospholipids) and biocompatible - biodegradable polymer (poly-lactic-co-glycolic). The entrapped drug is located within the anhydrous environment and therefore fully protected from both enzymatic and long-term water-exposure-related degradation.

PolyPid formulation nanostructure was characterized by different physical methods including wide angle X-ray analyses (WAXS) differential scanning calorimetric (DSC) and SEM. WAXS analyses show a strong signal in the range of 1.3-1.8 2Θº, suggesting that the polymer and lipid is a highly organized nanostructure.

The principle lipid in PolyPid technology formulations is phosphatidylcholine, which constitutes more than 85% of the overall lipid mass. It was found that the length of the acyl chains (14, 16 and 18 carbons, respectively) can significantly alter the release rate of a drug during the prolonged (4 weeks), zero-order release phase, but did not alter the release profile as demonstrated by the release of antibiotic (doxycycline).

PolyPid's first focus is BonyPid™, a local delivery platform designed to treat bone morbidities (orthopaedic and dental). BonyPid™ loaded with antibiotics was tested in humans for the treatment of contaminated or infected severe open long-bone fractures.

Gustilo type III open fractures are associated with a high infection rates in spite of instituting standard of care (SOC) consisting of intravenous antibiotics, irrigation and debridement (I&D), and delayed wound closure. Locally-delivered antibiotic has been proven to assist in reducing infection in open fractures. The aims of this study are to determine the effectiveness and safety of the new implantable and biodegradable antibacterial BonyPid™ in preventing bacterial infections and initiating bone growth in open fractures.

The osteoconductive antibacterial BonyPidTM used is a synthetic bone void filler (comprised of ≤1 mm β-tricalcium phosphate granules) coated by a thin layer (≤20 µm) of PolyPid nanotechnology formulation. Upon implantation, the coating releases doxycycline at a constant rate for a predetermined period of 30 days. One BonyPidTM vial of 10 grams contains 65 mg of formulated doxycycline.

After approval, sixteen subjects with Gustilo type III open tibia fractures, were implanted with the BonyPidTM immediately on the first surgical intervention (I&D), followed by external fixation. Patients had periodic laboratory, bacteriology and radiology follow-up.

Six months results showed that no infection developed and only one BonyPidTM implantation was needed with no subsequent I&D, in the target tibia fracture. Immediate soft wound closure was done in 6/16 subjects following implantation. Out of 10 remaining subjects, 3 needed soleus muscle transfer-skin grafting and 7 required delayed primary closure; by skin grafting (5) or suturing (2). Early callus formation was seen at 8-12 weeks post-surgery, followed by bone healing seen from 16 weeks onwards. Safety of implantation was remarkable, with only one deep infection at a fibular open fracture without BonyPidTM implantation. One BonyPidTM related adverse event caused delay in skin healing due to excessive granules in the superficial soft tissues.

CONCLUSION

BonyPidTM demonstrate the advantages of long lasting, controlled release rate and protected reservoir a PolyPid nanotechnology in both efficacy and safety profile. BonyPid™ is effective in reducing bone infection and promoting early callus formation, resulting in early bone healing. In contrast to any other non-antibiotic bone void fillers, BonyPidTM is safe for immediate implantation into contaminated/infected severe open-bone fractures. Results support that one month release of doxycycline in a controlled manner provides an effective way for treating open fractures. This new local antibiotic delivery system is applicable in unmet medical situations associated with localized infections.

PolyPid platform technology is also used for developing of coatings for medical devices. Orthopaedic Hydroxyapatite coated metals, metal screws and pins were coated by thin layer of PolyPid formulation releasing antibiotic in a constant controlled rate for up to several months.

UNDERSTANDING INFLAMMATORY REACTIVITY OF NANOMATERIALS: FOCUS ON BIODEGRADATION

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Inflammation is a complex protective response to harmful stimuli including pathogens, damaged or dying cells, and other irritants. However, inflammation is a double-edged sword insofar as chronic inflammation can lead to destruction of tissues thus compromising the homeostasis of the organism. Recent studies have shown that nanomaterials – including carbon nanotubes – can trigger activation of the inflammasome complex in macrophages resulting in secretion of pro-inflammatory cytokines. In addition, inflammatory cells – neutrophils and eosinophils – are capable of peroxidase-mediated degradation of carbon nanotubes, with mitigation of the pro-inflammatory effects. Neutrophils produce so-called neutrophil extracellular traps (NETs), chromatin fibers studded with granule proteins, to ensnare bacteria and recent work has revealed that NETs also capture and degrade carbon nanotubes. Taken together, these findings underscore that inflammation is both good and bad.

FURTHER READING

Shvedova AA, Pietroiusti A, Fadeel B, Kagan VE. Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. Toxicol Appl Pharmacol. 2012;261:121-33.
Bhattacharya K, Andon FT, El-Sayed R, Fadeel B. Mechanisms of carbon nanotube-induced toxicity: focus on inflammation. Adv Drug Deliv Rev. 2013 [in press].

TALKING TO MEMBERS OF VENTURE CAPITAL – HOW TO GET SUCCESS

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Beside the scientific challenges of nanotechnology lead by the up-to-date existing uncertainties in risk and safety issues, a strong co-operation between stakeholders on business and research level is required. To deal with important questions, to bear in mind that an application or a product should have impact to solve social issues, and that via the scientific results – in best case – sozio-economic value can be achieved, will be some key elements for sustainable development of nanotech-sector. It will be important to also enhance the social potential of nanotechnology resulting in benefits for medicine and pharmacy and strengthening of regulators, national authorities and industry. Recent research showed that direct investments seem to be the most welcome way of financing research. Therefore, researchers should be aware of the importance to also attract investors such as venture capitalists, etc. via their scientific work. However, investors need to understand the potential of the scientific results; otherwise they will not identify the ability to gain value out of the investment.
Within this contribution some of the above mentioned key challenges will be addressed: e.g. what can be the right questions, and according to this, the right answers from researchers. Additionally, within this talk the so called tool "Nano-Deals generator", developed in the NANOFORCE project, will be introduced, providing assistance to researchers and investors, to facilitate the communication aiming on project and business development, and to foster the market implementation of nanotechnology application.

NANOMEDICINE AGAINST MALARIA: USE OF POLY(AMIDOAMINE)-BASED NANOVECTORS FOR THE TARGETED DRUG DELIVERY TO PLASMODIUM

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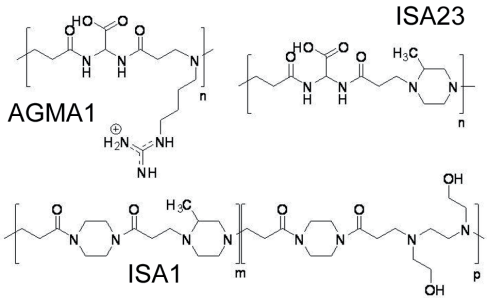
More than 40% of the world's population lives at risk of contracting malaria, with most recent estimates indicating several hundred million clinical cases and 665,000 deaths in 2010, of which the large majority are children below 5 years. The recent call for elimination and eradication of the disease requires research from multiple fronts, including developing strategies for the efficient delivery of new medicines. When taking a blood meal, the female Anopheles mosquito inoculates Plasmodium sporozoites that in the liver infect hepatocytes and proliferate into thousands of merozoites; these invade red blood cells (RBCs), where they develop first into rings, and then into the late forms trophozoites and schizonts. Schizont-infected RBCs burst and release more merozoites, which start the blood cycle again. Because the blood-stage infection is responsible for all symptoms and pathologies of malaria, Plasmodium-infected RBCs (pRBCs) are a main chemotherapeutic target.
Since antimalarial drug delivery currently relies on soluble compounds with little or no specificity for pRBCs, the administration of most drugs requires high doses. However, unspecificity of toxic drugs demands low concentrations to minimize undesirable side-effects, thus incurring the risk of sublethal doses favouring the appearance of resistant pathogen strains. Targeted nanovector systems can fulfill the objective of achieving the intake of total amounts sufficiently low to be innocuous for the patient but that locally are high enough to be lethal for the parasite. The development of novel drug delivery systems is not only less expensive than developing new drugs, but may also improve release of antimalarials at the desired rates. Nanomedicine, which uses nanosized tools for treatment of disease, has not yet been extensively applied to malaria, but the administration of antimalarial compounds would largely benefit from a method based on nanoparticles of sufficiently small size to be formulated for oral intake. Ideally, such nanoparticles should be able to penetrate pRBCs with the aid of targeting molecules small and of low cost. The nanoparticles themselves must be economically affordable since the final product is expected to be used in malaria endemic regions, which in most cases are located in developing areas with low per capita incomes. Finally, the nanovector must be capable of carrying antimalarial compounds.
In a previous work[1;2], we had shown a 10-fold improvement in the in vitro activity of immunoliposome-encapsulated antimalarial drugs. However, although antibodies have an exquisite recognition of their corresponding antigens, some of their properties limit their role as targeting elements in antimalarial therapeutic strategies. First, antibodies are relatively expensive to produce, which is an undesirable characteristic when designing medicines to be used in developing countries. Secondly, they are highly immunogenic unless genetically engineered humanized forms are used. Finally, as a result of the high variability of Plasmodium proteins exposed on pRBC surfaces, any antibody will likely lose targeting efficacy as the parasite switches to new antigenic repertoires.
Polymers offer virtually unlimited diversity in chemistry, dimensions and topology, rendering them a class of materials that is particularly suitable for applications in nanoscale drug delivery systems. Polymeric nanoparticles are made of natural or artificial polymers that range in size between 10 and 1000 nm, and if adequately targeted they can be used to deliver highly localized drug doses into specific cell types or tissues. In the case of biodegradable polymers, which

decompose into products that can be completely eliminated by the body, significant advantages are their history of safe use, proven biocompatibility, a high surface/volume ratio, and ability to control the time and rate of polymer degradation and drug release, thus increasing the half-life of bioactives. Poly(amidoamine)s (PAAs) are a family of synthetic polymers exhibiting a combination of properties imparting them a considerable potential in the biomedical field, being usually biocompatible, degradable in water and bioeliminable. They display membrane disruptive capacity in response to a decrease in pH, conferring endosomolytic properties in vitro and in vivo. These characteristics led us to explore PAAs as nanovectors for the targeted delivery of antimalarial drugs.

AGMA1 (Figure 1), a prevailingly cationic amphoteric PAA obtained by polyaddition of (4-aminobutyl)guanidine (agmatine) to 2,2-bis(acrylamido)acetic acid (BAC), was studied as a potential DNA carrier and transfection promoter. In spite of its cationic character, AGMA1 is neither toxic (maximum tolerated dose is ca. 500 mg kg-1) nor hemolytic in the pH range 4.0-7.4 and circulates in the blood for a long time without preferentially localizing in the liver. A second amphoteric PAA exhibiting an interesting combination of properties is ISA23, derived from the polyaddition of 2-methylpiperazine to BAC. ISA23 is approximately as biocompatible as dextran, and after intravenous injection it is not captured by the reticulo-endothelial system and has also a prolonged permanence in blood. The polycationic polymer ISA 1 is formed by Michael type polyaddition of bis(acryloyl)piperazine, 2-methylpiperazine and bis(hydroxyethyl-ethylenediamine).

PAA-derived nanovectors combine into a single chemical structure drug encapsulating capacity, antimalarial activity, low unspecific toxicity, high biodegradability, specific pRBC targeting, and affordable synthesis cost, all of which makes of them good candidates for oral administration formulations that can bring nanomedicine into the antimalarial therapeutic arena[3].

A



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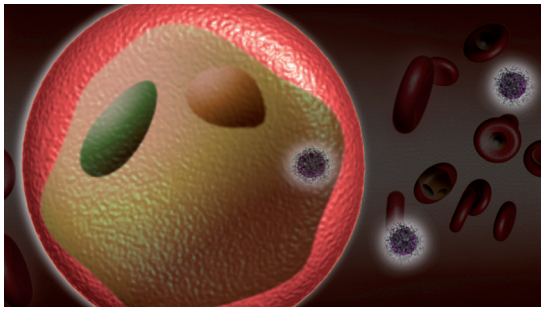

***Figure 1**. A, Chemical structures of AGMA1, ISA1, and ISA23. B, Schematic view of the application of polymeric nanocapsules to the targeted delivery of antimalarial drugs.*

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1. Urbán P, Estelrich J, Cortés A, Fernàndez-Busquets X. A nanovector with complete discrimination for targeted delivery to Plasmodium falciparum-infected versus non-infected red blood cells in vitro. J.Control.Release 2011;151:202-211.
2. Urbán P, Estelrich J, Adeva A, Cortés A, Fernàndez-Busquets X. Study of the efficacy of antimalarial drugs delivered inside targeted immunoliposomal nanovectors. Nanoscale Res.Lett. 2011;6:620.
3. Fernàndez-Busquets X, Urbán P, Ranucci E, Ferruti P, Fenili F, Manfredi A, and Mauro N. Amphoteric polyamidoamines in the treatment of malaria. Patent EP12192633.1, 2012

DEVELOPMENT OF NEW MULTIFUNCTIONAL NANO-CONSTRUCTS BEARING ANTIBODY-DERIVATIVES FOR HER2-POSITIVE CELLS TARGETING IN BREAST CANCER

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BACKGROUND

Among the various kinds of tumors, breast cancer represents a target of primary interest, both from a diagnostic and a therapeutic point of view. In this context, a powerful device is a target-specific magnetic nanoparticle, able to be at the same time an efficient contrast agent for MRI detection and a good delivery system for anticancer drugs. Recently, we have designed a versatile molecular superparamagnetic nanocomplex, functionalized with the commercial anti-human epidermal growth factor receptor 2 (HER2) trastuzumab (TZ) by the intermediation of a recombinant low-molecular weight monodomain of protein A. These nanocrystals were demonstrated to be effective in selectively targeting HER2 in MCF7 breast cancer cells both in vitro[1] and in vivo.[2]

Nowadays, many efforts are devoted to design nanosystems which can be localized at cancer cells for an extended period of time, in order to optimize diagnostic sensitivity and therapeutic efficiency. Recent studies were aimed to correlate the physicochemical properties of nanomaterials (i.e. size, shape and surface charge) with the kinetics of internalization, the intracellular localization and the rate of degradation in target cells.[3] Indeed, an investigation of how nanoparticle properties affect its delivery in cancer cells is essential to redesign products able to accumulate efficiently at the tumor.

Three different hybrid multifunctional nanoparticles (MNP) were developed (Figure 1) consisting in an iron oxide nanocrystal core coated with a highly soluble amphiphilic polymer and functionalized with TZ, a TZ half-chain or an anti-HER2 scFv.[4]

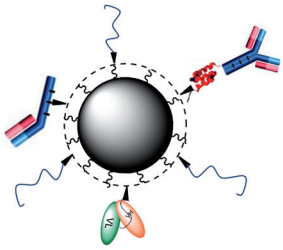

***Figure 1**: PMA-coated Fe304 nanocrystals, peghylated and biofunctionalized with MNP-TZ, -HC or scFv*

AIM

Aim of this study is to investigate the dependence of targeting efficiency of HER2-directed multifunctional nanoparticles engineered with TZ-derivatives from the structural features of the immobilized homing ligands. This goal has been achieved by in vitro, in vivo and ex vivo experiments to compare the targeting potential of the nanoconstructs toward a HER2-positive breast cancer model (MCF7). Accurate cellular and subcellular analyses were also performed to characterize the interaction of the nanoconstructs with the tumor cells.

RESULTS AND DISCUSSION

The in vitro assessment of HER2 targeting efficiency showed that all three fluorescent TZ-variants were able to target HER2-positive MCF7 breast cancer cells (Figure 2). Moreover, confocal images revealed that, while MNP-TZ were mainly distributed in aggregates in proximity of cell surface after 1 h of incubation at 37°C, MNP-HC and MNP-scFv readily spread out into the cytoplasm toward nucleus (Figure 2B). The resolution of MNP-scFv signal appeared limited by the lower fluorescence intensity of these nanoparticles.

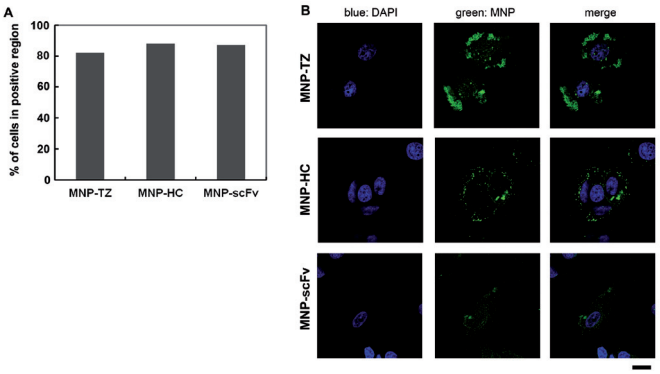

Figure 2. A) Percentage of cells in the positive region after flow cytometry assay. MCF7 cells were incubated 1 h at 37 °C with MNP-TZ, MNP-HC or MNP-scFv.
B) Confocal microscopy images of MCF7 cells, incubated for 1 h at 37 °C with FITC-labeled MNP-TZ, MNP-HC or MNP-scFv. Nuclei stained with DAPI. Scale bar: 10 µm.

Starting from the assumption that the ideal candidate for application in cancer diagnosis and therapy should be a nanosystem able to localize itself at cancer cells for an extended period of time, we decided to compare the behavior of the three nanoconstructs from 5 to 48 h after injection in MCF7 tumor-bearing mice (Figure 3).

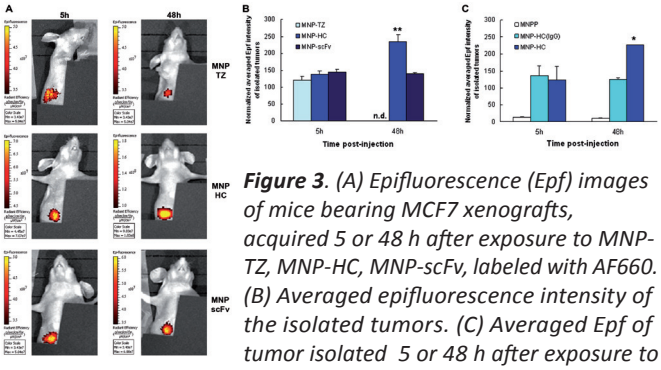

***Figure 3**. (A) Epifluorescence (Epf) images of mice bearing MCF7 xenografts, acquired 5 or 48 h after exposure to MNP-TZ, MNP-HC, MNP-scFv, labeled with AF660. (B) Averaged epifluorescence intensity of the isolated tumors. (C) Averaged Epf of tumor isolated 5 or 48 h after exposure to MNPP, MNP-HC(IgG) or MNP-HC. Epf values have been normalized on fluorescence intensity of injected solution in order to keep into account the differences in intrinsic fluorescence emission for each nanocomplex. Mean ± S.E. of 3 different samples for each experimental condition. *P<0,05; **P<0,01 vs. 5 h (Student's t-test).*

Figures 3A and 3B, shows that MNP-scFv is a more efficient probe compared to MNP-TZ, because its Epf in the tumor mass did not decrease over time, as occurred with the whole antibody. However, the best result has been obtained with MNP-HC, whose Epf in the 48 h xenograft was even higher than that recorded after 5 h. Therefore, while MNP-TZ followed the same mechanism of entry and fate in cancer cells previously described for similar TZ-engineered nanoarchitectures,2 regarding a receptor-mediated internalization followed by degradation of the organic shell by a lysosomal pathway, a comparable degradative process did not seem to concern MNP-HC and MNP-scFv within the first 48 h from injection.
To clarify the different behavior of MNP-TZ, MNP-HC and MNP-scFv in cancer cells, especially referred to their different timing of interaction and incorporation, a comparative analysis of endocytosis, trafficking and metabolism of the three nanoprobes in MCF7 cells was performed by transmission electron microscopy (TEM). The overall results demonstrated that MNP-TZ, whose low stability originated aggregates in serum of size up to 310 nm, were endocytosed by cell membrane at a slower rate respect to MNP-HC and MNP-scFv (not shown), in agreement with in vitro assays (Figure 2B). Once internalized, all three nanoconstructs were directed to the endosome-lysosome pathway, but significant differences were observed in degradation timing. In particular, MNP-TZ achieved the lysosomal system to be degraded within the following 48 h (Figure 4A), while MNP-scFv and MNP-HC remained within endosome-like vesicles for an extended period of time: at 48 h post-injection MNP-scFv were observed both in endosomes and in lysosomes (Figure 4C) and MNP-HC were only detected in endosomes (Figure 4B). We assumed that also MNP-HC would be finally driven to the lysosomal pathway, even though at a slower rate. The different timing in MNP-TZ, MNP-HC and MNP-scFv intracellular trafficking is not fully comprehensible, although we suppose it could be related to the different interaction of the peptide ligands with HER2 receptors.

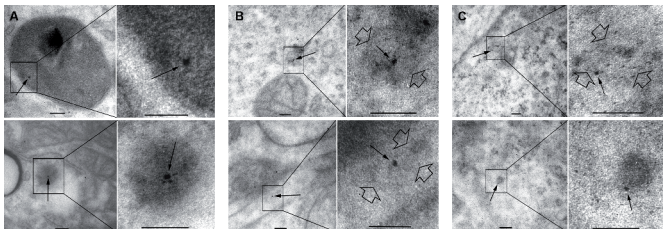

***Figure 5**. TEM images of MCF7 tumors isolated at 48 h postinjection of MNP-TZ (A), MNP-HC (B) or MNP-scFv (C). To the right of each single figure, the correspondent higher magnification image is reported. Nanoparticles and cell membranes are evidenced by small and big arrows, respectively. Bars: 100 nm*

Since the EPR effect could affect the strong accumulation of biofunctionalized MNP observed in mice xenografts (Figure 3A,B), we finally verified the real contribution of the active targeting to the delivery of nanoparticles in solid breast cancers. Therefore, we compared the accumulation in the tumor mass of MNP-HC to that of non-specifically-targeted nanoparticles, as unconjugated peghylated MNP (MNPP) or nanoparticles engineered with a non-specific IgG ligand (MNP-HC(IgG)). We demonstrated that only in the presence of specific HER2-directed ligands on MNP surface, the overall concentration of nanoparticles in solid tumors increased over time (Figure 3C).

CONCLUSIONS

1) the aggregation state of nanoparticle formulation affects their endocytosis rate by target cells; 2) the interaction of the different peptide ligands with HER2 receptors affects the intracellular fate of nanoparticles; 3) the extended period of accumulation of MNP-HC in the tumor cells makes this nanoparticle the ideal candidate for future application in breast cancer treatment; 4) a short-term localization of nanoparticles in vivo is given by EPR effect regardless of the presence of targeting ligands, even though only HER2-directed functionalization allows a long-term accumulation in cancer cells.

REFERENCES

[1] Mazzucchelli S, et al. ACSNano 2010; 4: 5693-5702.
[2] Corsi F, et al. ACSNano 2011; 5:6383-93.
[3] Chithrani BD and Warren CWC Nano Lett. 2007; 7:1542-50.
[4] Sommaruga S, et al. Appl. Microbiol. Biotechnol. 2011; 91:613-21.

DEVELOPMENT STAGES OF PARENTERAL NANOMEDICINE: FROM DISCOVERY TO CLINICAL PHASE 1

ANDREAS FISCH

Novartis Pharma AG Basel Switzerland

This talk is specifically addressed to young Nano-scientists from who want to learn about the basic requirements for development of parenteral nanomedicine for clinical development in the pharmaceutical industry.
In general, nanomedicin has to meet the same requirements as other new therapeutics: defined and reproducible quality, safety and efficacy. However, to due the more complex structure, those basic requirements are more cumbersome to be addressed in an appropriate way.
In vivo performance i.e. efficacy and safety of parenteral nanomedicine is crucially linked to the quality of the starting materials, the surface properties of the nanocarriers, the way the drug substance is integrated into the nanomedicine, eventual linker and spacer design and the in vitro and in vivo stability of the final drug product. Thorough characterization of the starting materials as well as the surface of the nanomedicine ifself is key but at the same time challenging. A reproducible and predictive in vitro release test should

be the basis for formulation optimization.
In addition, the process of making the nanomedicine critically defines its in vivo performance and its suitability for commercial production. Especially the scaling up risks cannot be underestimated. Assurance of sterility and absence of pyrogens is sometimes also difficult to achieve.
A high level overview on in vitro and in vivo efficacy and safety tests as well as on basic GLP pre-clinical testing is given.
Finally it has to be stated that since nanomedicine has to compete and be superior to current medicine including already established nanomedicine like liposomes not only in terms of clinical outcome but also with regards to safety and production costs. Therefore, all attempts must be taken to reduce complexity of the nanomedicine and its manufacturing process.
The take home message of this presentation is that success of parenteral nanomedicine in the clinics and on the market must be ensured already at the bench by using development concepts based on pharmaceutical development standards and regulatory guidances.

WHAT TO LOOK AT TO ACHIEVE IN EUROPEAN PROGRAMMES APPLICATIONS

HEICO FRIMA
European Commission, Directorate-General for Research & Innovation

The European Union 7th Framework Programme for Research & Technological Development (FP7) (2007 – 2013) will soon comes to its end as far as launching Calls for Proposals is concerned. Projects funded from FP7 Calls for proposals will continue running and will deliver their results over several more years, with the last FP7 projects ending in the year 2018.
The Nanomedicine research community has been very active and successful in the various themes and priorities of FP7. The FP7 NMP (Nanotechnology, Materials and Production Technologies) Programme supports an estimated 85 nanomedicine projects with € 400 million EU funding. These projects develop novel biomaterials for implants and regenerative medicine, novel nano-enabled diagnostics and therapy of diseases, and smart implants using bio-nano-info convergence. A solid pre-clinical nanoscience and nanotechnology base for application in medicine has meanwhile been established in European R&D laboratories.
Further nanomedicine research and development work will be undertaken in the next framework programme Horizon 2020 (2014 – 2020). Horizon 2020 has a proposed budget of € 80 billion. The first Calls for Proposals of Horizon 2020 will be published early in the year 2014. Horizon 2020 has three main priorities: 'Excellent Science', 'Industrial Leadership' and 'Societal Challenges' and each of these has several specific objectives. The priorities in Horizon 2020 are all bound by the same legal and contractual framework, including similar basic rules and management principles. For instance, funding of the projects takes place through Calls for Proposals that are based on an annual workprogramme and the proposals are evaluated by independent experts through peer review using a common evaluation system. The same set of basic IPR and funding rules apply. Together, the funding opportunities in Horizon 2020 span the innovation cycle from early frontier research to rather close to market activities.
Nanomedicine developments are complex and involve multiple actors. It takes many years from the initial project idea to the introduction of a new product to the market and the clinic. Therefore it is very likely that a new product development needs several R&D projects, covering different development and funding phases. Along the way, different funding sources will be needed and the consortium will probably change composition.
With a project idea in mind, it is therefore first of all important to reflect on the stage of development of the R&D and on the overall R&D and future exploitation aims. What is the global state-of-the-art in the field and what is the IPR situation? Is it an early stage frontier research project, is it pre-clinical research or is the development already in the clinical testing phase? What is the opinion of medical regulatory specialists on your novel nanomedicine product concept? What is the size and scope of the project or of the problem to be solved? Is an EU research project the best choice, or could the project be funded by private sources such as venture capital, regional or national funding, or is maybe a combination of funding sources needed? What other organisations will be needed as partners to bring the project to a successful end?
Answering these questions will help you to identify the most suitable funding source(s) for submitting a proposal for funding. Study carefully the requirements and expectations of the call. Keep in mind that in European research programmes there is no point in submitting a proposal that does not properly meet the requirements and expectations of the call. It has to be stressed that the preparation of a proposal is a team work. Inputs will be needed from all the partner organisations, from specialists in various scientific and technical disciplines, from industrialists, end-users, marketing specialists and the organisations' administrations for providing financial and administrative data. The management of the partner organisations need to support the proposal. Make good use of available specialist advice and networks, such as from the European Technology Platform for Nanomedicine, EU projects and technology transfer departments or venture capitalists, National Contact Points and Helpdesks.
Usually there is strong competition for limited European project funding and only the best responding projects can be retained. It is certainly challenging, but the preparation of a proposal is also a very enriching experience

AFM BEYOND IMAGING TOWARDS PERSONALIZED MEDICAL DIAGNOSTICS

CHRISTOPH GERBER

Recently AFM technologies have entered the clinic in a variety of application. Here we use a micro-fabricated silicon cantilevers array platform that offers a novel label-free approach where ligand-receptor binding interactions occurring on the sensor generate nanomechanical signals like bending or a change in mass which is optically detected in-situ. It enables the detection of multiple unlabelled biomolecules simultaneously down to picomolar concentrations within minutes in differential measurements including reference cantilevers on an array of eight sensors. The sequence-specific detection of unlabelled DNA in specific gene fragments within a complete genome is shown. In particular the expression of the inducible gene interferon- a within total RNA fragments and unspecific back ground . This gives rise that the method allows monitoring gene regulation , an intrinsic step in shining light on disease progression on a genetic level. Moreover malignant melanoma, the deadliest form of skin cancer can be defected with this technology on a single point mutation level without amplification and in the background of the total RNA.

NANOPARTICLES FOR TRANSCUTANEOUS VACCINATION

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We recently introduced a new non-invasive transcutaneous vaccination strategy which uses nanoparticles (NP) to deliver encapsu-

lated antigens to the skin antigen presenting cells (APCs). [1] Infectious diseases impose a serious public health threat worldwide. HZI and HIPS work at the forefront to combat resistance, to improve our understanding of pathomechanisms and to develop new anti-infectives and vaccines. Among the strategies to fight infections only vaccination has the potential to eradicate the disease (WHO certified the eradication of smallpox in 1979). New, safe, efficient, and cheap vaccination strategies are desperately needed to meet the needs of a growing population in developing countries as well as the challenges of fast spreading infectious diseases due to global traffic (e.g. 2009 swine flu pandemics).
NPs which are applied onto the skin migrate into the hair follicle openings by a size-selective mechanism. [2] Inside the hair follicles antigen uptake by the rich pool of peri-follicular APCs is facilitated due to the absence of a stratum corneum (SC) barrier in the lower follicular orifice. Therefore this delivery strategy does not require application of any further measures which would reduce the skin barrier (such as tape stripping or abrasion etc.). We showed that transfollicular delivery of the nano-encapsulated model antigen ovalbumin (OVA) elicited similar proliferation of OVA specific CD4+ T cells as an i.m. injection of the same dose of soluble antigen. [1] Nano-encapsulation into polymeric NPs improved the delivery of OVA into the hair follicles on excised pig ears by a factor of at least 2.5-3 compared to OVA in solution and protected OVA from cleavage or aggregation so that it maintained its biological activity to a high degree during storage and shipping. [1]
As an important outcome of the study we observed in vitro as well as in vivo an intrinsic adjuvanticity of the NPs which was also influenced by the polymer material used. [1] The effect was even enhanced upon combination of nano-encapsulated OVA with known adjuvant molecules such as BPPCysMPEG (TLR2/6 agonist, Th1 dominated) [3], or cyclic di-AMP (Th1/Th2/Th17 balanced) [4] (unpublished results).
Acklnowledgements: This work was financially supported by a starting grant of the “Bill-and-Melinda-Gates Foundation” Grand Challenges Exploration Program.

[1] A. Mittal, A.S. Raber, T. Ebensen, K. Schulze, S. Weissmann, C.A. Guzman, U.F. Schaefer, C.M. Lehr, S. Hansen, Non-invasive delivery of nanoparticles to hair follicles - a perspective for transcutaneous immunization, Vaccine, accepted (2012).
[2] A. Patzelt, H. Richter, F. Knorr, U. Schaefer, C.M. Lehr, L. Dahne, W. Sterry, J. Lademann, Selective follicular targeting by modification of the particle sizes, J Control Release, 150 (2011) 45-48.
[3] B. Fuchs, S. Knothe, S. Rochlitzer, M. Nassimi, M. Greweling, H.D. Lauenstein, C. Nassenstein, M. Muller, T. Ebensen, A.M. Dittrich, N. Krug, C.A. Guzman, A. Braun, A Toll-like receptor 2/6 agonist reduces allergic airway inflammation in chronic respiratory sensitisation to Timothy grass pollen antigens, International Archives of Allergy and Immunology, 152 (2010) 131-139.
[4] T. Ebensen, R. Libanova, K. Schulze, T. Yevsa, M. Morr, C.A. Guzman, Bis-(3›,5›)-cyclic dimeric adenosine monophosphate: strong Th1/Th2/Th17 promoting mucosal adjuvant, Vaccine, 29 (2011) 5210-5220.

NEXT-GENERATION DRUG SCREENING, DEVELOPMENT AND DISCOVERY

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G protein-coupled receptors (GPCRs) are the most widely represented protein superfamily in the human genome. Drugs targeting GPCRs account for the majority of best-selling prescription pharmaceuticals in an annual $80B market making GPCRs an excellent bet for future drug discovery successes. However only a small proportion of GPCRs are currently targeted with drugs largely because of the difficulties in screening GPCRs for novel drugs. Currently, screening of GPCRs for new drugs requires cell based systems in large scale plate based assays which are expensive and laborious.
Additionally, the choice of cell type and endpoint signaling assay can bias the types of drugs identified. We are developing a novel drug screening technology that will allow ultra-high throughput screening of G protein-coupled receptor (GPCR) binding/activation by potential drug compounds. By exploiting the advances in single molecule detection and the ability to stably isolate fully active GPCRs in solution, the entire screening process can be performed in a nano-fluidic chip, where electric signals are used to determine the potential success of a drug. Importantly by isolating the receptors in solution we are able to exclude potential false positive “hits” that are inherent in cell based systems where endpoint assays can be activated by non-specific interactions.
Guided by numerical simulations, initial experimental results demonstrate the ability to electrically detect binding events of targets and compounds. This technique represents a fundamental shift in how drug screening can be performed: it removes the difficult and slow step of incorporating cell-based assays into the screening scheme by reducing the readout to an electrical measurement. We believe our next generation drug development technique will open up completely new horizons and revolutionize drug screening and discovery, similar to what the 1000-Dollar-Genome is expected to do to next-generation DNA-sequencing.

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SENSING SEPSIS: TECHNOLOGICAL ADVANCES IN SEPSIS DIAGNOSIS

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Sepsis remains a major cause of death in intensive care units and is reportedly responsible for more deaths than prostate cancer, breast cancer and AIDS combined. An estimated 18 million people contract sepsis every year. Because the clinical symptoms and laboratory signs currently used are not specific enough, sepsis diagnosis is often delayed. This delay in diagnosis and the correspondingly late administration of adequate therapy has a major impact on survival: Recently published studies have revealed that for every hour delay in the administration of appropriate (antibiotic) therapy there is an associated 7% rise in mortality (Dellinger, 2008).
The present contribution will discuss new biomarkers and technological approaches to facilitate rapid diagnosis of sepsis aimed to improve risk stratification and optimize treatments for severely ill patients. The presentation will focus on the use of nanoparticles and bioparticles for biomarker detection in clinical samples. Newly developed theranostic approaches are aimed to allocate hospital resources and ICU beds more accurately and cut down on the unnecessary use of antibiotics translating into drastically reduced costs.

Dellinger, R.P., Levy, M.M., Carlet, J.M., Bion, J., Parker, M.M., Jaeschke, R., Reinhart, K., Angus, D.C., Brun-Buisson, C., Beale, R., et al. (2008). Critical Care Medicine 36, 296-327.

NOT YET REALIZED NANOAPPLICATIONS FOR TRANSDERMAL ROUTE

MICHAEL HORSTMANN

Heterogenicity characerizes this interesting application area. Classically, for the actual absorption of nanoparticles via the skin, to a limited extent, hair-shafts have proven to be responsible for some transport, as has been shown by working groups both in Saarbruecken and Berlin.
Nanoscaled particles could specifically profit from poration and newer generation microneedle approaches. This technique does not allow for direct drainage to circulation, but repeated needle

movements increase the likelihood of particle presence close to absorptive sites in the epidermis.
After short discussion of powder injection possibilities, the finally most "solidly grounded" aspect will be explained: Taking advantage of solubility boosting effects by leveraging of the thermodynamic potential of API nanostructures. Technologies are at time only theoretically presented, in which nanoparticles of API are conserved in hydrophilic environment, and embedded in adhesive, lipophilic polymers to employ their effect via swelling and supersaturation.

A short summary and outlook will finish the lecture.

SCIENTIFIC INTRODUCTION: WHERE NANOMEDICINE AND TARGETED MEDICINE MEET

PATRICK HUNZIKER
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COMPREHENSIVE TARGETING IS THE AVENUE TO A PERSONALIZED, HIGHLY EFFECTIVE, INNOCUOUS, AND COST-EFFECTIVE MEDICINE OF THE FUTURE

Arguably, the current era of "one-size fits all" medical strategies is associated with suboptimal efficacy, unnecessary toxicity, and wasting of resources in healthcare.
Why ? The currently dominating paradigm of medical care, named "evidence-based medicine" claims that ultimate truth for medicine is found in randomized multi-center trials and meta-analyses, in particular those focusing on mortality. The assumption is that statistics describing average efficacy of a therapy in a large cohort included in a clinical trial will be predictive for the benefit of this therapy in future individual patient and will therefore represent the best therapeutic choice. This will typically include a significant number of individuals at low risk, with a harm/benefit ratio and cost/benefit ratio in a range that few events are avoided in this subgroup, but many side effects are induced, and financial expense is substantial; likewise, those patients with highest risk may not get sufficiently intense therapy to circumvent an adverse clinical course. This neglect of the huge variability in genetic and phenotypic setup of mankind (reflected in genomics, epigenetics, proteomics etc) which is of major importance for disease course and therapeutic efficacy, relegates knowledge about individual variations in pathophysiology, anatomic presentations and temporal course in a given disease ("the art of medicine") to second class information. As the limitations of such a "one size fits all" approach are increasingly evident[1], we need to progress beyond "evidence based medicine" towards a "knowledge-based medicine" that embraces a broader view by giving adequate weight to all knowledge about a patient and his disease, integrating cohort information with molecular information about the individual.

TARGETING INDIVIDUALS INSTEAD OF AVERAGED COHORTS

We need to learn how to integrate genetic setup, molecular presentation, and clinical variables with randomized trial information in a way that patient selection, drug selection, drug dose, drug duration result in an optimal individual benefit, and with the lowest individual side effect profile . Only such a personalized approach based on molecular information has the chance of improving both, quality AND cost effectiveness of today's medicine in parallel.

TARGETING THERAPIES TO THE RIGHT ORGANS, CELLS, AND RECEPTORS, AND PATHWAYS

In the past decades, biotechnology, systems biology and nanomedicine have developed in separate tracks, but it is increasingly evident that their combination will be of particular benefit to clinical medicine:
Medical biotechnology, definable as the use of biomacromolecules, cells, and biological processes for a medical purpose, has been successfully applied in a number of medical challenges, visible in the successful clinical introduction of insulin, growth factors, antibody therapies (and certain cell therapies). In a typical scenario, a specific ligand/target receptor interaction is inhibited by a monoclonal antibody to achieve a desired therapeutic effect, an application usually termed "targeted therapy", in particular when used in cancer therapy. The term "targeted therapy" is also used for inhibition of such tumor-specific pathways by small molecular inhibitors.
Nanomedicine has been based on insights on supramolecular chemistry, molecular biology, physics and material sciences. It has developed the capability of designing complex nanosize, supramolecular objects composed from a broad range of materials including polymers, metals, lipids, biomacromolecules and small drug molecules. This allows the design of multifunctional nano-objects with separation of concerns like receptor docking and biologic effect on the target, a strategy for which the term "targeted delivery" is typically used. Beyond the basic concept of such delivery, a major interest in nanomedical approaches is the possibility to embed complex functionality, like biosensors and triggered functionalities, into these nano-objects.

TARGETING: COMPLEMENTARY VALUE OF NANOMEDICAL AND BIOTECHNOLOGICAL APPROACH

The greatest potential of those apparently separate fields is, however, in their interaction: For example, peptides and antibodies are excellent ligand molecules on nanocarriers built from synthetic materials: nanocarriers permit a much higher "payload per ligand ratio" compared to antibody drug conjugates. Conversely, nanomedical carrier technologies appear to be the enabler for future breakthroughs in biotechnology like the targeted use of nucleic acid silencing technologies (RNA inhibition) in clinical application.

MEDICAL TARGETING OF THE FUTURE BASED ON SYSTEMS BIOLOGY OF DISEASE AND INDIVIDUALS

Medical targeting of the future will combine disease- and patient-specific genomics and gene expression analysis, make use of systems biology approaches to integrate this information into a disease model at the level of the target cell, will choose or assemble suited multifunctional carrier/payload combinations and will exploit theragnostic capabilities of nanomedical objects for therapy and disease monitoring in combination.
For medical biotechnology as well as for nanomedicine, understanding specific diseases in specific individuals based on a systems approach, including elucidation of suited cellular targets that yield therapies with optimum benefit, minimum side effect and translate into improved cost efficacy of medicine are the way to advance together.
Such a medicine of the future will overcome the simplistic "evidence-based" one-fits-all approach and replace it by a comprehensive "knowledge-based" medicine that puts the individual again to the centre, delivering individually tailored therapies that are powerful, innocuous and cost-effective.

[1]Joshua J. Goldman and Tiffany L. Shih. The Limitations of Evidence-Based Medicine—Applying Population-Based Recommendations to Individual Patients. Virtual Mentor. January 2011, Volume 13, Number 1: 26-30.

THE ERADICATION OF ATHEROSCLEROSIS

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A major point on the agenda of the medicine of the future is the eradication of atherosclerosis.
Atherosclerosis is a major cause of morbidity, the leading cause of death, and an important driver of healthcare costs. This presentation looks into pathophysiologic features potentially permitting targeted management of atherosclerosis, and discusses requirements for successful application of targeted delivery and/or targeted therapy of atherosclerosis. It summarizes current efforts in the field and evaluates the value of molecular imaging, targeted therapeutic modalities and theragnostic modalities.

IG-001 – A NON-BIOLOGIC NANOPARTICLE PACLITAXEL FOR THE TREATMENT OF OVARIAN CANCER

LARN HWANG, Chao Hsiao, Kouros Motamed, Vuong Trieu. IG-DRASOL, Inc. Fountain Valley, CA 92708 USA.

INTRODUCTION

IG-001(Genexol-PM), like nab-paclitaxel, is a polymer bound nano-particle paclitaxel. IG-001 utilizes biodegradable di-block copolymer composed of methoxy poly (ethylene glycol)-poly (lactide) to form nanoparticles with Paclitaxel containing hydrophobic core and a hydrophilic shell. (Fig.1) IG-001 is being developed for difficult to perfuse hypoxic tumors such as pancreatic cancer and ovarian cancer.

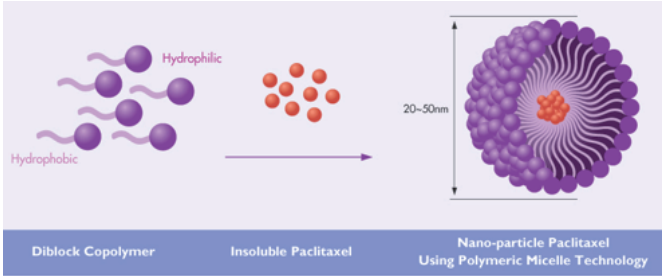

***Fig. 1**: IG-001- a cremophor- free polymeric micelle formulation of paclitaxel*

METHODS

Clinical efficacy was evaluated across multiple cancer indications including metastatic breast cancer (MBC), non small cell lung cancer (NSCLC), pancreatic cancer and ovarian cancer. Phase I ovarian trial design: six patients/dose level were treated with IG-001 at 220, 260, and 300 mg/m2. Phase II ovarian trial design: randomized, two-arm trial, primary advanced epithelial ovarian cancer consisting of 100 pts (50/each arm). Control Arm: Solvent based paclitaxel 175mg/m2 IV + Carboplatin 5 AUC IV, 3 weeks, 6 cycles and Experimental Arm: IG-001 260mg/m2 IV + Carboplatin 5 AUC IV 3 weeks, 6 cycles. Phase III ovarian trial design- planning phase to consist of therapeutic drug monitoring and biomarker testing.

RESULTS

Clinical trials conducted with IG-001 demonstrated significant anti-tumor activities comparable to historical data for Abraxane. Across three cancer indications (MBC, NSCLC, and Pancreatic Cancer), IG-001 was more active than historical Taxol. Since Pancreatic Cancer is known to be poorly-perfused, IG-001 activity in this indication is consistent with it being able to penetrate poorly-perfused tumors (Table 1).

Activity of IG-001 was investigated in animal model and in phase I-III trials. In a preclinical xenograft study, 10 animals per group of nude mice were implanted subcutaneously with SKOV3 (ovarian carcinoma). Animals were treated on q4x3 schedule. IG-001 at equitoxic dose was more effective than Taxol (Figure 2).

Metastatic Breast Cancer	NEXT-GEN SOLVENT-FREE		SOLVENT-BASED		
	IG-001 (n=41) (Ph2 Korea)	Abraxane®' (n= 97) (US PI)	Taxol® (n=235) (US PI)	Taxotere® (n=408) (US PI)	Taxotere® (n=161) (US PI)
	1st line 300 mg/m² q3w	1st line 260 mg/m² q3w	2nd line/6 mo recur 175 mg/m² q3w	Anthra-pretreated patients	Alkylating agent pretreated
ORR	59%	42%	28%	28%	45%
PFS	9 M	7 M	4.2 M	4.3 M	6.5 M
OS	>20M	18 M	11.7 M	11.4 M	14.7 M

Advanced NSCLC	NEXT-GEN SOLVENT-FREE			SOLVENT-BASED	
	IG-001 (n=69) (Ph2 Korea)	Abraxane® (n=43) (Ph2 US)	Abraxane® (n=521) (US PI)	Taxol ® (n=195) (US PI)	Taxotere® (n=408) (US PI)
	1st line 230-300 mg/m² q3w + Cis	1st line 260 mg/m² q3w mono	1st line 100 mg/m² weekly + Carbo	1st line 250 mg/m² q3w + Cis	1st line 75 mg/m² q3w + Cis
ORR	38%	15.5%	33%	23%	31.6%
PFS	5.8 M	6.0 M	6.3 M	4.9 M	4.9 M
OS	21.7 M	11.0 M	12.1 M	10.0 M	10.9M

Advanced Pancreatic Cancer	+ GEMCITABINE		
	IG-001** Ph1 (Russia)	Abraxane® ‡	Abraxane®
	1st line ph 1 220-300 mg/m²q3w+ 1250 mg/m² Gem N=18	1st line ph 2 125 mg/m²+ 1000 mg/m² Gem wkly N = 44	1st line ph 3 (MPACT') 125 mg/m² + 1000 mg/m² Gem wkly N= 861
ORR	31.3%	48%	23% (vs. 7%)
PFS	5.6 M	--	5.5 M (vs. 3.7M)
OS	>7 M	12.2 M	8.5 M (vs. 6.7 M)

***Table 1**. Clinical efficacy of IG-001 versus MBC, NSCLC, and Pancreatic Cancers*

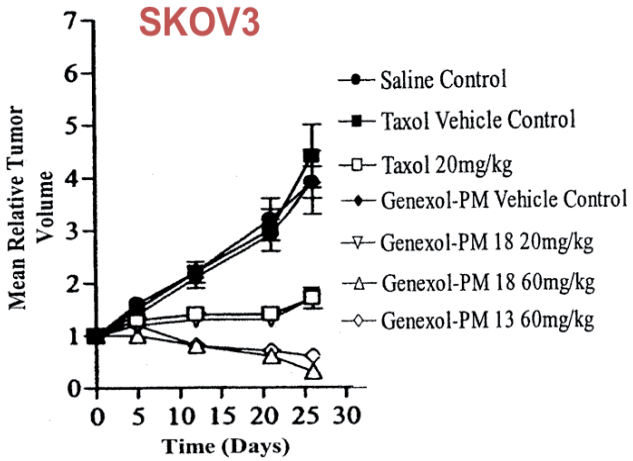

***Fig. 2**: Comparison of IG-001 vs. Taxol in SKOV3 xenograft study.*

In the multi-institutional Phase I clinical trial of cremophor-free, polymeric micelle formulation of paclitaxel with carboplatin for patients with advanced epithelial ovarian cancer: 1) the primary objective was to determine MTD (Maximum Tolerated Dose) and Recommended Dose for Phase II Trial; and 2) the second objectives were Dose Limiting Toxicity and Safety. DLT was shown as grade 4 myalgia in one patient at 300 mg/m2. MTD was not determined in this phase I trial (over 300mg/m2). Response rate: 94.1% (16/17) by investigator/94.4% (17/18) by reviewer, not different according to the dosage (p=1.00). Recommended dose for Phase II Trial of Cremophor-free paclitaxel in combination with Carboplatin : 260mg/m2 (considering the safety and the dose in phase III trial in breast cancer). Phase II is ongoing with enrollment completed and data to be reported at the meeting. Phase III trial is designed to incorporate Paclitaxel TDM and biomarker testing to fully personalized IG-001: 1) Personalized Dosing by Point-of-Care Therapeutic Drug Monitoring (TDM)—Point-of-care device allowing for self-sampling and self-monitoring of blood paclitaxel level (personalized dosing would reduce toxicity while increasing efficacy); and 2) Patient Selection by Point-of-Care Biomarker Testing—Biomarker testing by point-of-care device to identify responders and non-responders. The innovative aspect of this phase III design is the rapid & quantitative point-of-care lateral flow test to allow for personalized dosing (Pac-TDM) and biomarker testing (PD biomarkers, predictive and prognostic biomarker testings). These tests eliminate the burden of frequent venipuncture, eliminate the need for well-equipped processing and storage facilities, most useful at home, primary care physician's office, and most importantly, no dilution or manipulation of the sample is required due to our expanded dynamic range technology.

CONCLUSIONS

IG-001 is effective across multiple cancer indications. Clinical development of IG-001 in ovarian cancer is ongoing. Phase I data was encouraging and phase II data forthcoming. IG-001 could be considered to be superior to Cremophore based paclitaxel because of the possibilities of delivering higher paclitaxel dose without additional toxicities. Phase III trial design incorporating therapeutic drug monitoring and biomarker testing should further improve on IG-001 clinical activities.

ENDOCYTOSIS AND INTRACELLULAR TRANSPORT OF NANOPARTICLES: PRESENT KNOWLEDGE AND HOW TO PROCEED

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Nanoparticles (NPs) have proved promising results both as delivery vehicles for therapeutics and as imaging agents for various diseases such as cancer. Importantly, as most molecular targets of the drugs are inside the cell, the NPs should be taken up by the diseased cells in order for the drugs to efficiently reach their target

of action. Based on our long experience in studies of endocytic mechanisms, I will introduce the various endocytic pathways and where the NPs end up within the cells. The challenges and pitfalls of studying cellular uptake and intracellular transport of NPs by using pharmacological inhibitors, mutant proteins, siRNAs and fluorescence microscopy experiments will be critically evaluated and improvements will be suggested. Moreover, the issue of judging whether the NPs are actually internalized or merely adsorbed to the cell surface will be discussed. Active targeting of the diseased cells by coupling of ligands against overexpressed cell surface molecules appears as an attractive strategy for increasing the specificity of drug delivery and hence also for reducing adverse effects on healthy cells. I will highlight studies of targeted NPs that have revealed some general challenges in obtaining the expected efficiency and specificity of cellular interaction, i.e. relating to effects of multivalent binding of ligand to the NP and the role of a protein corona forming on the NPs. Studies reporting delivery of NPs to the cytosol by crossing the cell membrane or by endosomal escape will be evaluated. Experimental setup of studies addressing accumulation and degradation as well as disturbances of cellular processes and toxicity of internalized NPs will be discussed.

NANOMEDICINES FOR TARGETED DRUG DELIVERY COMBAT ATHEROSCLEROSIS

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Atherosclerosis is a multi-factorial disease and versatile tools and approaches have been applied for its effective treatment. It is characterized by a low-grade inflammatory process in the vascular wall, triggered by endothelium dysfunction that leads through a cascade of events to the eventual formation of atheromatous plaque and arterial stenosis. Different types of cells and molecules participate in the process making more difficult to recognize the potential molecular targets within the plaques. Hence, the injured endothelium, platelet hyperactivity, macrophage-mediated processes, matrix modeling events, coagulation factors and dysregulated metabolic activities constitute the disease-specific targets.
The rise of nanomedicine over the last decade has provided new types of polymeric nanosystems that have multifaceted advantages in drug delivery. They can be used for targeted delivery of lipophilic drugs and even insoluble ones, improved bioavailability, and enhanced stability of therapeutic agents against enzymatic degradation. Decorating the surface of nanoparticles with targeting molecules against biomarkers of the disease facilitates their specific delivery and accumulation within the pathological tissue. In this talk an overview of the recent advances in nanomedicine that has provided novel insights to the disease diagnosis and treatment will be given in line with different nanotechnology enabled approaches to advance the cardiovascular stents. Especially, theranostic nanoparticles either to detect the vulnerable plaques or to serve as drug nanocarriers for drug eluting stents will be presented for the efficient delivery of their therapeutic payloads at the atherosclerotic lesions.

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TARGETING LUNG CANCER USING RNAI AGAINST TUBB3

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Lung cancer is the most common cancer in the world and non-small cell lung cancer (NSCLC) accounts for 80% of all cases. The majority of patients are diagnosed with advanced stage disease that has often metastasised and is poorly responsive to treatment. In the advanced forms five-year survival rates are less than 13%. A small proportion of NSCLC cases have genetic mutations that are amenable to targeted therapies but this has yet to be translated to increased survival rates. NSCLC is commonly treated with radiation therapy and/or a platinum based DNA damaging agent and a microtubule-targeted agent. The tubulin/microtubule system is an important target in the treatment of numerous cancers1. High expression of the tubulin protein, βIII-tubulin (encoded by TUBB3 gene) is strongly linked with laboratory and clinical resistance and more aggressive disease in NSCLC2. We have previously shown that RNAi mediating gene silencing can sensitise NSCLC cells to not only tubulin-binding agents but also to DNA damaging agents 3. Importantly, silencing βIII-tubulin not only increased in vivo drug sensitivity but also decreased tumour formation in NSCLC. Collectively this data highlighted the TUBB3 gene as a promising target for NSCLC therapy 4. Our goal has been to develop approaches to silence TUBB3 in vivo and chemosensitise NSCLC tumours to current therapy. To this end we have established an orthotopic human xenograft model of NSCLC that recapitulates the tumour environment and closely mimics the human disease state. We are using both siRNA and shRNA approaches to silence the TUBB3 gene in vivo. For our siRNA studies we have optimized and evaluated a dendrimer based delivery system that can efficiently and potently silence TUBB3 in vitro and in vivo. For prolonged knockdown we are using a shRNA vector and a commercially available polyethylenimine polymer system for in vivo delivery. Both siRNA and shRNA targeting of TUBB3 can efficiently silence expression of this gene in orthotopic lung tumours. Importantly, in vivo suppression of the TUBB3 gene sensitises the NSCLC tumours to chemotherapy, highlighting this as a promising therapeutic strategy for the treatment of NSCLC.

1. Kavallaris M. Microtubules and resistance to tubulin-binding agents. Nat Rev Cancer. 2010;10:194-204.
2. Seve P, Dumontet C. Is class III beta-tubulin a predictive factor in patients receiving tubulin-binding agents? Lancet Oncol. 2008;9:168-75.
3. Gan PP, Pasquier E, Kavallaris M. Class III beta-tubulin mediates sensitivity to chemotherapeutic drugs in non small cell lung cancer. Cancer Res. 2007;67:9356-63.
4. McCarroll JA, Gan PP, Liu M, Kavallaris M. betaIII-tubulin is a multifunctional protein involved in drug sensitivity and tumorigenesis in non-small cell lung cancer. Cancer Res. 2010;70:4995-5003.

IN-VIVO TUMOR IMAGING USING A NOVEL RNAI-BASED DETECTION MECHANISM

WOLFGANG KEMMNER

A new concept of tumor imaging is introduced using a siRNA-based probe that is capable of amplifying the specific endogenous Protoporphyrin IX (PpIX) based fluorescence emission within cancerous tissues. PpIX is under extensive study as a photosensitizer for tumor detection and photo-dynamic therapy. In previous studies we described a preferential accumulation of the fluorescent heme precursor PpIX in gastrointestinal tumor tissues (Moesta T et al., Cancer Res, 2001) and demonstrated that enhanced PpIX based fluorescence can be induced by down-regulation of the activity of the FECH enzyme using a specific siRNA sequence (Kemmner W et al., FASEB J, 2008). Photodynamic therapy, which needs a high concentration of PpIX depends on a sufficient intracellular concentration of the precursor 5-ALA, requires exogenous addition of this acidic substance. However, 5-ALA addition carries a risk for the patients. Thus, there is a need to reduce the 5-ALA concentration and to improve the specificity of the treatment. Meanwhile, we were able to demonstrate that PpIX-fluorescence within the tumor tissue

can be induced by FECH-siRNA carried by folate-coupled liposomes or dendritic polyglycerolamine nanoparticles in conjunction with a low amount of 5-ALA (Wan K et al., Nanomedicine:NBM, 2012). Xenografted tumors approximately 3 mm in diameter were easily recognized by enhanced emission of PpIX-dependent fluorescence (Figure 1). On the one hand, the specificity of the treatment is increased, because FECH is silenced only within the tumor due to selective penetration supported by the carrier. On the other hand, addition of 5-ALA could be reduced significantly to 5 mg 5-ALA / kg body weight, which is about 20-40 times less than that used in older studies.
At the moment we are evaluating different types of delivery systems for their efficiency to transport FECH siRNA by measuring the intracellular concentration of newly formed PpIX. Carriers that can transport FECH siRNA in a highly efficient manner may also be suitable for other siRNA-based probes. Thus, this novel intracellular detection mechanism allows the selection and optimization of highly efficient carriers for various siRNA molecules.

INBORN ERRORS OF METABOLISM – A GROWING GROUP OF RARE DISEASES

STEFAN KÖLKER

INBORN ERRORS OF METABOLISM

The term "inborn errors of metabolism" (IEM) was coined by Sir Archibald Garrod in the early 19th century, at that time comprising not more than four disorders. Today, this group includes more than 500 rare IEM, and its number is constantly increasing due to diagnostic advantages, such as in the field of 'omics'. Deficiency of specific organelles, enzymes and transporters are the underlying cause of these diseases resulting in incomplete degradation of amino acids, carbohydrates, fatty acids and complex molecules as well as lack of energy substrates and other important endproducts (e.g. neurotransmitters, cholesterol, bile acids, cofactors). The clinical presentation of affected individuals is highly variable. It ranges from dysmorphism due to disturbed intrauterine development (e.g. Smith-Lemli-Opitz syndrome), acute neonatal decompensation and multi-organ failure due to accumulation of toxic metabolites (e.g. organic acidurias) or impaired energy metabolism (e.g. mtDNA depletion syndromes), to progressive deterioration of single organs (e.g. Leber's hereditary optic nerve atrophy) in adults.

METABOLIC TREATMENT: TODAY

In general, metabolic treatment aims to improve the biochemical and clinical phenotype of patients with IEM. This can be achieved by dietary treatment (e.g. reduction of precursors of toxic metabolites, maintenance of anabolism), pharmacological detoxification or substrate reduction (e.g. stimulation of physiologic or alternative detoxification pathways), upstream inhibition of the deficient pathways (e.g. reversion of tyrosinemia type I in type III by application of nitisinone), increasing of the deficient enzyme activity (e.g. cofactor supplementation, enzyme replacement, chaperones, gene therapy), application of precursors of lacking endproducts (e.g. L-DOPA), transplantation (e.g. liver, liver/kidney, bone marrow, hematopoetic stem cells), and gene therapy (in animal models). At present, 83 medicines are used for various IEM (Alfadhel et al. Arch Dis Child 2013), most of them with grade 4 level of evidence. During the last two decades, greatest therapeutic achievements (with high level of evidence) were made for patients lysosomal storage defects. This includes the development of enzyme replacement therapy, substrate reduction therapy, and bone marrow or hematopoetic stem cell therapy.

METABOLIC TREATMENT: TOMORROW

Despite significant therapeutic improvements, symptomatic treatment is still the only available option for many IEM. One major obstacle in the treatment of IEM is transport across biological barriers such as the blood-brain barrier (BBB). Molecules that penetrate the BBB must be lipophilic and less than 500 Da in size which significantly limits the number of potential therapeutic tools. As a consequence, the brain compartment is often spared and thus neurological symptoms may progress while the disease course can be halted or slowed in other organs. The development of nanomedicine strategies for IEM by exploitation of endocytotic pathways would be an excellent opportunity to overcome recent therapeutic limitations for IEM. By use of (ICAM-1-)targeted nanocarriers, enzymes, oligonucleotides (for RNAi), chaperones, and small pharmacologic molecules could be reliably be delivered to the 'deep' metabolic compartments like the brain. Furthermore, the use of nanomedicine should be considered as an important strategy to improve currently available therapeutic strategies such as enzyme replacement therapy in that it helps to reduce the applied dosages, dose-dependent adverse events, immunologic inactivation, and costs.

THERMOSENSITIVE LIPOSOMES FOR ENHANCED DRUG DELIVERY TO TUMORS

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Liposomes have been successful as a nanomedicine for the delivery of chemotherapeutic drugs and are currently applied clinically in the treatment of various types of cancer. Liposomes offer the advantage of a high drug payload, decreased drug toxicity and enhanced drug accumulation at tumor sites. Increased accumulation is due to the somewhat leaky tumor vasculature that allows liposome extravasation. However, between different types of tumors and even within one tumor, vascular permeability and thus liposome extravasation differs greatly. Furthermore, upon accumulation of liposomes in the tumor area, chemotherapeutic drug bioavailability is low, and drug release is slow and uncontrolled. At present, these are the major challenges for liposomal chemotherapy.
Mild hyperthermia (HT), the heating of tumor tissue to temperatures of up to 43°C, has been developed in the past decades as an established and efficacious treatment modality in combination with chemo- and radiotherapy. The aim of our work is to use HT to further improve liposomal chemotherapy in two ways. First, HT increases vascular permeability in solid tumors leading to increased intratumoral liposome accumulation. Second, we use optimized PEG-coated thermosensitive liposomes (PEG-TSL) to trigger drug release intratumorally upon HT (1,2).
We used intravital optical imaging and whole body optical imaging to proof liposome extravasation upon HT in different tumor models (5). Besides depending on passive tumor accumulation we also designed cationic TSL for active dual targeting of tumor vasculature and tumor cells (4). To monitor liposome tumor accumulation and triggered drug delivery following the intravascular (2,3,6) or interstitial drug release approach (2,5) we studied fluorescently labeled liposomes and fluorescent drugs by intravital microscopy.
HT strongly enhanced liposome accumulation and intratumoral penetration. Cationic TSL specifically bound to tumor vasculature and tumor cells, where they could be triggered to deliver their drug contents. Delivery of doxorubicin (Dox) and its penetration into tumor tissue and delivery to tumor cells and tumor vascular endothelial cells was demonstrated. Treatment of BLM melanoma with Dox-PEG-TSL and HT was compared to lysolipid-based Dox-TSL (3) and proved more efficacious (6).
The combination of hyperthermia with thermosensitive liposomes therefore provides a promising approach to further improve chemotherapy for solid tumors. In order to be successful optimal combinations of both need to be found and applied to increase levels of liposome extravasation and to improve drug bioavailability by heat-triggered release.

1. Li L, ten Hagen TL, Schipper D, Wijnberg TM, van Rhoon GC, Eggermont AM, Lindner LH, Koning GA. Triggered content release from optimized stealth thermosensitive liposomes using mild hyperthermia. J Control Release. 2010 Apr 19;143(2):274-9
2. Koning GA, Eggermont AM, Lindner LH, ten Hagen TL. Hyperthermia and thermosensitive liposomes for improved delivery

of chemotherapeutic drugs to solid tumors. Pharm Res. 2010 Aug;27(8):1750-4.
3. Manzoor AA, Lindner LH, Landon CD, Park JY, Simnick AJ, Dreher MR, Das S, Hanna G, Park W, Chilkoti A, Koning GA, ten Hagen TL, Needham D, Dewhirst MW. Overcoming limitations in nanoparticle drug delivery: triggered, intravascular release to improve drug penetration into tumors. Cancer Res. 2012 Nov 1;72(21):5566-75
4. Dicheva BM, Hagen TL, Li L, Schipper D, Seynhaeve AL, Rhoon GC, Eggermont AM, Lindner LH, Koning GA. Cationic Thermosensitive Liposomes: A Novel Dual Targeted Heat-Triggered Drug Delivery Approach for Endothelial and Tumor Cells. Nano Lett. 2012 Jun 13. doi: 10.1021/nl3014154
5. Li L, ten Hagen TLM, Bolkestein M, Gasselhuber A, Yatvin J, van Rhoon GC, Eggermont AMM, Haemmerich D, Koning GA. Improved intratumoral nanoparticle extravasation and penetration by mild hyperthermia. J. Controlled Release. 2013, 167(2):130-137
6. Li L, ten Hagen TLM, Hossann M, Suss R, van Rhoon GC, Eggermont AMM, Haemmerich D, Koning GA. Mild hyperthermia triggered doxorubicin release from optimized stealth thermosensitive liposomes improves intratumoral drug delivery and efficacy. J. Controlled Release. 2013 Mar 21;168(2):142-150

LOOKING AND LISTENING TO LIGHT FROM LIPOSOME NANOSTRUCTURES FOR CANCER THERANOSTICS

KOSTAS KOSTARELOS

The discovery of novel diagnosic and theranostic tools against cancer greatly depends on merging progress made in technology development of imaging modalities (hardware, image analysis) and advances in nanomaterial fabrication. Some of the most promising modalities that allow deep tissue imaging are those using optical signal detection at the near-infrared region with minimal background interference. As such optical imaging technologies mature and begin to be adopted in clinical practice, probes with appropriate signals and the capacity to target and accumulate in tumor tissue will be needed. Recently, we have proposed liposomes as clinically-established nanoscale platforms for the design of multi-spectral optical probe systems for multispectral optoacoustic tomography, fluorescence and bioluminescence imaging in vivo. 'Looking and listening' to light emitted from newly engineered liposome systems from within tumor tissue will be shown to allow visualisation of their exact localisation, release of their therapeutic cargo (siRNA) and reveal features of the tumor vasculature architecture.

FUNCTIONALIZED NANOPARTICLES AND NEGATIVELY CHARGED LIGANDS AS INHIBITOR FOR VIRAL VECTORS

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[2]European School of Molecular Medicine (SEMM) and Università degli Studi di Milano, Italy
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Viral diseases are becoming an increasing burden on the global health system as they are frequently causing chronic disease and requiring permanent treatment or leave the patients handicapped. In the present study we investigated the interaction of surface-functionalized ("patchy" and non-structured) nanoparticles of different sizes or single ligands with different viruses such as e.g. lentivirus, herpes simplex 2 or adenovirus. Moreover, we included a nanotoxicology assay in order to face safety issues of the nanoparticles in the light of a potentially use as antiviral drug.
The experiments with the enveloped lentivirus mainly confirmed previous findings by other groups1-5 that the transfection efficacy of enveloped viruses can be efficiently blocked by sulphonated nanoparticle surfaces or direct interaction with the sulphonated ligand molecules. Nanotoxicology assays indicate that the nanoparticles express a very low toxicity profile towards their target cells. In vivo immunogenicity screening didn't reveal any adverse effect up to extremely high concentrations (0.5 mg/kg pig). Additionally we found that the same NPs are inhibitory against a broader range of viruses and not only against one type. This indicates that mechanism of inhibition is more general and can provide a platform as general antiviral non-toxic drug.

REFERENCES

1. Baram-Pinto et al. (2009) Bioconjugate Chem., 20, 1497–1502
2. Baram-Pinto et al. (2010) Small, 6, 1044–1050
3. Di Gianvincenzo et al. (2010) Bioorg. Med. Chem. Lett., 20, 2718–2721
4. Witvrouw et al. (1997) Gen. Pharmacol. 29, 497-511
5. Moulard et al. (2000) J. Virol. 74, 1948-1960

PASSIVE VS. ACTIVE TARGETING

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Many different drug delivery systems and drug targeting strategies have been evaluated over the years, especially for the treatment of cancer. Examples of routinely used drug delivery systems are liposomes, polymers, proteins and micelles, and examples of commonly employed drug targeting strategies include passive targeting, active targeting to cancer cells, active targeting to endothelial cells and triggered drug release. In the present lecture, I will briefly address the principles and (pre-) clinical progress made with passively and actively targeted drug delivery systems. In particular, the high variability observed in EPR-mediated passive drug targeting, the often over-interpreted potential of active drug targeting, and the relatively disappointing clinical performance of tumor-targeted nanomedicines will be addressed. Strategies to overcome these shortcomings, based on the use of nanotheranostics and non-invasive imaging techniques, as well as on nanomedicine-based combination therapies, will also be discussed.

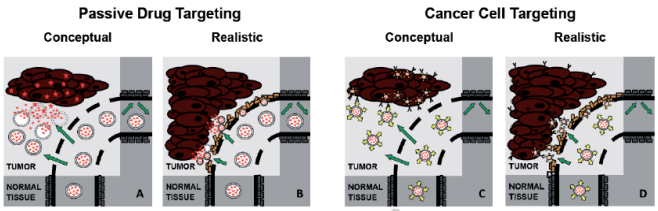

***Figure 1**: Conceptual and realistic models for passive and active drug targeting*

PREPARATION OF MULTIFUNCTIONAL MULTIMODAL NANOPARTICLES USING SPECIFIC AMPHIPHILES: CLICK CHEMISTRY FOR NANOPARTICLES

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Hybrid imaging using multifunctional multimodal nanostructures gives us the opportunity of implementing synergy to the nanostructures for in vivo theragnostic use. Huge surface area of nanostructure makes it possible to label multiple ligands or chelators. Porous inner structure is also enables nanostructure to be used as carrier. We developed an easy one-step method to label nanostruc-

ture with multiple ligands, chelators, so-called 'click chemistry for nanoparticles' (NPs).
The method was facile encapsulation of NPs, quantum dot and iron oxide as examples, using functionalized amphiphiles. The functionalized amphiphiles were synthesized conjugating C18 alkyl chain with each functional molecule, such as NOTA, cRGDyk, and sugars and so on for their suitable uses. These amphiphiles and Tween60 solution was mixed vigorously with NPs. This method improved following difficulties while modifying surface of NPs; first, we could adopt various molecules 'at the same time' in contrast to other previous stepwise methods, and second, encapsulated NPs have desirable characteristic of hydrophilicity and probable stealth effect. Most importantly, this method could be applied to various other hydrophobic NPs (nanosilica, UCNP and others).
As the first example, we developed PET/Fluorescence imaging probes using Quantum Dots (QDs) [1]. We obtained high tumor specific NPs (Fig. 1). We made encapsulated QDs with two sugars – Mannose, Lactose. Those two types of NPs showed the differences in specificity. Both encapsulated QDs showed high uptakes to liver and especially QD655T-Man showed high uptakes to spleen. In ex vivo study we confirmed specific accumulation of QD545T-Lac to liver and QD655T-Man to Kupffer cells of liver and spleen using confocal fluorescence imaging. In another study, Iron Oxide (IO) NPs was labeled as probe for PET/MRI imaging [2]. We confirmed specific uptakes of IO NPs at lymph nodes suitable for PET and MRI imaging (Fig. 2). Distinctive blue pigment demonstrated the existence of ferric iron biopsied from the popliteal lymph nodes.
We are expecting wide use of this method for labeling NP probes with various ligands and chelator according to the experimental needs. Various NPs can be combined with various radioisotopes, targeting molecules and ligands for each user's specific purposes.

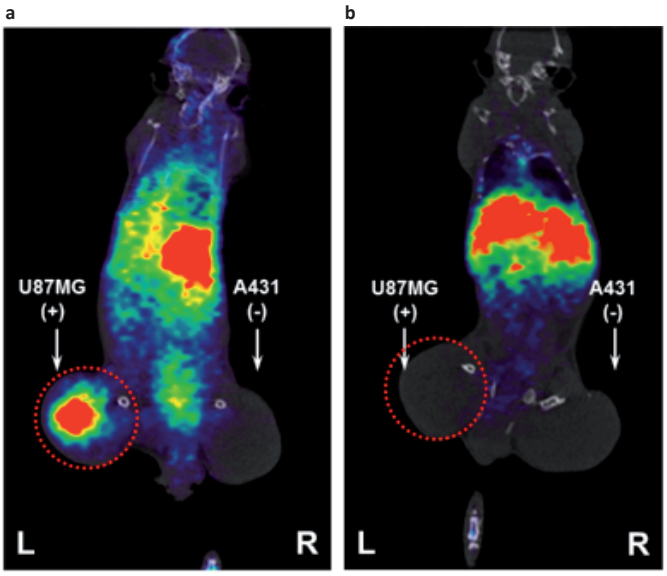

Figure 1. In vivo images of ^{68}Ga-NOTA-QD655T-Man in tumor bearing (U87MG(+), A431(-)) mouse, a) uptake in U87MG tumor site and b) blocking study

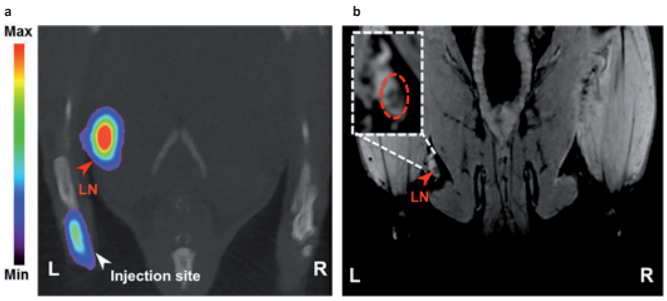

Figure 2. In vivo images of ^{68}Ga-NOTA-IO-Man showing lymph node uptake after s.c. injection into a mouse left food pad, a) PET/CT and b) ^{T2}-weighted MRI

[1] Lee YK, et al (2012), J Nucl Med, 53, 1462-70.
[2] Yang BY, et al (2012), EANM 2012, OP530.

NBTXR3, A REAL NANOTHERAPEUTIC IN PHASE I DEVELOPMENT FOR PATIENTS WITH SOFT TISSUE SARCOMA

LAURENT LEVY
CEO of Nanobiotix

Nanobiotix is a clinical-stage nanomedicine company pioneering novel approaches for the local treatment of cancer. The Company's first-in-class proprietary technology, NanoXray, is at the forefront of a new era of nanomedicine, where nanoparticles are not just a vehicle for targeted drug delivery, but have become the principal active element. NanoXray products enhances radiotherapy efficacy in the tumor without increasing healthy tissues damages to provide a new, more efficient treatment for cancer patients. NanoXray products are compatible with current radiotherapy treatments and are meant to treat a wide variety of cancers via multiple routes of administration. Nanobiotix's lead product, NBTXR3 based on NanoXray, is undergoing phase I trials in patients with advanced Soft Tissue Sarcoma (STS) at the Institut Gustave Roussy, France.
Nanobiotix published positive interim results regarding the safety of NBTXR3 in the first group of patients in November 2012. This is an encouraging milestone in the clinical validation process of NBTXR3.
The Company has partnered with PharmaEngine for clinical development and commercialization of NBTXR3 in Asia. The Company is based in Paris, France.

REGULATORY REQUIREMENTS (GMP / GLP / GCP)

MARKUS LIMBERGER

Before new chemical or pharmaceutical products like drugs or the relevant raw materials and active pharmaceutical ingredients are allowed to be launched into the market, the manufacturer is committed to proof the therapeutic efficacy and safety. It has to be shown that no side effects on humans or environment will be caused by the substances.
This will be assured by a broad range of tests and quality assurance activities.
A good guidance for this are the GXP guidelines (GMP, GLP, GCP, GDP, GCLP, GCCP). They will cover the different phases of the development and lifecycle of the product.

POTENTIAL IMPACT OF DERMAVIR NANOMEDICINE IN HIV THERAPY

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Genetic Immunity, Budapest, Hungary and McLean, VA

BACKGROUND

HIV infection is presently treated with combination of antiretroviral drugs (HAART). However, HAART offers durable virus suppression for only 25% of the HIV-infected Americans. Cytotoxic T lymphocytes (CTL) are exploited to control HIV replication. We have been developing DermaVir therapy that induces CTL. However, the worldwide diversity of HIV together with the extreme HLA polymorphism presents an unprecedented challenge to clear virus-producing cells by CTL.

METHODS

We developed esztiMINER engine for personalized data-mining and prediction of CTL specificity and efficacy, calibrated the epitope-prediction algorithms with 1,335 experimentally-proven HLA-restricted T cell epitopes and determined 99% reliability.

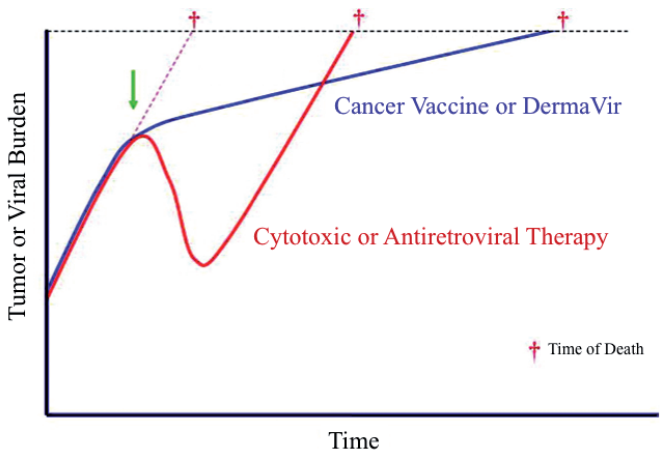
RESULTS

Clinical and preclinical studies with DermaVir consistently demonstrated no treatment-limiting toxicities and delayed treatment effect in reducing viral load, consistent with CTL-mediated mechanism of action. To predict the likely responder to DermaVir we

modeled the percentage of cross-reactive epitopes (CR) recognized by DermaVir-induced CTL on the patient's cells expressing HIV-antigens. We found extreme personal diversity in HLA-restricted T cell epitope pattern. We showed that HLA polymorphism in combination with HIV evolution leads to highly variable DermaVir-induced CTL efficacy in patients, CR ranging between 20-50%. To address both HIV and HLA variability we digitally optimized a vaccine for a target patient population and invented a method for effective immunization with a family of related vaccines that digitally optimized for the broadest CTL recognition of infected cells.

CONCLUSIONS

DermaVir is a personalized therapy. We have clinical evidences for the exclusion from DermaVir treatment the likely non-responders with CR<35%. CR represents a novel, rapid, reliable and accurate genetic marker that predicts the efficacy of vaccine-induced CTL in every individual by linking the mechanism of action with clinical efficacy. Our results imply that developing of personalized DermaVir therapy is essential for efficacy.



Adopted from Madan R A et al. The Oncologist 2010;15:969-975

Model for achieving survival benefit by vaccine-induced CTL in HIV and cancer. *Existing chemotherapy/HAART (red line) affects tumor growth/virus replication, which may result in a dramatic but transient response. Following discontinuation of therapy, both the tumor growth and viral load returns to the pre-treatment value. In contrast, DermaVir therapy (blue line) alters the immune responses of the host. This may not cause an immediate or dramatic change in tumor burden or viral load, but continued cumulative slowing pressure on the rate of tumor growth or viral burden, especially if started early in the disease course, may lead to substantially longer overall survival. The arrow (green) indicates the initiation of treatment.*

DEVELOPMENT OF BIODEGRADABLE NANO-FIBER BASED SYSTEMS FOR DRUG DELIVERY

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There are clinical needs for the development of biodegradable polymeric matrices for implants to release their therapeutic payloads in a controllable manner and to promote tissue regeneration simultaneously. Electrospun nanofibers show great promise for developing novel drug delivery systems due to their special characteristics of high porosity and high surface area in line with their simple, cost-effective top-down fabricating process. Even more, when nanomedicine combines with bioelectronics, conducting polymers may act as new types of materials for scaffolds that induce tissue regeneration. In this talk, the development and characterization of electrospun drug loaded biodegradable scaffolds are presented and their feasibility to serve as delivery vehicles for e.g., anti-inflammatory drugs, is investigated. The morphology of the drug-loaded electrospun poly (caprolactone) (PCL) matrices was assessed by Atomic Force Microscopy and SEM studies whereas drug release kinetics studies were performed. Conducting Poly (3, 4-ethylenedioxythiophene) (PEDOT) based scaffolds were also synthesized to promote cell proliferation, especially for tissue regeneration in bioelectronics field. Cytotoxicity and cell viability studies follow to test their safety and effectiveness.

In overall, the nanofibrous scaffold made of biodegradable polymers that can degrade harmlessly within the human body has evolved as a new perspective for controlled drug release from implant surface enabling its better integration with the surrounding tissues.

Acknowledgments: This work has been financially supported by the ROleMak Project of the European Commission under the 7th Framework Program, FP7-REGPOT-2011-1.

MICROWAVE AND SONOCHEMICAL TECHNOLOGY TO PRODUCE ACTIVE BONE REGROWTH SCAFFOLDS

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3: Faculty of Materials Science, Warsaw University of Technology, Poland.
4: Department of Histology and Embryology, Center of Biostructure Research, Medical University of Warsaw, Poland.

In this paper we present a technology of nano-hydroxyapatite (HAP) production, its unique biocompatibility, and how we can coat 3D printed polymer scaffolds for bone regrowth with the nanoparticles. The coating changes a hydrophobic surface of the polymer into a hydrophilic one, and is expected to increase the scaffold biocompatibility.

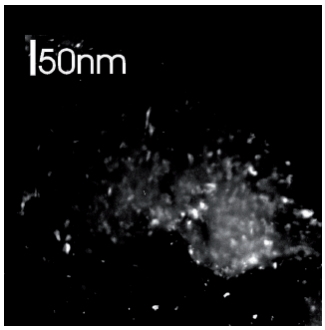
GOHAPTM NANOPARTICLES SYNTHESIS

The nano-HAP powder prepared is characteristic in very small grain size, controlled in the range 5 -25 nm, plate like structure, hexagonal crystalline structure, and deviation from Ca/F ratio – calcium deficiency, which can also be controlled. To prepare the precursors for the synthesis, a standard procedure described in detail in [,] was used: The starting material were calcium hydroxide (Ca[OH]2) and phosphoric acid (H3 PO4 , 85% solution. The reactants were used in a molar ratio Ca/P of 1.67. However, the solution was treated in a closed pressure vessel in a microwave reactor (ERTEC, Wroclaw, Poland) microwave power strongly concetrated at the bottom of the reaction vessel. A power density of 5 W/mL permitted to heat the solution up to appr. 110oC in 90 seconds, and abruptly stop the reaction, or to continue it further up to 600 sec. When the reaction was stopped after 90 sec, the finest nanostructure was obtained, and the Ca/F ratio was 1.57, while for long synthesis time it reached 1,67, i.e. the stoichiometric composition (Table 1). Note, that natural HAP in the human and animals bone is calcium deficient. The nano-HAP in question was registered in the Polish patent office as GoHAPTM. The powders are resorbable according to norm ISO 10993-14: biological evaluation of medical devices – identification and quantification of degradation products from ceramics. Extension of synthesis time leads to decrease of resorbability (Fig.2).

Table 1*: Effect of the microwave radiation time on the specific surface area, density, grain size, and Ca/P ratio for the GoHAP powder*

(min)	(m2 /g)	(g/cm3)	(nm)	Ca/P
1.5	236	2.91	9	1.57
2.5	174	2.94	12	1.65
5.0	99	2.96	21	1.66
7.5	81	3.00	25	1.67
10.0	63	3.03	32	1.67

1 Highly biocompatible, nanocrystalline hydroxyapatite synthesized in a Solvothermal process driven by high energy density microwave radiation , D. Smolen et al, International Journal of Nanomedicine 2013:8 653–668
2 D. Smolen et al., Patent P-369906, filled 15.11.2011



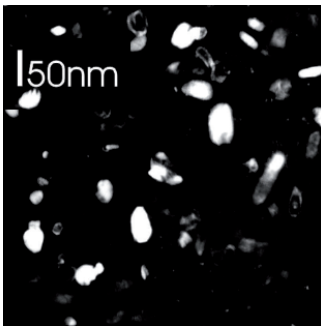


Fig. 1. *Transmission Microscopy image of GoHAPTM after 90 sec synthesis [2]. The white bar corresponds to 50 nm.*

Fig. 2. *Transmission Microscopy image of GoHAPTM after 450 sec synthesis [3].*

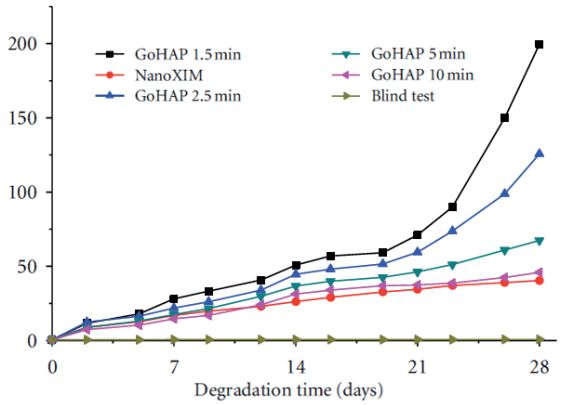


Fig. 3. *Effect of synthesis time on the resorbtion rate [3].*

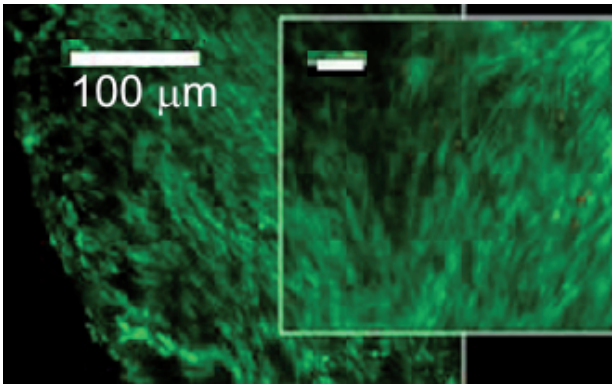


Fig.4. *Fluorescent microscopy of cells 14 days after charging to an osteoblastic medium on pellets made from GoHAPTM [1].*

3 Hydroxyapatite Nanopowder Synthesis with a Programmed Resorption Rate, D. Smolen, T.Chudoba, S. Gierlotk, A.Kedzierska, W. Lojkowski, K.Sobczak, W. Swieszkowski, K.J. Kurzydlowski, Journal of Nanomaterials, Volume 2012, Article ID 841971, doi:10.1155/2012/841971

COATING A 3D PRINTED SCAFFOLD WITH GOHAPTM NANOPARTICLES

3D printed scaffolds made from PCL were immersed in a colloidal suspension of the GoHAP nanoparticles. Further, the suspension introduced into a High Power Ultrasound generator. High power ultrasound cause cavitation in water, i.e, growth of water vapour boubles. When they reach a critical dimension, they collapse, leading to creation of spots with pressures reaching hundreds of MPa or more. If a nanoparticle is in close to such a spot, the pressure gradient will throw it with high kinetic energy. If on its way a surface is present, the particle will be strongly attached to it. This coating technology was developed by Gedanken et al [e.g.]. We applied it to coat PCL (policaprolacton) scaffolds: pellets and 3 D structures. Coating was possible even at average temperature of 25oC. The adhesion of the coating to the polymer was good for thin layers. Delamonation was observed if the polymer pellet was coated with a layer thicker than 1 mm, and the pellet was bent. However even in that case, a thin layer of GoHAP remained strongly bonded with the PCL pellet (Fig 5 and 6)

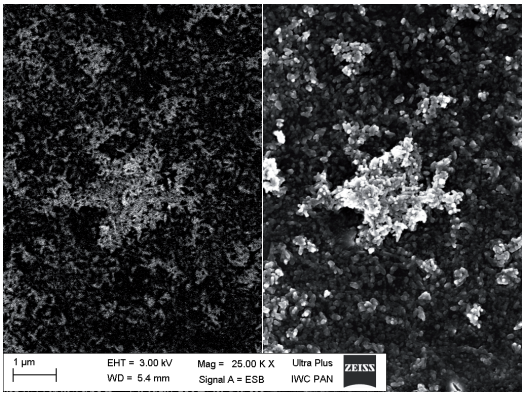


Fig.5. *Scanning Electron microscopy image of the GoHAP layer on PCL (left: BSE image, right SE image)*

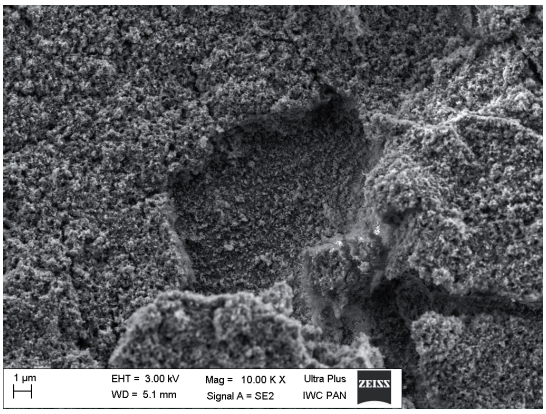


Fig. 6. *Scanning Electron microscopy image of the GoHAP layer on PCL showing delamination but a remaining adhering layer.*

CONCLUSION

High energy density techiques: microwaves, ultrasounds, open new oportunities in nanotechnology for medical appliactions. Microwave solvothermal syntheis premits to obtain highly biocompatible nano-HAP particles (GoHAP), and ultrasound technolgy to coat polymer scaffolds with them. This opens new perspectives to improve polymer safooflds bio-compatibility.

Acknowledgement: This work was supported in part by the project Bioimplant, Sonosca and CePT.

4 Ultrasound radiation as a “throwing stones” technique for the production of antibacterial nanocomposite textiles, Perelshtein I, Applerot G, Perkas N, Grinblat J, Hulla E, Wehrschuetz-Sigl E, Hasmann A, Guebitz G, Gedanken A. ACS Appl Mater Interfaces. 2010 Jul;2(7):1999-2004. doi: 10.1021/am100291w.

ABUMIN TRAFFICKING IN TUMOUR TISSUES MEASURED BY MRI: A NEW TOOL TO PREDICT THE DISTRIBUTION OF MACROMOLECULAR DRUGS IN CANCER LESIONS

ALESSANDRO MAIOCCHI, Bracco Imaging S.p.A., Via Ribes 5, Colleretto Giacosa (TO), 10010, Italy

About 30 therapeutic monoclonal antibodies (mAbs) have been approved by the US FDA and ~350 are currently in the clinical pipeline. Among the mAbs in clinical use, the 50% targets antigens relevant to cancer. The inhomogeneous delivery of mAbs-based drugs in solid tumors was recognized as one of the major cause of resistance to treatments.

Indeed a number of scarcely or heterogeneously-perfused tumors are, encountered in the clinical practice, which exhibit a resistance to pharmacological treatments with macromolecular anticancer drugs which is essentially due to the inadequate delivery within the pathological area or tissue to be treated. Among them are, for instance, larger or later-stage solid tumors, often comprising an external layer typically displaying more dense and hyper-permeable microvascularisation, and an internal core, commonly including necrotic areas scarcely vascularised and hard to be penetrated by macromolecular therapeutic agents.
A phenomenon that accounts for some parameters facilitating the delivery and the accumulation of macromolecular solutes within a tumor mass is the Enhanced Permeability and Retention (EPR) effect, firstly reported and discussed by Maeda and co-workers. The EPR effect relates to the functional abnormalities in the tumor tissue, which in turn result in an increased permeability of the blood vessels with an extensive extravasation of blood plasma components, such as macromolecules, nanoparticles and lipidic particles. Moreover, the slow venous return in tumor tissue and the poor lymphatic clearance mean that macromolecules are retained in the tumor, whereas extravasation into tumor interstitium continues.
However the distribution of systemically administered antibodies in tumor tissues is also related to antigen density, antigen-binding kinetic, and dose. Monoclonal antibodies extravasating in tissues showing high antigen density and low diffusivity can be strongly immobilized in the perivascular space from the so called 'binding site barrier'. This is an additional limitation to the homogeneous distribution of an antibody that could be amplified by the impairment of the convective transport of fluids due to the high interstitial fluid pressure in the interior of the tumor tissue. All these factors can affect differently the transport and biodistribution of a mAb depending on tumors and patients resulting very often in an unpredictable (and costly) therapeutic outcome.
A need, therefore, still exists for improved diagnostic methods allowing the non-invasive verification of the existence in the tumor tissue to be treated of those conditions permitting an effective delivery of macromolecular drugs, so as to discriminate resistant tumors and, hence, patients, who can hardly benefit from a therapeutic treatment using macromolecular drugs (or pro-drugs) due to an insufficient or impaired distribution thereof in the tissue to be treated.

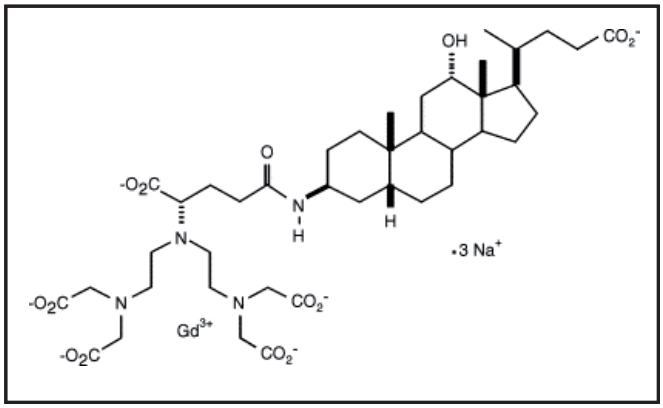


***Figure 1**: Molecular structure of B22956/1*

In this work a Dynamic Contrast Enhanced MRI (DCE-MRI) method using an albumin-binding Gd(III)-complex (B22965/1, Figure 1) was developed to derive surrogate biomarkers reporting on the bioavailability of a mAb at the tumor tissues. The non covalent binding to albumin with a MRI contrast agent enable the labeling of the albumin for a time longer enough to appreciate the extravasation of the macromolecule without increasing too much the blood life time of the Gd(III) complex, a fundamental requirement to reduce the risk of toxic effects. The proposed method was validated with an extensive quantitative histological analysis of a fluorescent albumin distribution in the tumor tissues correlating the amount of extravasated albumin with the DCE-MRI derived parameters using B22965/1. When a currently used MRI contrast agent which does not bind to albumin was used instead of B22965/1, no correlation with the extravasation of albumin in tumor tissues was found. Moreover it was demonstrated that the DCE-MRI parameters derived from the analysis of the wash-in/wash-out MRI signal curve measured in the tumor tissues can be correlated with the distribution of Cetuximab® (CTX) in a human epidermoid carcinoma A431 tumor xenograft model. In addition the possible effect of Paclitaxel® (single iv injection 24 h before the mAb administration) in modulating CTX delivery in the same tumor was also assessed. The histological results showed a greater perivascular accumulation of CTX in untreated tumors while PTX treated tumors showed better CTX penetration also in the lower perfused tumor regions apparently reducing the limiting effect of the 'binding barrier site'(Figure 2).

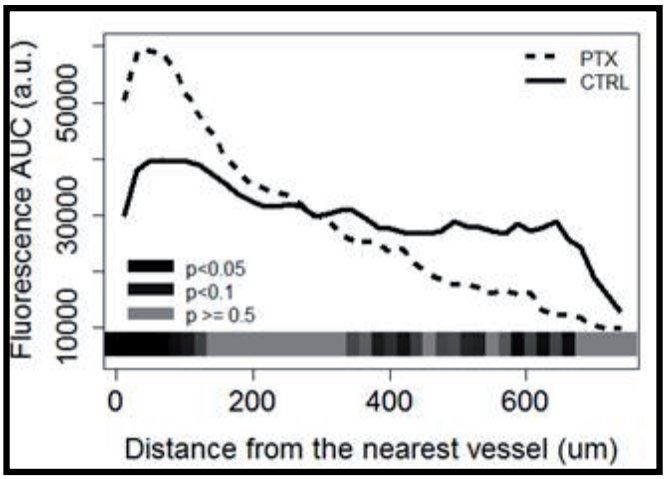


***Figure 2**: CTX distribution in tumor tissue*

On the other side DCE-MRI showed that PTX can induce a change in the physiopathological state of the tumor enhancing perfusion and increasing extravasation of the albumin-binding contrast agents in the extravascular space with respect to untreated tumors.
The comparison of DCE-MRI parameters and the histological data suggested that it is possible to stratify tumors with favorable and unfavorable CTX distribution providing the basis for the development of a non invasive imaging tool to be used for patient stratification and macromolecular targeting therapy selection.

DIAGNOSIS AND TREATMENT OF VULNERABLE ATHEROSCLEROTIC PLAQUES – THE "NANOPLAQUE" AND FP7 "NANOATHERO" PROJECTS

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Atherosclerosis is the underlying cause in cardiovascular disease, the leading killer in developed countries. With sudden cardiac death as the first symptom in approximately half of the cases, identifying individuals at risk is critical. Current methods for diagnosing atherosclerosis rely on estimating the degree of arterial stenosis; however, vulnerable plaques, which do not correlate to degree of stenosis, are responsible for the majority of cardiac deaths. Within the scope of these projects, we are using nanotechnology as a platform to improve the diagnosis and therapy of vulnerable atherosclerotic lesions.
Specifically, within the NanoPlaque project we are characterizing, developing and testing biomarker-coated nanocarriers (e.g. liposomes). Globular adiponectin (gAd) and interleukin-10 (IL-10), a Type II cytokine, are two of the few known anti-inflammatory molecules and play key roles in suppressing inflammatory responses. As atherosclerosis (AS) is a sub-acute inflammatory condition of the aortic vessel wall, we identified IL-10 and gAd as promising targeting molecules for vulnerable AS plaques. Further, we have established constructs using either pegylated stealth liposomes or proticles as a base and then adding these biomarkers.
A high payload of contrast agents (e.g. iron, gadolinium) can be transported specifically and safely by this nanocarrier system to AS lesions.

Within the EU FP7 large-scale NanoAthero project, 16 partners from 10 European countries coordinated to create the "Nanomedicine for Atherosclerosis" consortium. It is aimed at studying and developing the clinical feasibility of nanomedicine for targeted diagnosis and treatment of atherosclerosis. Coordinated by Inserm, the project has just obtaining 10 million euros of funding over the course of five years from the European Commission.

NANOPARTICLES FOR THERAPY OF ALZHEIMER DISEASE

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Alzheimer Disease (AD) is characterized by progressive and irreversible neurodegeneration of higher nervous functions, leading to loss of cognitive functions and autonomy and eventually to death. In spite of the substantial progress in research, there remains an urgent need to identify effective therapies and early detection strategies, in order to avert a financially overwhelming public health problem.
The accumulation in the brain of large amounts of β-amyloid (Aβ), a peptide fragment of the membrane-associated amyloid precursor protein (APP), plays a central role in AD. This peptide is released from cells in a soluble form and progressively aggregates forming oligomers, fibrils and ending with extracellular plaques, detectable post-mortem in AD brains.
NAD project (large cooperative; 19 partners, funded by the E.C. , FP72007-2013, G.A. n° 212043) is aiming to the therapy and diagnosis, even combined, of AD, by creating different multiple-functionalized nanoparticles, able to bind, detect and remove Aβ from the brain and from the blood.
Within the frame of the project, different types of NPs have been synthesized (liposomes, SLN, polymeric PHDCA) specifically functionalized to bind Aβ with high affinity: acidic lipids (Kd 6-60 nM), a curcumine derivative (Kd 2-10 nM)[2], monoclonal anti-Aβ antibodies (Kd 200-300 pM). These NP proved their potential to be used for the therapy of AD by preventing, in vitro, Aβ aggregation, neurotoxicity of Aβ peptide, and Aβ-induced Tau phosphorylation [1-3].
Functionalizations for crossing the BBB have been performed and tested using radiochemical techniques on a BBB in vitro transwell model utilizing brain capillary endothelial cells, either human (hCMEC/D3) or murine (RBE4): i) Antibody anti-Transferrin-receptor: ii) modifying peptides derived from ApoE; iii) HIV Tat-1 peptide. Double functionalization (both for binding Abeta and for crossing the BBB) enhances NP cell uptake and transport across the BBB model without affecting the affinity for Abeta. Different effects have been reported depending on the BBB ligand utilized, on ligand surface density and on the strategy utilized for chemical ligation to NPs. The crossing of the BBB has been also been tested in vivo following radioactive lipids inserted into liposomes, and showing the ability of specific functionalization to enhance barrier crossing. Experiments in vivo carried out on animal models of Alzheimer disease (single APPswe and double APPswe/PS1 TG mice) suggest that treatment with double-functionalized NP can decrease the content of Abeta in brain.
Taken all together, these results open new possibilities for the treatment of Alzheimer Disease.

TETRAPHENYLPORPHYRIN AND TETRAPHENYLCHLORIN TETHERED CHITOSAN BASED CARRIERS FOR PHOTOCHEMICAL INTERNALIZATION

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Photochemical internalization (PCI) is a novel technology, which utilizes selected photosensitizers in combination with light excitation, to produce ROS mediated damage to endosomal membranes1. Thereby endocytosed hydrophilic drugs and macromolecules can released reach their target of action before being degraded in lysosomes. PCI has been shown to enhance the biological activities of a variety of macromolecules that do not readily penetrate through the plasma membrane including gene encoding plasmids and chemotherapeutic agents such as bleomycin.
Most hydrophilic photosentizers (PS) do not insert well into cell membranes and are therefore not suitable for PCI, whereas amphiphilic PS like TPPS2a and AlPc2a are most effective in PCI. They can be inserted into plasma membranes but are unable to penetrate through this barrier and will therefore be taken into cells by endocytosis. The current clinical candidate for PCI is meso-tetraphenylchlorin disulfonate (TPCS2a, Amphinex®). A recent Phase I/II study of Amphinex based PCI of bleomycin in patients with local recurrence or advanced/metastatic cutaneous and subcutaneous malignancies resulted in complete clinical regression of target tumors in 87.5% (14 of 16) of patients completing the first assessment and in 72% (8 of 11) of patients completing the second assessment.
One of the common problems associated with photodymamic therapy (PTD) in clinical application is skin photosensitivity and unfavorable biodistribution of the PS. Polymeric nanocarriers have shown tumor selective accumulation due to the enhanced permeation and retention (EPR). Chitosan (CS) (poly-β-1-4-N-glucosamine) is a non-toxic, biocompatible and biodegradable polymer which has been investigated for various drug delivery applications.
The aim of the current study was to develop chitosan based macromolecular nanocarriers for hydrophobic photosentizeres and investigated their application in PCI. We have recently developed the diTBDMS protection strategy for synthesis of chitosan derivatives2,3 and this was utilized in the current project. The highly lipophilic PS meso-tetraphenylporphyrine (TPP) was covalently attached to N-bromoacetyl-DiTBDMS-CS and these materials were then reacted with either trimethylamine or 1-methylpiperazine in order to introduce a hydrophilic cationic moiety. These reactions were carried out in organic solvent in order to enable quantitative and reproducible attachment of functional moieties to the polymer backbone. Finally the TBDMS protecting groups were removed and water soluble carriers were obtained. The structures of the carriers and the intermediates in the synthesis were confirmed by 1H-NMR, FT-IR and solid state 13C-NMR UV-Vis, Fluorescence and NMR investigations showed that the carriers formed nanoparticle like structures in aqueous solution with a core of semi-solid π-stacked photosensitizers. Dynamic light scattering study revealed the average size of nanoparticles was in the range 100-300 nm. Due to their amphiphilic nature, in these structures could unfold lipophilic environment. The efficacy of the carriers for photochemical internalization (PCI) mediated gene delivery was evaluated in vitro using the HCT116/LUC human colon carcinoma cell line. The efficacy of transfection was comparable to what could be achieved by the reference compound and current clinical candidate TPCS2a.
These initial investigation showed chitosan based nanocarriers TPP could be used PCI but TPP has limited utility as it can only be activated by light with relatively short wavelengths. Chlorins are preferred over porphyrins for photodynamic therapy as they offer use of light with deeper penetration in organic tissue due to their increased absorption in the farthest-red side band (600-800 nm) and also generates better singlet oxygen quantum yields which is also important for in vivo treatment.
Chlorin based carriers were therefore synthesized following this proof-of-concept with the TPP-carriers. Synthesis of TPC derivatives is much more challenging than synthesis of TPP derivatives but by utilizing diTBDMS chitosan and the same approach as before the synthesis of TPC-carriers was successfully achieved. Dynamic light scattering confirmed the formation of nanoparticle like structure with a 140-200 nm diameter. These carriers could be activated at 650 nm (red region) light and were therefore suitable candidates PCI based cancer treatment. The carriers were also evaluated in preliminary in vivo experiments using tumor bearing Hsd:Athymic nude-Foxn1nu female mice. Untreated control and animal treated with TPCS2a + bleomycin animals exhibited strong fluorescence after 11 and 15 days. This indicated the presence high number of living cancer cells in the tumor. In contrast, for the animals treated with the TPC-nanocarriers + bleomycin there was only weak fluo-

rescence in only one of the animals at day 11, and there was no detectable fluorescence after 20 days. These results showed TPC-nanocarriers, like TPP-nanocarriers, induced a strong PCI effect. Further studies have also confirmed a significant EPR effect.
We have now demonstrated that diTBDMS chitosan can be used for highly reproducible chitosan based nanocarriers for TPP and TPC. These carriers form nanoparticle like structures in aqueous solution but can unfold in contact with the cell membrane to insert the lipophilic PS moieties into the endocytic membrane. These carriers exhibit strong PCI effect are therefore promising for PCI based cancer therapy and photochemical transfection.

REFERENCES

(1) Berg, K.; Selbo, P. K.; Prasmickaite, L.; Tjelle, T. E.; Sandvig, K.; Moan, D.; Gaudernack, G.; Fodstad, O.; Kjolsrud, S.; Anholt, H.; Rodal, G. H.; Rodal, S. K.; Hogset, A. Cancer Res 1999, 59, 1180.
(2) Runarsson, O. V.; Malainer, C.; Holappa, J.; Sigurdsson, S. T.; Masson, M. Carbohyd Res 2008, 343, 2576.
(3) Song, W. L.; Gaware, V. S.; Runarsson, O. V.; Masson, M.; Mano, J. F. Carbohydrate Polymers 2010, 81, 140.

OPENING REMARKS AND NCL OVERVIEW

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The Nanotechnology Characterization Laboratory (NCL) conducts preclinical efficacy and toxicity testing of nanoparticles intended for cancer therapeutics and diagnostics. The NCL is a collaborating partnership between the U.S. National Cancer Institute (NCI), the U.S. Food and Drug Administration (FDA), and the National Institute of Standards and Technology (NIST). As part of its assay cascade, NCL characterizes nanoparticles' physical attributes, their in vitro biological properties, and their in vivo compatibility using animal models. The NCL also looks at trends across nanoparticle platforms, parameters that are critical to nanoparticle biocompatibility, and develops assays for preclinical characterization of nanoparticles. The Opening Remarks and NCL Overview presentation will provide an overview of the NCL, discuss observed trends, and illustrate how physical parameters influence nanoparticle biocompatibility and toxicity.

IMAGE-GUIDED HEAT-INDUCED DRUG DELIVERY PLATFORM

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INTRODUCTION

The development of activable (i.e. heat-sensitive) nanocarriers encapsulating drugs presents a promising approach for cancer treatment. Nanocarriers accumulation at the tumor site (via the EPR effect) and control of drug release (via an "on/off" external activation source) may have considerable benefit by increasing treatment efficacy while reducing side effects and toxicity. Further, the possibility to monitor both nanocarrier accumulation at the tumor site and drug release using current clinical imaging techniques may be of particular relevance to follow and quantify the drug delivery process.
Within the European project "Sonodrugs" we investigated the opportunity of both triggering the drug release from new nanocarriers (temperature and pressure-sensitive) using High Intensity Focused Ultrasound (HIFU), and monitoring the drug release profile at the tumor site by Magnetic Resonance Imaging (MRI) (De Smet et al., Journal of Controlled Release, 2011, 150, 102–110).
A new versatile thermosensitive liposome was designed and developed to efficiently encapsulate a drug (doxorubicin) and a contrast agent (superparamagnetic iron oxide nanoparticles).

RESULTS

Figure 1 shows a typical cryo-transmission electron microscopy (TEM) image of doxorubicin-loaded thermosensitive magnetoliposomes (DOX-loaded TSM), showing iron oxide nanoparticles (black arrow) and doxorubicin crystals (white arrow) within the liposomes.

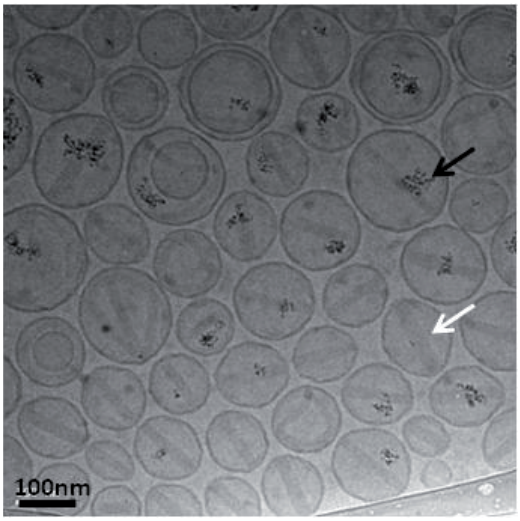

***Figure 1**: Cryo-transmission electron microscopy (TEM) image of DOX-loaded TSM, showing iron oxide nanoparticles (black arrow) and doxorubicin crystals (white arrow)*

The doxorubicin (DOX) release profile was assessed by fluorescence measurements following incubation of the DOX-loaded TSM in PBS/fetal bovine serum 50/50 medium at increasing temperature. Release of doxorubicin at 43°C (60% released after 15 min) was observed, corresponding to the main gel-to-liquid crystalline phase transition temperature (Tm) of the lipid composition. Moreover, no marked drug release was observed upon aging the DOX-loaded TSM solution at 37°C (below Tm).
In vitro cell viability assay (MTT) was performed on human umbilical vein endothelial cells (HUVEC). Cells were incubated 4h at 37°C with DOX-loaded TSM at increasing DOX concentration. No marked toxicity of DOX-loaded TSM was observed up to the maximum tested DOX concentration of 63µM, suggesting an efficient retention of the drug within TSM at 37°C.
In vivo biodistribution of 14C-DOX-loaded TSM (injected dose of doxorubicin: 5mg/kg) in B16F10 melanoma tumor engrafted in C57BL6 mice, 24 hours after a single intravenous injection showed about 16%, 19% and 16% of the injected dose (ID) per gram of organ in the liver, spleen and kidneys respectively (14C-quantification using a liquid scintillation counter). Tumor accumulation showed about 4% ID of 14C-DOX per gram of tumor.
Histological observations were performed 60 days after a single intravenous injection of DOX-loaded TSM (injected dose of doxorubicin: 5mg/kg) in C57BL6 mice to assess potential in vivo toxicity. Perl's Prussian staining of iron oxide nanoparticles confirmed a high accumulation mainly in the spleen and to a lower extent in liver and kidneys. No marked abnormalities of the tissues except some mild tubular atrophy in the kidneys were observed, which could be attributed to the presence of iron oxide nanoparticles.
Drug release monitoring explorations were performed using MRI at 1.5 T (Lorenzato et al., Contrast Media Mol. Imaging, 2013, 8(2), 185-192). Upon heat activation of the TSM nanocarrier above Tm, a marked positive contrast enhancement was observed on Magnetic Resonance (MR) images. On contrast, no change in the MR images was observed for non-thermosensitive magnetoliposomes (NTSM) and free superparamagnetic iron oxide nanoparticles (free NP) (Figure 2). The signal changes were assessed by longitudinal and transverse relaxivity measurements, r1 and r2* respectively. The MRI

signal modification observed for the TSM nanocarriers above Tm was attributed to permeabilization of TSM membrane.

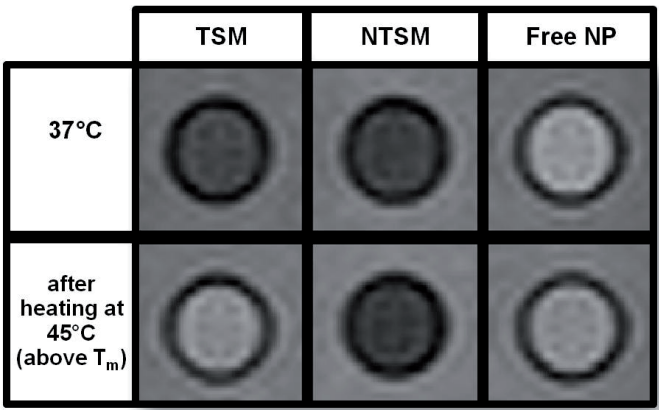

***Figure 2**: Evolution upon heating of Magnetic Resonance (MR) signal (T1-weighted gradient echo images) at 1.5 Tesla of iron oxide encapsulated in thermosensitive (TSM) and non-thermosensitive magnetoliposomes (NTSM), and free nanoparticles (free NP). The iron oxide concentration is 20µg/mL for all samples.*

CONCLUSION AND PERSPECTIVES

An image-guided heat-induced drug delivery platform was developed within the Sonodrugs consortium.

Our first prototype, a doxorubicin-loaded thermosensitive magnetoliposomes (DOX-loaded TSM) was designed. No marked in vitro toxicity of DOX-loaded TSM was observed in HUVEC cell lines up to a doxorubicin concentration of 63µM (highest tested dose). In vivo, the designed TSM showed preferential accumulation in the reticulo endothelial system organs (liver and spleen) and clearance organs (kidneys) with no major sign of toxicity. Accumulation within tumor, 24 hours after a single intravenous injection of the drug-loaded TSM showed about 4% ID of 14C-DOX per gram of tumor. Finally, membrane permeabilization could be monitored upon heating the TSM above Tm using MRI.

Further in vivo experiments are now required to monitor the nanocarrier accumulation and drug release at the tumor site by MRI and to evaluate the anti-tumor efficacy of DOX-loaded TSM under HIFU activation. .

Another anti-tumor agent, irinotecan, a prodrug of SN38 which is currently used in the treatment of colorectal cancer, was also successfully encapsulated and retained in the TSM. Preliminary in vitro evaluations of irinotecan-loaded TSM are currently planned.

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NANOGEL STAR POLYMERS AS INTERESTING SOFT COLLOID MATERIALS FOR BIOMEDICAL APPLICATIONS

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Polymers have numerous biomedical applications including delivery of therapeutic materials, imaging applications, tissue regeneration, antimicrobial action and others. We have developed a bottom up route to nanogel core star polymer amphiphiles with control over size, functionality, end groups and molecular architecture. These materials are interesting because they do not need self assembly as required for micelles, liposomes and polymersomes and are composed of a number of molecular nanocompartments. The nanogel star particles can either be biostable or biodegradable depending on the desired application and composition. These materials encapsulate hydrophobic materials such as therapeutic materials and/or dyes at the 10-15% level without covalent bonding. The ligand-containing outer shell also sequesters magnetic particles for MRI studies and ligates ions such as copper 64 for PET. In this regard, the particles have multifunctional capabilities. The outer shell can also be used to generate an inorganic shell around the polymer such as functional silica or catalyze the electroless deposition of a gold shell. The later leads to a surface plasmon resonance absorption shifted into the near IR suitable for inducing hyperthermic processes in cells upon irradiation. We have also studied the antimicrobial properties of suitable substituted nanogel star polymers derivatives primarily for touch surface applications. The incorporation of hydrophobic dyes in the inner shell increases the efficacy of these polymeric materials for antimicrobial applications when used in the presence of a visible light source.

DISC-SHAPED POINT-OF-CARE PLATFORM FOR INFECTIOUS DISEASE DIAGNOSIS

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ABSTRACT

Malaria is one of the most prevalent infectious diseases in tropical areas of the world. According to the World Health Organisation (WHO) 2010 records, there were 174 million malaria cases reported, 80% of which were in Africa, leading to 600 000 deaths. The patients present themselves with acute fever as the only clinical symptom, which complicates the diagnosis because in Africa and other tropical regions acute fever is the clinical indication of several other diseases, such as dengue and chikungunya (viral infections), as well as typho and pneumonia (bacterial infections). In fact, it is reported that approximately 30-40% suffering from fever are given anti-malarial drugs while in reality they suffer from another disease. Furthermore, there is an increased risk for spread of the aforementioned diseases to developed countries due to globalization, climate change, immigration and returning travelers from malaria endemic regions, which raises the need for diagnostic efficiency.

Existing diagnostic methods are mostly the microscopy smear tests (specially for malaria) and Rapid Diagnostic Tests-RDTs based on immunodetection. These tests, however, have often been disputed for their reliability and accuracy. Despite their low cost, the fact that they are specific to only one single disease marker often necessitates the use of more than one RDTs, thus, increasing the overall cost. The DiscoGnosis project develops a point-of-care diagnostic platform, aiming to detect several diseases with similar clinical symptoms in a rapid, specific and automated way. A number of novel technological approaches are implemented towards this scope, described below along with the advantages they offer:

ASSAYS

Apart from malaria, the diagnostic panel includes dengue, chikungunya, typho and pneumonia. Such diverse panel requires that the diagnosis is based on highly specific molecular marker detection. Depending on the time elapsed from the onset of the disease, different molecular identification is necessary. Therefore, both, nucleic acid targets and protein markers are monitored in order to span a large diagnostic window. The implementation of LAMP technology for nucleic acid amplification and detection offers high specificity due to its multi-primer nature. The low energy demand of LAMP due to its isothermal profile is particularly advantageous compared to PCR in point-of-care diagnostics.

ANALYTICS

Magnetic beads are used as carriers to form a bead array that will be probed by an integrated camera. Fluorescent nanoparticles (Quantum Dots – QDots) at various colors/wavelengths will be conjugated on the magnetic beads, acting as the identification and quantitation agents. The detection optics are based on single-

wavelength UV excitation of the QDots and subsequent fluorescent imaging with an integrated camera. This approach enables multiplexity and allows several targets to be detected in a single reaction chamber with one "shot".

AUTOMATION

Assay and analytics components are converged in a disc-shaped microfluidic platform, which uses centrifugal and capillary forces to manipulate fluids. The advantage of this approach is the lack of external pumping instrumentation and interface with tubes since the centrifugal microfluidics are essentially self-driven. The sample handling is based on the concept of unit operations, i.e., individual microfluidic structures each serving a discrete fluidic function and altogether interfaced appropriately on the disc. Within this concept, all operations are carried out on disc, for example plasma isolation for protein analysis, cell lysis and nucleic acid extraction and purification for genetic analysis. Towards the full automation, pre-storage of reagents is a core objective: enzymes, primers, amplification components are dry-stored on the disc and re-hydrated; liquid buffers are pre-stored in special "packets" called "stick-packs", which open controllably at will (based on centrifugal burst pressure) to release the buffers.
Production: The fabrication of disposable cartridges is based on micro-thermoforming, i.e., the modification of the standard thermoforming technology (used for producing blister packages for food and/or pharmaceutical purposes) into microstructure-compatible production process. The typical steps for fabricating the final kits are: thermoforming; filling with dry/liquid reagents; sealing; cutting and packaging. The polymer-foil nature of the microfluidic cartridge allows adaptability to low-cost scalable production in large batches.

VALIDATION

The developed system will be validated in clinical settings at the areas of need, in local hospitals in Africa via partners' established contacts. The development steps take into account local particularities such as high temperature and humidity, frequent electricity cuts etc. In parallel, the results will be compared with the "gold standard" methods used for the examined diseases to assess the reliability of the system. Finally, the clinical interpretation of the acquired multi-marker diagnostic data will be done by the clinical experts of the consortium.
The project runs since November 2012 for three years. In light of commercialization, most experimental processes (e.g., fabrication, assays, micro/nanoparticle production) will be quality-control approved and the final device will be CE-certified. Further information about the project is available at www.discognosis.eu.

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A MICROBIAL APPROACH FOR BY-PASSING POLYMERIC NANOPARTICLE-MEDIATED INFUSION REACTIONS

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The role of complement in idiosyncratic reactions to nanomedicine infusion is receiving increasing attention. With increasing development of therapeutic and functional nanomaterials, and because of the high structural complexity of these entities and/or the use of peptides, proteins or nucleic acid components as payload or targeting ligands, the incidence of acute allergic reactions will increase and such outcomes may impose further health and financial burden. Strong emphasis has now been placed on nanomaterial properties and surface fabrication methodologies that do not trigger complement cascade. The interaction between nanomaterials and the complement system, however, is complex and regulated by inter-related factors that include chemical composition, reactivity, morphology and shape, hydrodynamic size, surface characteristics and surface defects. Each of these parameters may affect complement activation differently through the binding of different complement initiating molecules. These issues are raising challenges for controlled engineering of nanoparticle surfaces with polymers that was previously considered to be inert and safe. Currently, slowing of the rate of nanomedicine infusion, along with anaphylaxis preventable measures (e.g., administration of antihistamines, corticosteroids, bronchodilators) usually help in preventing complement activation-related allergic-like reaction and is effective in most subjects. These measures, however, are not specific and severe reactions may still occur in some individuals. Alternative strategies must therefore be sought for safer administration of nanomedicine. Accordingly, we have now taken lead from a number of endogenous as well as microbial strategies to minimize complement activation by nanoparticles and these will be discussed.

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MINING GENOME INFORMATION FOR NEW STARTING POINTS IN PERSONALIZED CANCER NANOMEDICINE

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For cancer therapy, the future will likely experience an increasing convergence of targeted medicine and nanomedicine. The situation, however, is still complicated by the molecular complexity of cancer, even within a particular subtype, the plasticity of cancer cells, which gives rise to drug resistance, and the recently emerged issue of cancer stem cells. On the other hand, novel techniques enabled the comprehensive molecular fingerprinting in diverse cancer types. A large repertoire of novel changes in cancer is expected to be provided by efforts such as the International Cancer Genome Consortium (ICGC) and techniques are under development, which may enable molecular fingerprinting of the patient`s individual tumors in clinical settings. The large number of molecular changes in cancer, however, still needs to be systematically scanned for those, which may provide new starting points for the development of the next generation of anti-cancer drugs.
We will present a strategy to close this gap by allowing for standardized analyses of genes with molecular alterations in cancer. We applied this on substantially large gene sets of 100 or more candidates and filtered out new modulators of breast cancer growth and for metastasis formation. Consecutively, this information is converted into systematic high-throughput screens to identify targeted nanodrug components with selective killing effects. As integral part of these strategies, we implement evaluation of effects on cancer stem cells. The present state of the art will be discussed.

FIRST IN HUMAN GOLD NANOPARTICLES FOR TRANSBUCCAL DELIVERY OF INSULIN IN PHASE 1 CLINICAL TRIAL: SAFETY AND BIO-DISTRIBUTION

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Last year, a First-in-Human study with ultra-small (<5 nm), proprietary, gold nanoparticles carrying regular, human insulin (GNP-I) was successfully concluded. Midatech sponsored the study, in partnership with MonoSol Rx (Warren, NJ). The nanoparticles, specifically

designed to bind bioactive peptide hormones such as insulin, were embedded in polymeric, muco-adhesive film and allowed efficient and safe transbuccal administration of GNP-insulin. To obtain clinical trial (CTA) approval a significant number of pre-clinical safety studies were performed on the GNP. These studies, performed in mice, rats, and dogs showed absolutely no indications of toxicity when orally applied in doses that were up to 6,000-fold the dose intended for human use. A very thorough bio-distribution and excretion study of the GNP was performed in rats which were treated for 14 days with 1.5 mg GNP/kg followed by a 14-day recovery period and revealed no treatment-related negative effects. The GNP were partially absorbed in the gastro-intestinal tract, but no accumulation of GNP in plasma or in any of the 7 major organs could be detected. It was also demonstrated that the kidneys were the only organs with significant GNP concentrations, confirming their role as the final collecting organ prior to urinary excretion. The majority of the orally administered GNP were excreted with the faeces. The bio-distribution of insulin and GNP was also studied in minipigs following intravenous infusion of GNP-I. At multiple time points blood was sampled from seven blood vessels entering and leaving major collecting organs. The results showed that the insulin quickly detached from the nanoparticles, did not accumulate in the organs and demonstrated shorter half-life in plasma than free, human insulin used as a control.

FABRICATION AND CHARACTERIZATION OF THREE-DIMENSIONAL BONE SCAFFOLDS

BERT MÜLLER

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Alpha-beta-tri-calcium phosphates are promising biomaterials to fabricate complex-shaped three-dimensional scaffolds for reconstructing larger osseous defects. Three-dimensional printing allows not only tailoring the macroscopic shape of patient-specific constructs but also the pore structures especially on the micrometer level to optimize cell attachment and migration. As a result, the mechanical properties of nature-analogue designed scaffolds can be controlled [J. Yoon, H. Deyhle, U. Gbureck, E. Vorndran, F. Beckmann, and B. Müller: Three-dimensional morphology and mechanics of bone scaffolds fabricated by rapid prototyping, International Journal of Materials Research 103 (2012) 200-206]. The obtained scaffolds were quantified by means of sophisticated software applied to the acquired tomography datasets. Here, the distance transform supports the evaluation of the printing quality and the porosity [F.C. Fierz, F. Beckmann, M. Huser, S.H. Irsen, B. Leukers, F. Witte, Ö. Degistirici, A. Andronache, M. Thie, and B. Müller: The morphology of anisotropic 3D-printed hydroxyapatite scaffolds, Biomaterials 29 (2008) 3799-3806], the radial density distribution as well as the accessibility of the scaffold by virtual spheres representing migrating cells. Therefore, the most promising designs can be selected for the specific implants in planned clinical studies. Analyzing the stress–strain curves of the sets of well-prepared scaffolds, the Young's moduli and the ultimate compressive strengths were uncovered. To reach the mechanical properties of jawbones, the scaffolds were treated with phosphoric acid according to a series of protocols. Depending on the design and applied treatment, the compressive strengths of the bone scaffolds lie between 30 and 60 MPa and their Young's moduli are between 50 and 140 MPa. Hence, we can start preparing optimized prototypes of bone scaffolds with dedicated porosities down to the nanometer scale for clinical trials.

CONTROLLED DRUG RELEASE VIA THERMO-LABILE NANOPARTICLES FOR CANCER TREATMENT

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This presentation presents the material science and materials engineering concepts that went into the design and testing of the Low Temperature-Sensitive Liposome (LTSL). Figure 1 is a schematic of the structure. Discussion centers on the roles of each of the components that make up the composite membrane; how the molecular and nanostructures that they form might influence the already anomalous permeability at the phase transition of the bilayer; and how this thermally-sensitive Drug Delivery System leads to ultra-fast, heat-mediated, triggered, intravascular release of pre-loaded doxorubicin. The LTSL has been characterized in vitro through a series of experiments including: carboxyfluoroscene release; dithionite permeability; and doxorubicin release as a function of LTSL composition and temperature. Basically, these experiments have shown that the low-temperature, thermally sensitive liposome (LTSL) retained small drug molecules like doxorubicin at 37oC, and rapidly released doxorubicin in less than 20s in response to applied heating equivalent to mild hyperthermia (HT) in the range of 40-42°C. Here is what we think is happening mechanistically:

Drug Retention and Leakage at 37oC

At 37oC, the in vivo preclinical data from Banno et al [32] showed that despite its unusual efficacy, the formulation does not retain drug all that well in the blood stream. As depicted in Figure 2A, the usually excellent drug retention of the more traditional lipid formulations is compromised by the need to trigger its release. There is still a pH gradient across bilayer and doxorubicin is still under the influence of the protonated-unprotonated equilibrium at pH 5.5. However, its retention in the liposome both in vitro and in vivo could hinge critically on the ability of the bilayer to retain hydrogen ions.

Thermally Triggered Drug Release at 41oC

With MSPC and DSPE-PEG2000 in the bilayer, as depicted in Figure 2B, the bilayer in the phase transition region acquires enhanced permeability through a purported MSPC pore. As the transition temperature is approached and the grain boundaries begin to melt, lateral lipid transport could well be increased and could allow more lysolipid to assume its preferred curvature (i.e. as a convex micelle), relaxing the planar bilayer structure by forming lysolipid-lined nanopores. With a few mol% of DSPE-PEG2000 in the bilayer, this MSPC pore appears also to be stabilized by PEG-lipid. As a consequence, the hydrogen ion gradient rapidly equalizes, DoxH+ comes out in seconds (large red arrow), as does any remaining embedded Dox in the bilayer.

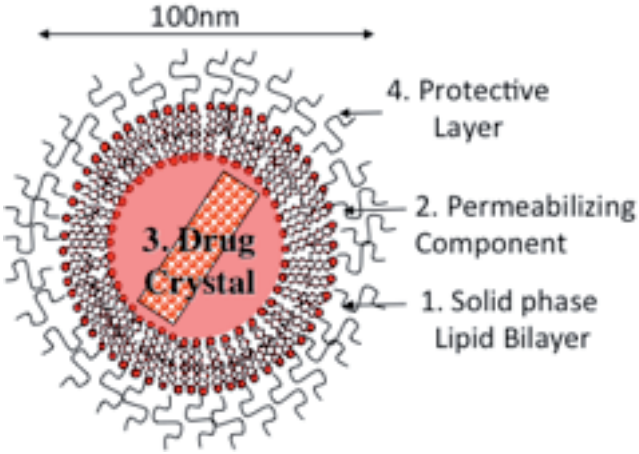

***Figure 1**. Component design of the 100nm diameter LTSL, comprising: 1. an encapsulating solid phase lipid bilayer; 2. A permeabilizing component; 3. an encapsulated drug; and 4. a protective PEG-layer.*

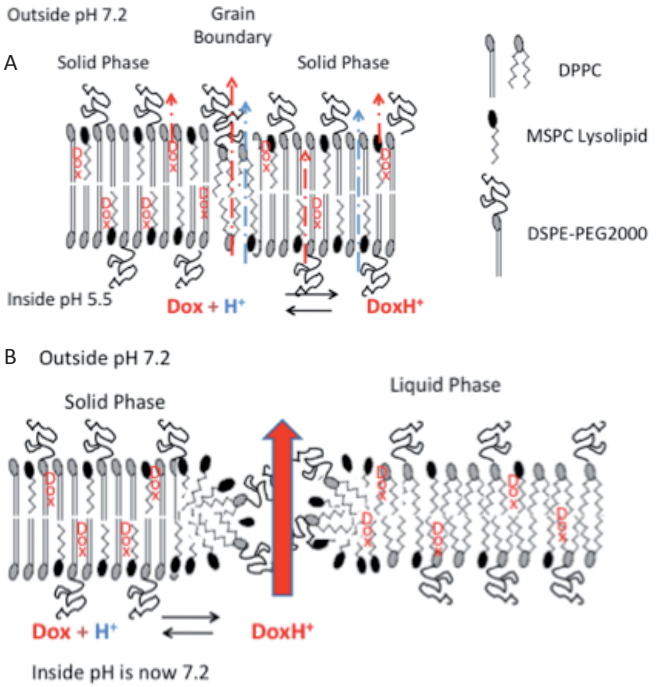

***Figure 2**. Purported mechanism for drug retention, leakage and thermally triggered release. A) Retention and possible leakage at 37oC; B) release at 41oC.*

In Vitro Behavior: Drug Release vs Membrane Composition

Liposomes composed of DPPC:MSPC:DSPE-PEG2000 released drug at the phase transition temperature at a rate that was expected from theory as modeled by Mouritsen et al. [18, 82], --relatively slowly. However as shown in Figure 3, compared to the DPPC:MSPC:DSPE-PEG2000 liposomes a massive increase in bilayer permeability now occurs at the phase transition just as a result of including the lysolipid, MonoStearoylPhosphatidylcholine (MSPC) Figure 3 shows just how fast doxorubicin is released from the LTSL when the MSPC bilayer concentration is at ~10mol% or higher. Over 80% of the drug is released in the first minute, and, for the slightly higher MSPC of 12-15 mol%, the drug comes out as fast as it can be measured, with ~90% released in the first 20s. As described next, this ultrafast release was essential for the liposome to achieve its function of releasing drug in a tumor vasculature so that the freed drug could diffuse into and penetrate the tumor interstitium, --release time was faster than transit time of liposomes through the tumor.

In Vivo Behavior: Drug Release and Penetration deep into the tumor interstitium

Release of drug penetrating deep into the tumor interstitium, is controlled by where, when, and for how long mild hyperthermia is applied [2] in relation to the liposome administration. As shown in Figure 4, real-time confocal imaging of doxorubicin delivery to the FaDu xenograft in window chambers, illustrates that intravascular drug release from LTSL-Dox massively increased the amount of free drug in the interstitial space [9]. This increased both the time that tumor cells were exposed to maximum drug levels and the drug penetration distance, compared with free drug or traditional pegylated liposomes.

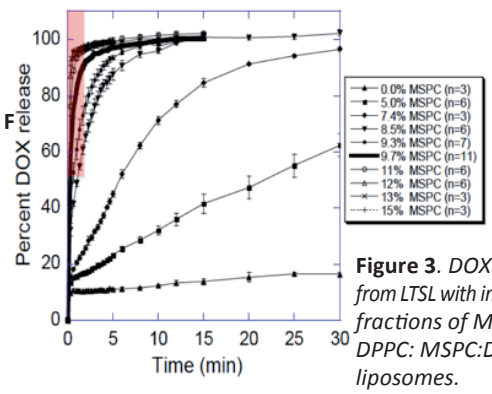

Figure 3. *DOX release at 41.3°C from LTSL with increasing membrane fractions of MSPC in the DPPC: MSPC:DSPE-PEG2000 liposomes.*

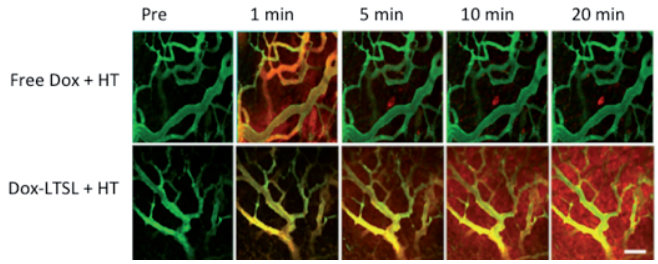

***Figure 4.** Tumor uptake of doxorubicin vs time. Time sequence images of blood vessels (green) and doxorubicin (red) for Pre-injection, and at 1, 5, 10, and 20 min after injection. Shown are injections to a warmed Tumor (42oC) of: Free Doxorubicin (Free Dox + HT); and the Doxorubicin loaded LTSL (Dox-LTSL + HT). Scale bar = 100 µm.*

Clinical Trials

This formulation, as ThermodoxÒ, has been used in a completed 700 patient phase III human clinical trial in liver cancer (HEAT study), is in a phase II trial in chest wall recurrence of cancer (DIGNITY study), and is also in a phase I trial of patients with colorectal liver metastases (ABLATE study) [5].

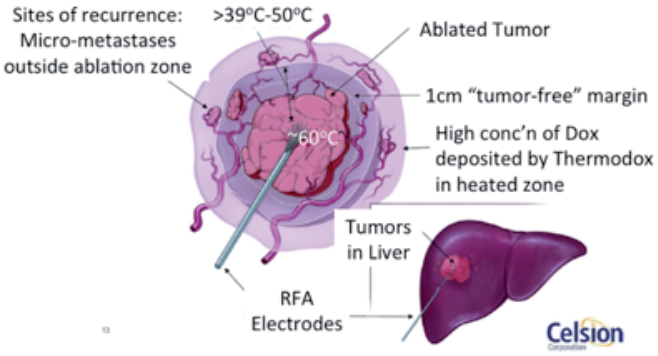

***Figure 5**. Radio Frequency Ablation of liver tumor + Thermodox®*

Phase III Liver Cancer

As depicted in Figure 5, the idea for the trial was to try to take advantage of the heating provided by standard RFA and see if this central heating could provide a high enough (just mild hyperthermic) temperature and release drug in the tumor periphery of large liver tumors. The hypothesis was that, because the temperature necessary to cause drug release (41°C-42oC) is over 15°C lower than the ablation temperature (>55°C), as the ablation temperature drops off from the center out to the periphery, there has to be a zone where temperatures are in the range 50oC – 39oC. As established in its in vitro performance [21], Thermodox® releases drug at significant rates with a peak at 41oC - 42oC. And so, the thinking was, if that zone happens to coincide with the periphery, then, when infused into the blood stream, Thermodox® will deposit high concentrations of doxorubicin throughout this heated tumor interstitium, and perhaps kill the dangerous micrometastases in the growing periphery of these deadly tumors. Unfortunately the top-line data, showed that, ThermoDox® in combination with radiofrequency ablation (RFA) did not meet the primary endpoint of the Phase III HEAT Study in patients with hepatocellular carcinoma (HCC), also known as primary liver cancer. Specifically,….. the HEAT Study did not meet the goal of demonstrating persuasive evidence of clinical effectiveness that could form the basis for regulatory approval in the population chosen for study [61]

Phase I-II Chest-Wall Recurrence

The Phase I-II RCW trial did however show more positive results. There were several instances of stable disease, partial response, and two complete responses for a dose escalation of 20 mg/m2 – 30 mg/m2. As reported by Vujaskovic, [57] several patients in this trial achieved either partial or complete responses. As shown in Figure 6, for one patient, after only four cycles, her widely disseminated chest wall tumor had completely disappeared. This test dose (30 mg/m2) was only 60% of the expected maximally tolerated dose, and so there were no side effects of the drug. A second patient had a similar complete response at 30 mg/m2 [57].

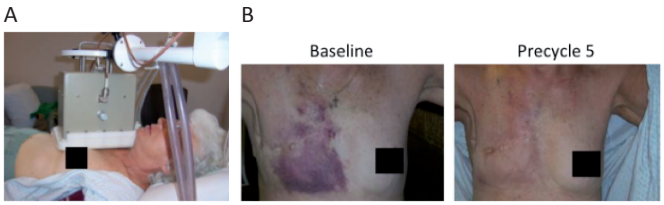

Figure 6. *A) Treatment of Chest Wall Recurrence of Breast Cancer, using a BSD-500 PC System to achieve a temperature goal of 40–42°C. B) Same patient before treatment and pre-cycle 5.*

LTSL and then Thermodox® is a bench to bedside story that addresses both overcoming limitations in nanoparticle drug delivery and also the importance of research implementation in translation to the clinic. With additional research and preclinical studies underway, including exploring the use of High Frequency Ultrasound (HiFu) as a heating modality, and a range of other drugs, imaging agents and biological modifiers poised for encapsulation, the LTSL could provide a new paradigm for drug and agent delivery for the treatment of localized tumors, --"rapid triggered drug release in the tumor bloodstream and deep penetration of drug into the tumor tissue".

While the phase III trial in liver cancer failed to meet its required primary endpoints for progression free survival, data is still being analyzed. Preclinical and now clinical research shows that it is essential for all future trials of this particular formulation, that the whole tumor should be heated to the desired temperature (41oC-42oC), and maintained at that temperature while Thermodox® is infused for a minimum of 20 mins, and probably an hour, i.e., heat first and then infuse drug.

References Cited

NON-INVASIVE AND HIGH-RESOLUTION OPTOACOUSTIC IMAGING TECHNIQUES OF NANOMEDICINES

ANTONIO NUNES[1], Nicolas Beziere[1], Juan Salichs[1], Volodymyr Ermolayev[1], Panagiotis Symvoulidis[1], Daniel Queiros[1], Neus Lozano[2], Kostas Kostarelos[2], Vasilis Ntziachristos[1]

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The introduction of imaging modalities for pre- and post-operative diagnostic, as well as for real-time intra-operative visualization, is expected to enhance the effectiveness of diagnostic tools for tumor patients. Recently, optoacoustic imaging has emerged as a versatile technology for the study and monitoring of anatomical, functional and molecular changes in tumors. Optoacoustic imaging is a promising modality that bridges the gap between optical imaging, with an optical absorption generated contrast, and ultrasound imaging, with its ultrasound-based detection system. After illumination by laser light pulses of short duration (10-100ns) a transient photo field is established in the tissue. Thermoelastic expansion of photoabsorbers then occurs, leading to the generation of acoustic waves that can be further detected by specific acoustic transducers. By detecting the pressure waves emitted by molecules absorbing pulsed laser light, Optoacoustic imaging could provide information regarding the localization of absorbing molecules (endogeneous, such as hemoglobin, or exogeneous, such as fluorophores or nanoparticles) in a living organism. Recent developments of the methodology also include a multispectral and tomographic approach, often called Multispectral Optoacoustic Imaging (MSOT). MSOT have been reported as a novel and powerful imaging modality, which offers new capabilities for pre-clinical (and possibly clinical) cancer research.

We have developed and explored MSOT, in particular mesoscopic MSOT, to simultaneously visualize in vivo the in-depth molecular imaging of absorbing compounds at high spatial and temporal resolution and the development of tumor vasculature. Particularly, we have investigated the dynamic enhancement of vasculature, characterization of the enhanced permeability and retention effect in tumors, as well as the oxygenated and deoxygenated hemoglobin mapping through the whole tumor.

Nanoparticle-based tracers were intravenously administered into different SC tumor-bearing mice. Optical excitation was provided by an optical parametric oscillator laser with ns pulse duration at repetition rates of 20Hz and a tunable range of 700-900nm. The ultrasonic detection was provided by a high-frequency, cylindrically focused transducer with a central frequency of 15MHz, allowing acquisition of coronal plane images of the tumor. The fiber bundle and transducer array were stationary, and the animal bed was rotational (360o) to acquire the entire tumor volume.

Further to the investigation of the distribution of the probe, we also studied their permeability and retention effect in tumors. We correlated the in vivo signal detected acoustically with the one obtained after ex vivo cryo-slicing, to determine the localization and extravasation of the probe into the tumor. Our results demonstrated the ability of mesoscopic MSOT to offer high resolution and fidelity imaging of optical contrast with a direct impact on oncology research. This study highlighted that MSOT is a powerful resource for biological applications, in particular for diagnosis or theranostics approaches in cancer research.

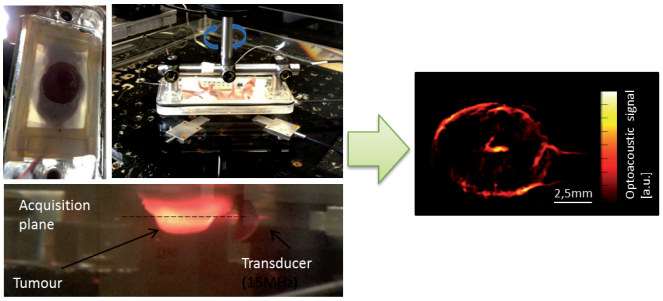

Figure 1*: High resolution MSOT System (left panel). Whole body image of a mouse during the scan in the mesoscopic MSOT camera showing the key components of the system. Mouse was placed in a rotational bed and 4 optical fibers homogenously illuminated the tumor volume (right Panel). An example of an optoacoustic image using mesoscopic MSOT.*

COMPUTATIONAL DESIGN OF EPITOPE SPECIFIC PEPTIDES, ANTIBODIES AND SMALL MOLECULES

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For a drug candidate to be successful it must target a protein that has a drugabble binding site, namely a surface patch or a cavity to which the drug can bind stably and selectively with sufficient affinity. But do druggable binding sites on proteins share common characteristics? And if such commonalities exist, can they be used to assist drug development? Large-scale analyses of druggable interfaces revealed that their characteristics indeed differ significantly from those of non-druggable interfaces. These analyses also highlight the specific characteristics of protein-small molecules interfaces and the very different characteristics of protein-peptides or protein-antibody interfaces. Using machine learning algorithms and modeling tools these typical characteristics of each type of interfaces can be used to predict from structure, and sometimes even from sequence, whether a given protein will bind a given small molecule or a given peptide or antibody. Experimental in-vitro results show that this approach can lead to the engineering of target specific peptides and antibodies. They are also successful in detecting novel protein-small molecule interactions, which may allow for drug repurposing and for identifying off targets.

CONTROLLED (BIO-) CONJUGATION OF INORGANIC NANOPARTICLES FOR TARGETED DELIVERY

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Arguably targeting is one of the biggest problems for controlled drug delivery. In the case that drugs can be directed with high efficiency to the target tissue, side effects of medication are drastically reduced. Colloidal inorganic nanoparticles (NPs) have been proposed and described in the last 10 years as new platforms for in vivo delivery. However, though NPs can introduce plentiful functional properties (such as controlled destruction of tissue by local heating or local generation of free radicals), targeting remains an issue of intense research efforts. While passive targeting of NPs has been reported (the so-called enhanced permeation and retention, EPR effect), still improved active targeting would be highly desirable. One classical approach for active targeting is mediated by molecular recognition via capture molecules, i.e. antibodies (Abs) specific for the target. In order to apply this strategy for NPs, they need to be conjugated with Abs against specific bio-markers. Though many approaches have been reported in this direction, the controlled bioconjugation of NPs is still a challenge. In this article the strategies of controlled bioconjugation of NPs will be reviewed giving particular emphasis to the following questions: 1) how can the number of capture molecules per NP be precisely adjusted, and 2) how can the Abs be attached to NP surfaces in an oriented way. Solution of both questions is a cornerstone in controlled targeting of the inorganic NPs bioconjugates.
Despite these promising applications, any new class of materials also bears a potential risk for human health and the environment. The advantages and innovations of these materials must be thoroughly compared against risks to evaluate each new nanomaterial. Although nanomaterials are often used intentionally, they can also be released unintentionally either inside the human body, through wearing of a prosthesis or the inhalation of fumes, or into the environment, through mechanical wear or chemical powder waste. This possibility adds to the importance of understanding potential risks from these materials. Because of fundamental differences in nanomaterials, sound risk assessment currently requires that researchers perform toxicology studies on each new nanomaterial. However, if toxicity could be correlated to the basic physicochemical properties of nanomaterials, those relationships could allow researchers to predict potential risks and design nanomaterials with minimum toxicity.
We will describe the physicochemical properties of nanoparticles (NPs) and how they can be determined and discuss their general importance for cytotoxicity.

REFERENCES

1. P. Rivera Gil, D. Jimenez de Aberasturi, V. Wulf, B. Pelaz, P. del Pino, Y. Zhao, J. de la Fuente, I. Ruiz de Larramendi, T. Rojo, X.-J. Liang, W. J. Parak, "The Challenge To Relate the Physicochemical Properties of Colloidal Nanoparticles to Their Cytotoxicity", Account of Chemical Research 46, 743–749 (2013).
2. J.-M. Montenegro, V. Grazu, A. Sukhanova, S. Agarwal, J. de la Fuente, I. Nabiev, A. Greiner, W. J. Parak, "Controlled (bio-) conjugation of inorganic nanoparticles for targeted delivery", Advanced Drug Delivery Reviews, http://dx.doi.org/10.1016/j.addr.2012.12.003.
3. M. Chanana, P. Rivera Gil, M. A. Correa-Duarte, L. M. Liz-Marzán. W. J. Parak, "Assessing the Physicochemical Properties of Protein-Coated Gold Nanoparticles in Biological Fluids and Cells before and after Proteolytic Digestion", Angewandte Chemie, in press.

PHYSIOCHEMICAL CHARACTERIZATION

ANIL PATRI
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The NCL has assessed nearly 300 nanomaterials, including liposomes, dendrimers and other polymers, quantum dots, gold colloids, metal oxides, and fullerenes. Out of the many physicochemical properties that define nanoparticles, we have found that size, surface charge, and drug loading, are among the most important influencers of biocompatibility. Specific NCL physicochemical characterization methods include microscopy (AFM, TEM, and SEM), static and dynamic light scattering, and separations methods such as size exclusion chromatography, field flow fractionation and capillary electrophoresis. This talk will cover the strengths and limitations of some of these techniques for characterizing different types of nanomaterials, including strategies for determining appropriate tests for batch-to-batch consistency, surface characterization, etc.

LUMINESCENT NANOPARTICLES FOR IMAGING IN CELLS AND BLOOD FLOW

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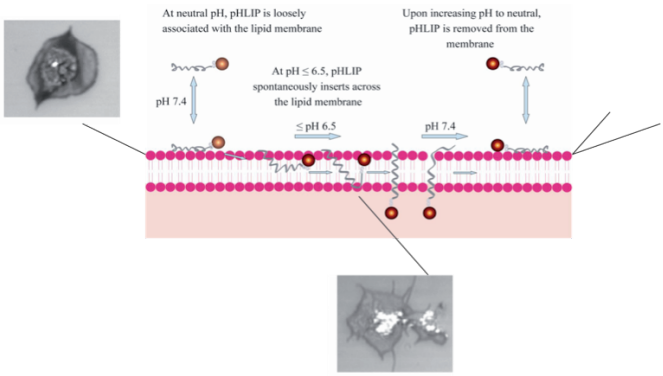
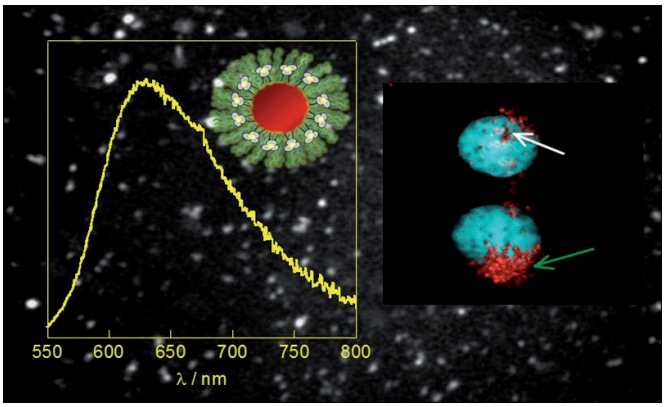
Fluorescence detection is a popular method to achieve high sensitivity in bioanalytical applications. However, single molecule luminescent labels have limited efficiency and cannot probe multiple interactions. It is a challenge for synthetic chemists to introduce multiple fluorescent labels on a reporter group without engaging in multiple step syntheses. Gold nanoparticles provide an attractive scaffold for the attachment of multiple fluorescent labels.[1-4] For imaging applications, tracing nanoparticles is important to probe events in the nanoscale and specific biomolecular interactions. They also provide a scaffold for the attachment of different labels (luminescent and MRI active) as well as potential drugs for controlled delivery. We use an approach of coating nanoparticles with metal complexes that luminescence in the visible and near infra red in a region from 500 nm to 1400 nm depending of the choice of the metal centre. One set of metal complexes involves lanthanide metals with characteristic emission band profiles in the visible (Eu,Tb, Sm, Dy) and the near infra-red areas of the spectrum (Nd, Yb, Er), offering significant advantages for intracellular imaging applications using wide-field fluorescence microscopy with long-lived signals far away from any background emission. We have used a pH sensitive peptide for targeted delivery of luminescent nanoparticles in platelets.[1]



For blood flow applications, we aimed to design nanoparticles with ruthenium and iridium complexes using a new approach of coating the particles. This approach has led to a high loading of he nanoparticle with metal complexes and has provided particles from 10 to 100 nm in diameter suitable for imaging applications of blood flow and cells.[5,6]



REFERENCES

[1] A. Davies, D. J. Lewis, S. P. Watson, S. G. Thomas and Z. Pikramenou Proc. Nat. Acad. Sci., 109, 1862, (2012).
[2] D. J. Lewis, C. Bruce, S. Bohic, S. P. Hammond, D. Arbon, Z. Pikramenou and B. Kysela Nanomedicine, 5, 1547, (2010).
[3] A. C. Savage and Z. Pikramenou Chem. Comm., 47, 6431, (2011).
[4] D. J. Lewis, T. M. Day, J. V. MacPherson, Z. Pikramenou Chem. Commun. 1433 (2006).
[5] D.J Lewis, V. Dore, M. J Goodwin, A. C Savage1, G.B Nash, P.Angeli and Z.Pikramenou Meas. Sci. Technol. , 23, 084004, 1-9 (2012).
[6] N. J. Rogers, S. Claire, R. Harris, G. Zikeli, S. Farabi, I. B. Styles, N. J. Hodges, Z. Pikramenou manuscript submitted.

MULTILAYERED NANOTECHNOLOGY FOR PERSONALISED NANOMEDICINE: FROM DIAGNOSTICS TO THERANOSTICS

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The use of engineered nanoparticles (ENP) in the medical/clinical field is well-established , through the use, for example, of superparamagnetic iron oxide nanoparticles (SPIONs) as magnetic resonance imaging (MRI) contrast agents.
Current development in this field are leading to believe that modified nanoparticles will improve disease treatment through a more adaptive and personalized approach to medicine. Possible route of delivery will be the selective accumulation of nanoparticles around the targeted cell/tissue allowing for the diagnosis of diseases and either imaging or monitoring of treatment efficacy. Proteomics or genomics personalised to patient will then allow for personalised treatment.
Surface modifications of ENPs can be designed in layers in order to achieve multilayered theranostic ENP for personalized medicine applications. One example is the making of functional SPIONs formed by three main components: (1) an iron oxide core functioning as MRI contrast agent, (2) a biocompatible coating and (3) a therapeutic coating targeted with a pharmacogenomics biomarker. Compared to conventional pharmaceutical products, nanometric drug delivery systems can often lead to higher drug bioavailability with lower adverse side effects. In addition, targeting moieties decorating the ENP surface can direct efficiently the clinical treatment to the affected tissue/cell with optimal selectivity.
For accelerating the translation of these materials into clinical markets, the impact of multilayered ENP on human health should be also assessed and modelled systematically on the relationship between surface properties and ENP toxicity behaviour. It emerges the need for assessment strategy and decision making tools on the efficacy of this complex ENPs. This becomes a priority in order to deliver a commercially viable tool with direct potential on the categorization and labelling of potential ENP-based nanomedical products for personalized medicine.
ACKNOWLEDGEMENT: This work was partially supported by the EU FP7 NAMDIATREAM project (NMP-2009-LARGE-3-246479), MULTIFUN project (NMP-2010-LARGE-4-262943) and Science Foundation Ireland CRANN-CSET (Pathfinder project).

FLASH NANOPRECIPITATION ENABLES THE PRODUCTION OF NANOPARTICLE DRUG COCKTAILS AND MULTIVALENT TARGETED NANOPARTICLES FOR IMAGING AND DRUG DELIVERY

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We have developed a block-copolymer-directed, kinetically-controlled self-assembly process called Flash NanoPrecipitation (FNP) to produce 50-400 nm nanoparticles. The process involves controlling micromixing to effect supersaturations as high as 10,000 in 1.5 ms, and then controlling nucleation and growth rates to match block copolymer assembly rates. The rapid assembly enables the encapsulation of multiple drugs and imaging agents into the same nanoparticle, and the production of multivalent targeted nanoparticles. We will give examples of nanoparticle assembly and focus on targeting and imaging applications. Imaging using long wavelength fluorphores, MRI contrast agents, and SPEC agents will be presented. An example of TB treatment combining multiple drugs and targeting made in a single-step process shows the versatility of the FNP assembly. The FNP process enables the production of nanoparticle libraries with variable ligand density. Examples of small molecule (mannose for TB), folate ligand, and antibody targeting will be presented. For the mannose targeted nanoparticles we show a maximum ligand density for uptake into macrophages, and a reduce uptake beyond this maximum value. The ability to tune ligand density on the nanoparticle surface affords the possibility of create strong and specific binding using lower cost targeting constructs than in traditional antibody targeting. This has strong implications for the cost and effectiveness of nanoparticle constructs for targeted delivery. Finally, the block copolymer makes a dramatic difference in the circulation and clearance times in vivo. Even with the same 5K PEG steric stabilizing layer, the presentation on the nanoparticle surface can change clearance half times between 2 hours to 24 hours.

SITE-DIRECTED PROTEIN IMMOBILIZATION ON POLYMER SURFACES VIA SORTASE A TRANSPEPTIDATION REACTION

MICHAEL RICHTER, Tobias Heck, Phu Huy Pham, Linda Thöny-Meyer
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The site-directed covalent attachment of polypeptides and proteins to surfaces is challenging and of importance for the production of immobilized biocatalysts, functional protein microarrays and biosensors with high sensitivity. Only few approaches are currently available that allow for concomitant covalent and oriented immobilization of natively folded proteins. Among these, the sortase A (SrtA) from Staphylococcus aureus has emerged as a promising enzymatic tool for the covalent immobilization of functional proteins in a site-directed manner under mild reaction conditions. SrtA recognizes a specific amino acid sorting motif (LPxTG) at the target molecules and catalyzes a transpeptidation reaction between this site and free terminal amino groups such as oligoglycine modified acceptors like proteins, fluorescent probes and even polymeric surfaces.
Recently, variants of SrtA have been reported that show improved transpeptidation efficiencies over the wild-type enzyme for the coupling of low-molecular weight model peptides (Chen et al., PNAS 2012, 108, 11399). In our approach we used green-fluorescent protein (GFPuv) engineered with the LPXTG sorting motif in order to investigate the suitability of the SrtA variants for the site-directed coupling of functional proteins to solid support. Further results will be presented showing the optimization of target proteins as well as coupling conditions in order to minimize competing enzymatic side reactions during the immobilization such as hydrolysis of the sorting motif and crosslinking among target proteins (Fig. 1). An overview of the potential of this technology for diverse applications in

the medical fields will be given.

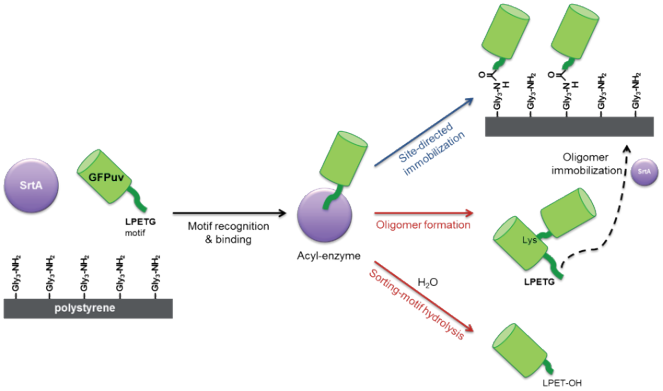

***Fig. 1**: Schematic representation of the SrtA-catalyzed immobilization of GFPuv target protein to polystyrene support and competing side reactions (red arrows).*

FOOD FOR THOUGHT CONCERNING THE IDEATION PROCESS FOR TRANSFORMING RESEARCH INTO APPLICATIONS

DOUGLAS K. R. ROBINSON, TEQNODE LIMITED, FRANCE

This short presentation is targeted at those who are (or may) consider translating research findings into products that will enter markets. It is not a presentation on how to create a company, manage your intellectual property or find investors, the focus is on the first steps in the ideation process which allows you to flesh out your thoughts around a preliminary idea for a technology innovation. My aim in this compacted time slot, is to provide insights that will allow you, I hope, to avoid some preliminary stumbling blocks in the organising of your ideas about your potential technology-lead innovation and developing an innovation strategy.

THEMES I WILL ELABORATE ON

Techno-push ideation approaches are only part of the picture. Technology-centred thinking leads to trouble, one has to thing further than the promising technology. Important are ways of thinking about the pathway through product concept development the manufacture process and arrival at the eventual users and need to be included in your thinking. Thus a charting of the innovation (chain-like) process is needed from the first concept to wider uptake and embedment in markets, identification of some of the key players in your journey is possible.

Will industry be in a position to absorb your product by the time it is nearing completion? Is the technology-production-system ready to receive your idea, or is some work needed? If work is needed, what steps could be taken already (especially in the nanomedicine and targeted therapy community).There are ways to find indications of this and I will mention a few from case examples.

Are killer applications, that stimulate breakthrough innovation, a myth? No, but rare. Often low performance applications are needed to bring radically new technologies into manufacture and markets, allowing for learning in the industrial-complex that will be part of the manufacture and distribution of the product. Also important are the users (both application producing firms: large pharma, medical device manufacturers etc.) and end users (healthcare providers, patients, clinicians etc.)

CONCLUSION

For those in research wishing to create a concept for application there are a number of things which I think can be useful to think about and that I hope you will take home and consider:

Stand-alone technology? Part of a configuration? Highly application specific? Or technology platform

Sketching out the Innovation Chain: who are the players to be involved, which aspects will play a role. Aids in identifying partners, sources of information and key production elements.

The current incumbent technologies (can you fit your tech in this existing regime? Can you stretch it to accept your technology…or is a totally new value chain and production community to be in place). Are your potential users easy to define? Single or multiple? Are they ready to receive? Engagement, interaction, involvement? Who, what, and when?Can visions of killer-applications be combined with low-performance (but still added value) applications to stimulate technology-production-community (those involved in manufacture, standards etc.) Allowing a step by step ramping up of your product (or product family)

Can there be redundancy (alternative back-ups) as you progress along this ramping up? Or put another way, can your technology at a specific stage of development be translated into different products? (examples are commercialisation of research in sensors, or materials which are developed for a specific application but may be transformed into a different product)

KILLING IT SOFTLY …WITH LIPIDS: ON THE NEW LETHAL TOPICAL VESICLES AGAINST PROTOZOA AND FUNGI

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The protozoa Leishmania braziliensis is the etiological agent of muco cutaneous leishmaniasis, an endemic zoonotic disease in South America that infects skin macrophages to end up destroying epithelial and mucosa tissue. The parasite is capable of taking up particulate material of size in the order of plasma proteins (< 10 nm diameter) by the flagellar pocket (FP). This type of endocytic uptake is limited to a small area (3 %) of the parasite body and seems to offers a steric restriction for the uptake of higher sized particulate material. The uptake of particulate material by fungi such as Candida spp on the other hand, is much less explored, but a similar steric constraint would be present. The penetration of particulate material across the intact skin is another example of steric restriction to be surpassed by vesicles that have to displace across hydrophilic nanochannels of less than 10 nm diameter. Our laboratory has developed different antifungal and antileishmania therapeutic and prophylactic strategies, by exploiting the sterically constrained endocytic uptake of parasites that colonize the skin surface and deeper layers combined with the high deformability of specially designed lipid nanovesicles. In this context we have found that in spite of their high size (~100 nm diameter) deformable nanovesicles have anti-protozoa activity per se and that if used for non occlusive topical delivery of anti mycotic, photodynamic agents to intracellular amastigotes or antigenic material to cells of the skin immune system, are more effective than their non deformable counterparts of similar size. Against Candida albicans and Candida parapsilopsis for instance, special deformable vesicles containing Amphotericin B have 33 and 8 folds lower MIC than Fungizone respectively, and 4 folds lower MIC than Ambisome. A low fluence rate of 0,2 J/cm2 on ultra low doses of photodynamic deformable vesicles at 0,01 mM ZnPC are capable of eliminating intracellular amastigotes of Leishmania braziliensis without killing host macrophages. In vivo, the same formulations substantially reduced the size of lesions in Swiss mice after 5 doses plus 30 minutes of sunlight irradiation. Finally, the topical application of a leishmania cell lysate within deformable nanovesicles targeted against skin macrophages on the intact skin of Balb c mice, produced antigen-specific systemic responses mediated by the induction of the pro-inflammatory cytokines TNFa and IL6. Remarkably, these results show that vesicles with special elasto mechanical properties can be used not only to replace injectables but also to be efficiently taken up by the complex endocytic machinery of eukaryotic pathogens.

NOVEL BIOMEDICAL NANOCARRIERS FOR A CONTROLLED INTERACTION WITH CELLS

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With the ever increasing production and use of engineered nanomaterials it is crucial that the interaction of such nano-sized materials with biological systems is understood. A lot of attention is paid to the material synthesis and characterization with a major focus on nano-properties and increasing attention to the nano-surface.
For fundamental but especially for the applied studies a central question addresses the state of the material. It is known that the cellular interaction of nanomaterials depends upon their physicochemical properties, such as size, shape, and surface charge. However, these properties might change in complex biological environments such as the cell culture media containing e.g. proteins, lipids, electrolytes, which in turn can fundamentally alter the nanoparticles' physicochemical properties. Such a property change can impact upon their cellular interaction in vitro and in vivo.
In addition, cellular systems are very complex and each cell type can react very differently to one and the same nanomaterial. To fundamentally address these questions, it is crucial to work with materials and cell culture systems, which are extremely well controlled and characterized before more complex studies with animals or humans can be considered.
Only by adjusting each individual step in this process, by concentrating on greater precision, and reproducibility, by standardizing materials and methods alike, it will be possible to improve the understanding of very complex systems, which in turn will facilitate closing the gap between scientific interest and commercial applications in the future.

TUMOR-HOMING PEPTIDE BOOSTS POROUS SILICON NANOVECTORS ACCUMULATION IN VIVO

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INTRODUCTION

Nanoparticulate delivery systems are designed to target anticancer drugs to tumors passively and/or actively.[1] These systems have been shown to increase the efficacy of anticancer drugs and reduce the side effects of chemotherapy.[2-4]
Porous silicon (PSi) particles are promising drug delivery vehicles due to their unique properties, such as high loading degree for different drug molecules and easy tuning of the particles' surface properties and pore size of the particles. These features enhance the drug dissolution behavior and/or allow the control of the drug release.[5-11]
The targeting moiety used in this study is a 9-amino acid long peptide (herein designated as CooP) that has been shown to home specifically to the intracranial murine brain tumor models of human glioma.[12]
Here, we aimed to prepare, characterize and test a PSi nanovector platform composed of THCPSi NPs functionalized with a tumor-homing peptide, CooP, and to evaluate their specificity to target the THCPSi NPs to MDGI-expressing MDA-MB-231 breast carcinoma cells in vitro and tumor xenografts in vivo. Flow cytometry and confocal microscopy were used to quantify the targeting efficiency and the uptake of the THCPSi-CooP NPs into MDGI-expressing MDA-MB-231 cells. Subsequently, we established subcutaneous MDGI-expressing tumors in nude mice and analyzed the biodistribution of the THCPSi-CooP NPs after intravenous injections. In addition, the homing efficiency and specificity of THCPSi-CooP into tumor site was evaluated. To our knowledge this is the first demonstration of the PSi-based materials biofunctionalized with a tumor-homing peptide to specifically target tumors in vivo.

EXPERIMENTAL SECTION

Preparation of THCPSi NPs: THCPSi NPs were prepared by the electrochemical anodization method as described elsewhere.[15] THCPSi NPs were labelled with tetramethylrhodamine-5-isothiocyanate (TRITC).
Preparation of THCPSi-CooP NPs: The particles were incubated in the peptide solution for 1-2 h under magnetic stirring at room temperature.
Physicochemical characterization of the THCPSi NPs: The structural properties (pore volume, specific surface area and pore diameter) of the THCPSi NPs were characterized by N2 sorption method and Attenuated Total Reflectance-Fourier transform infrared spectrometer (ATR-FTIR).
Stability of THCPSi-CooP NPs: The stability of the THCPSi NPs functionalized with CooP was studied in buffer (pH 7.4) at 37 ± 1 °C for 24 h and in human plasma after incubation at 37 ± 1 °C for 2 h.
Expression vectors, transfection and generation of stable MDGI-expressing MDA-MB-231 breast cell line: A stable MDA-MB-231 human breast carcinoma cells expressing MDGI as a c-Myc fusion protein (MDGI-c) were obtained by transfection with the pcDNA3-9E10 plasmid encoding the full-length MDGI protein.
Cell viability assay: MDA-MB-231 breast cancer cells were seeded in 96-well plates followed by addition of 50 µL of THCPSi and THCPSi-CooP NP suspensions (25, 50, and 100 µg mL–1). After 3 and 24 h incubations, the number of viable cells was assessed using the CellTiter-Glo™ reagent assay (Promega® Corporation, USA) according to the manufacturer's instructions.
Confocal microscopy: MDGI-GFP-expressing MDA-MB-231 cells were seeded in Lab-Tek™ 8-chamber glass slides and 200 µL of TRITC-labeled THCPSi and THCPSi-CooP NP suspensions (125 µg/mL) were added to the wells. After 3 h incubation, the particle containing suspensions were removed, fixed and analysed with Leica TCS SP2 AOBS confocal microscope.
Flow cytometry: Parental MDA-MB-231 and MDGI-expressing MDA-MB-231 cells were seeded in 12-well plates followed by the addition of 500 µL of THCPSi and THCPSi-CooP NP suspensions (75 and 125 µg/mL). After 3 h incubation, the cells were and analysed by flow cytometer (BD Biosciences, USA).
In vivo targeting studies: To establish a subcutaneous tumor xenograft derived from the MDA-MB-231 breast cancer cells expressing MDGI-c, athymic female Balb/c nu-nu mice were injected with 2 106 cells in PBS on the abdominal side of the mice. 50-days post-injections, after the tumors reached a volume of ~200–600 mm3, 150 µL of TRITC-labeled THCPSi and THCPSi-CooP NP suspensions (700 µg/mL) were injected into the tail vein of the tumor-bearing mice. Tumors and organs were collected, fixed and the tissue sections of tumors and organs were analyzed using an Axioplan epifluorescence microscope (Carl Zeiss AG, Germany).

RESULTS AND DISCUSSION

Characterization of THCPSi and THCPSi-CooP NPs: The specific surface area and the total pore volume for the THCPSi NPs were of 210 m2/g and 0.56 cm3/g, respectively. The average pore diameter of the THCPSI NPs was 10.7 nm and the Z-average particle size and ζ-potential obtained were 280 ± 18 nm and –35.9 ± 3.0 mV, respectively. After biofunctionalization with the CooP peptide, the Z-average particle size and ζ-potential values obtained were 181 ± 14 nm and –21.4 ± 1.2 mV, respectively.
Stability of THCPSi-CooP in buffer and human plasma: CooP biofunctionalization on the surface of THCPSi was stable for at least

24 h, and after several days no major detachment of CooP from the surface of the NPs was observed (data not shown).

Effect of NPs on viability of MDA-MB-231 cells in vitro: First, we studied the viability of the parental MDA-MB-231 cell line in the presence of THCPSi and THCPSi-CooP NPs. Either the THCPSi or THCPSi -CooP NPs did not induce toxicity to the MDA-MB-231 cells (results not shown).

Uptake of THCPSi-CooP NPs by the MDA-MB-231 cells: Confocal microscopy studies were performed with the THCPSi NPs labeled with TRITC and incubated with MDGI-GFP expressing MDA-MB-231 cells for 3 h. Confocal microscopy showed higher association of the THCPSi-CooP NPs with the MDGI-GFP expressing cells compared to the bare THCPSi NPs (Figure 1).

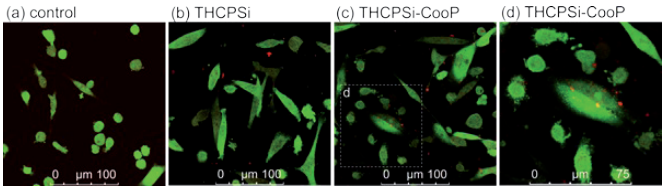

Figure 1. *(a) Confocal microscopy images of the MDGI-GFP-expressing MDA-MB-231 cells. (b) Cells were incubated with TRITC-labeled THCPSi NPs and (c) TRITC-labeled THCPSi-CooP NPs for 3 h at 37 °C. (d) Magnification of the selected region in (c), indicating association and internalization of THCPSi-CooP by the MDGI-GFP-expressing MDA-MB-231 cells.*

We next quantified the amount of THCPSi-CooP NPs internalized or associated with the MDGI-c expressing MDA-MB-231 cells by flow cytometry measurements. Comparison of the SSC-values showed that MDGI expression significantly ($p < 0.001$) increased the internalization of the THCPSi-CooP NPs in MDA-MB-231 cells (Figure 2) compared to the parental cells, which express negligible amounts of MDGI.

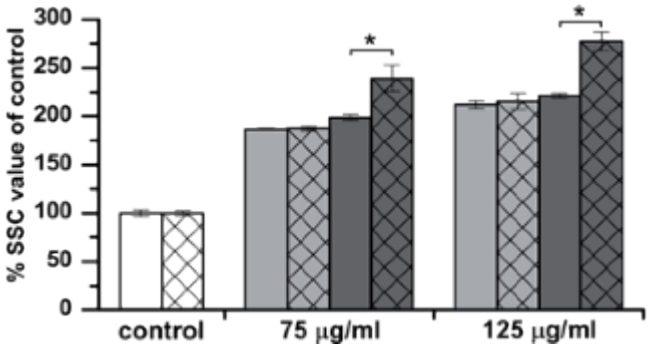

Figure 2. *SSC-values obtained from the flow cytometry dot plots of the treated cells were normalized to that of untreated cells (n ³ 4; mean ± s.d.). THCPSi-CooP NPs were taken up most efficiently by the MDGI-c expressing cells, which had significantly higher SSC-values compared to the control cells treated with the THCPSi-CooP NPs (*p < 0.001). There was no significant difference between the control and MDGI-expressing cells treated with the THCPSi NPs.*

Enhanced in vivo tumor targeting of THCPSi-CooP NP: To study the potential of the CooP peptide to target the THCPSi NPs to the tumors, we established an MDGI-c expressing MDA-MB-231 tumor cell line and first verified that the presence of MDGI increases CooP homing to these tumors. 105 µg of TRITC-labeled THCPSi or THCPSi-CooP NPs were administered intravenously via the tail vein into tumor-bearing mice and allowed to circulate for 1 h after which the animals were sacrificed. To study the tissue distribution of NPs, tumors and organs were collected and prepared for immunohistochemistry. Our results showed efficient homing of the THCPSi-CooP NPs to the tumor site (Figure 3a-b) when compared to the control THCPSi NPs (Figure 3c-d). Quantification of the accumulation of the NPs revealed ~3-fold ($p < 0.001$) more THCPSi-CooP NPs in the tumor tissue compared to the control THCPSi NPs (Figure 3e).

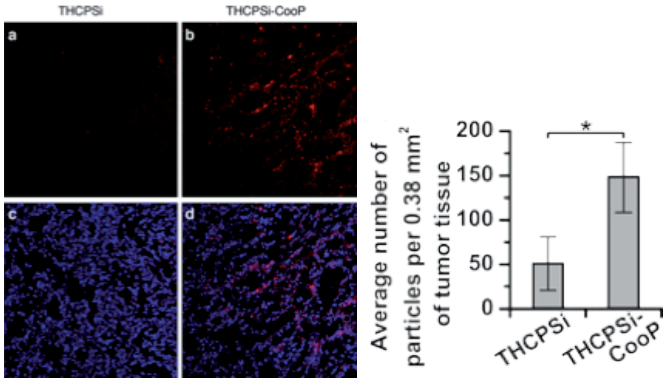

Figure 3. *Representative images of tumor tissue after intravenous injection of THCPSi (a and c) and THCPSi-CooP NPs (b and d). TRITC-labeled THCPSi and THCPSi-CooP NPs (red) were injected (105 µg) into the tail vein of tumor bearing mice (n ³ 2) and the accumulation of the NPs in the tumor tissue was assessed by examining the tissue sections under an epifluorescence microscope. The nuclei of the tumor tissues were visualized by using DAPI (blue in c and d). Scale bars are 200 µm. (e) Quantification of the average number of NPs per 0.38 mm2 of tumor tissue section (n ³ 4). The amount of accumulated THCPSi-CooP NPs in the tumor area was significantly higher compared to the control THCPSi NPs (*p < 0.001). Error bars represent s.d. (n ³ 2).*

In addition, biodistribution studies of THCPSi or THCPSi-CooP NPs were not detected in the kidneys after 1 h post-injection and the accumulation of the NPs in the lungs, heart) and brain was also negligible (results not shown).

CONCLUSIONS

We have demonstrated a simple and stable biofunctionalization of THCPSi NPs with a 9-amino acid long tumor-homing peptide (CooP), which targets the MDGI-expressing MDA-MB-231 breast carcinoma cells. The developed PSi nanovectors 181 nm in size were stable under physiological conditions in human plasma and did not cause any cytotoxic effects to the carcinoma cells. Expression of MDGI, the interacting partner for the CooP peptide, enhanced the cellular uptake of the THCPSi-CooP NPs in MDA-MB-231 cells compared to the control THCPSi NPs. After intravenous injections into nude mice bearing MDGI-expressing tumors, THCPSi-CooP NPs homed specifically to the tumor site whereas no major accumulation to the vital organs was observed. Our results provide the first proof-of-principle of targeted delivery of PSi nanovectors biofunctionalized with a tumor-homing peptide in vivo. Next, the THCPSi-CooP NPs carrying cytostatic drugs to the tumor tissue and the potential of this drug delivery system to suppress tumor growth will be evaluated.

ACKNOWLEDGEMENTS

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REFERENCES

[1] F. Danhier, O. Feron, V. Préat, J. Control. Rel. 2010, 148, 135-46.
[2] L. Brannon-Peppas, J. O. Blanchette, Adv. Drug Deliv. Rev. 2012, 64, 206-212.
[3] S. E. Mcneil, WIRES Nanomed. Nanobi. 2009, 1, 264-271.
[4] A. Z. Wang, R. Langer, O. C. Farokhzad, Annu. Rev. Med. 2012, 63, 185-198.
[5] L. M. Bimbo, E. Mäkilä, J. Raula, T. Laaksonen, P. Laaksonen, K. Strommer, E. I. Kauppinen, J. Salonen, M. B. Linder, J. Hirvonen, H. A. Santos, Biomaterials 2011, 32, 9089-9099.
[6] P. Kinnari, E. Mäkilä, T. Heikkilä, J. Salonen, J. Hirvonen, H. A. Santos, Int. J. Pharm. 2011, 414, 148-56.
[7] D. Liu, E. Mäkilä, H. Zhang, B. Herranz, M. Kaasalainen, P. Kinnari,

J. Salonen, J. Hirvonen, H. A. Santos, Adv. Funct. Mater. 2013, doi: 10.1002/adfm.201202491.
[8] H. A. Santos, L. M. Bimbo, V.-P. Lehto, A. J. Airaksinen, J. Salonen, J. Hirvonen, Curr. Drug Discov. Technol. 2011, 8, 228-249.
[9] M. P. Sarparanta, L. M. Bimbo, E. M. Mäkilä, J. J. Salonen, P. H. Laaksonen, A. M. K. Helariutta, M. B. Linder, J. T. Hirvonen, T. J. Laaksonen, H. A. Santos, A. J. Airaksinen, Biomaterials 2012, 33, 3353-3362.
[10] L. M. Bimbo, M. Sarparanta, H. A. Santos, A. J. Airaksinen, E. Mäkilä, T. Laaksonen, L. Peltonen, V.-P. Lehto, J. Hirvonen, J. Salonen, ACS Nano 2010, 4, 3023-32.
[11] J. Rytkönen, R. Miettinen, M. Kaasalainen, V.-P. Lehto, J. Salonen, A. Närvänen, J. Nanomater. 2012, 2012, 1-9.
[12] P. Laakkonen, E. Ruoslahti, G. Bergers, U.S. Patent #8188221, May 29, 2012.

DOES THE CELL ARCHITECTURE INFLUENCE ENGINEERED NANOMATERIAL UPTAKE? – AN EXPERIMENTAL APPROACH

ANGELA SCHIPANSKI, Carina Mouth, Tina Bürki, Markus Rottmar, Peter Wick, Katharina Maniura-Weber

Engineered nanomaterials (ENMs) harbor distinctive physicochemical properties (i.e. catalytic, thermal and optical properties), they do display an increased surface area to volume ratio, different geometries and surface characteristics. These unique characteristics render ENM`s attractive for a variety of applications in technical industry e.g. waste water treatment, or in cosmetic consumer products e.g. sunscreen or toothpaste. Thereby, ENMs are in direct contact with the human organism, and it is until present not completely understood how they interact with. As the size of ENMs corresponds to those of typical cellular components and proteins, the body may process ENMs as such. Experiments revealed predominantly an active cellular uptake of ENMs via different endocytic pathways comprising phagocytosis ("cell-eating") and pinocytosis ("cell-drinking"). The latter pathway can be subdivided into clathrin-mediated endocytosis (CME), caveolae-mediated endocytosis (CvME), and possibly alternative routes.
A prerequisite, for ENM incorporation, is the adherence of particles to the cellular surface. Interaction with the cell surface can be studied experimentally via different approaches, e.g. pharmacologically or mechanically. Endocytic pathways can be blocked using pharmacological inhibitors. This strategy must be treated with caution, as these inhibitors are not specific and may influence alternative internalization routes as well as the actin and the microtubule network. In addition, compensatory routes of uptake may be activated in cells upon inhibition of specific transport pathways. Moreover, most of these inhibitors interfere with vital cell functions and are therefore intrinsically toxic. On the other hand, cells can be incubated at lowered temperatures to interrupt endocytic processing. Temperatures around 20°C prevent progression of particles from early to late endosomes. Further temperature lowering to 4°C, for example, blocks all energy-dependent processes. It should be kept in mind, that temperature reduction does effect the phospholipid composition of the cell membrane, which in turns influences cellular uptake capacity as well as the uptake route.
Alternatively, a mechanical modulation of cell membrane properties being important for cellular uptake e.g. tension, contractility or actin/ tubulin network organization, might be an attractive approach to investigate ENM-cell surface interaction. Micro-patterned substrates are a perfectly suitable tool for this purpose. Hereby, small chemical islands (proteins of the extracellular matrix) promote cell adhesion on a cell repellent background (polydimethylsiloxane, PDMS) allowing for selective cell spreading. This in turn, results in single cells with well-defined and reproducible cellular shape, contractility, and intracellular organization as well as defined cell surface area (Fig.1). The characterization and evaluation of single cells thus is easily possible. Beyond that, a structural and biochemical microenvironment is generated resembling partially properties present in vivo, where cell-cell or cell-extracellular matrix contacts are current. All these parameters are lost when cells are cultured under conventional 2D conditions on tissue plastic dishes.

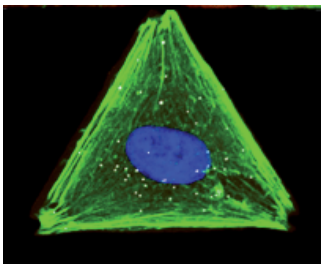

Fig 1. *Shape- normalised cell with internalized 240 nm sized ENMs; green: actin, blue: nucleus*

We studied single cells on T-, L-, Δ- geometry patterns with identical surface area but different actin network organization, force distribution and internal cell organization, in order to correlate ENM uptake with cell architecture. First, we identified suitable cell types, which adhere and spread nicely on micropatterns and are able to internalize ENM within a time period of 24h. Second, we chose well-characterized fluorescent ENMs being non-toxic and monodisperse in cell culture media. In addition, dye leakage from the ENM has to be ruled out. Fluorescently labeled polystyrol (fPS) beads of 240 nm fulfilled these criteria. Third, a semi-quantitative approach using image analysis procedure based on confocal laser scanning microscopy was established. Stacks of confocal fluorescence images of single cells interacting with fPS were used to determine the mean intensity of internalized fPS. To analyze the average location, amount and distribution of actin, fPS and nuclei, heatmaps were generated. Heatmaps were created by an overlay of 20-stacked images. Furthermore, subcellular localization of fPS by staining endosomal and lysosomal compartments is anticipated. This experimental setup provides an attractive model system to study cellular ENM uptake. Such know-how is much needed to exploit the beneficial potential of ENMs for pharmaceutical applications or to estimate their possible negative impact on humans. In addition, such a single cell analysis tool might facilitate systematic studies elucidating mechanisms underlying ENM uptake into cells.

UNIVERSITY VILLAGE "SMALL TALKS" SESSION

RUTH SCHMID

To be able to translate academic research efficiently and successfully into clinical applications and industrial products, intensive communication between university researchers and industry, clinicians and regulatory agencies is a must. The University Village and the Small Talk session at CLINAM, which gives university participants the possibility to highlight their research activities and get valuable feedback from industry, physicians and regulatory experts, is a unique instrument to foster this dialogue at an early stage of scientific research and to contribute to bring new innovations to the patient quickly in a highly regulated area. This type of sessions is of high value in the landscape of Open Innovation and will contribute to align academic research to patient needs.
The University Village and "Small Talks" Session has a bidirectional impact. Industry and clinicians will get insight into novel approaches, new research projects and initial outcomes of scientific nanomedicine research, while university participants will get feedback based on clinical needs, regulatory rules and industrial priorities to redirect and align their research for the best of the patients.
I encourage all University participants to grasp this unique possibility and all clinicians, industry participants and regulatory experts to attend the small talk session to jointly contribute to overcome the gap from laboratory research to bed-site.
I personally participated in the University Village and with a Small Talk at CLINAM 2011 and 2012 and will do so also in 2013. This opportunity to interact was the most important part of my participation at CLINAM, as it resulted in networking with many known and new people and several common proposals, both at national and international level.

CANCER STEM CELLS AS PREFERENTIAL TARGET FOR PERSONALIZED CANCER NANOMEDICINE

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In many solid tumors, resistance to therapy and metastatic disease seem to be sustained by the presence within the tumors of the cancer stem cells (CSC) capable of regenerating a tumor after chemotherapy and/or radiation treatment. In breast cancer, these cells correspond to a small fraction of cells within the tumor that express stem cell markers (CD44+/CD24-/low/lin-) which provides a useful target to the delivery of therapeutic agents to CSC. In this context, design of specific vehicles for the deliver of chemotherapeutic drugs with known antitumor activity against breast cancer targeting CSC is envisioned as a poweful new therapeutic anticancer strategy. To this end, specific CSC in vitro and in vivo models have been generated using specific bioluminiscent vectors to test different drug delivery systems (DDS). These, have been directed to the CSC compartment by using the CD44 receptor as a target. Such systems allow specific CSC-targeting and cell internalization, and together with the enhanced retention an permeability effect (EPR) improve accumulation of drugs into CSC. Interestingly, drug efficacy is also improved against CSC which seem to be less resistant to Chemotherapy when using nanomedicine drug delivery. This suggest, that use of DDS might reduce tumor resistance to conventional drugs.

UNSOLVED PROBLEMS IN CANCER

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Even though improved chemotherapeutic drugs and early diagnosis of cancer diseases have substantially increased life expectancy and overall survival of cancer patients in the last 20 years, still a big window of improvement is achievable. Several problems need still to be properly addressed to improve survival, such as metastasic spread of the disease which is still one of the most relevant clinical unsolved problems. Among them, better treatment regims and combination therapies, new combined drugs targeting different molecular pathways, new mechanisms of action, lower toxicities and more selective treatments that should decrease cancer cell resistance to current treatments.

A NEW, HIGH THROUGHPUT, LOW COST APPARATUS FOR CIRCULATING TUMOR CELLS COUNTING AND ANALYSIS (*)

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Counting Circulating Tumor Cells (CTCs) have been recently widely accepted as a diagnostic tool with prognostic values and for therapy monitoring. The only commercially available FDA-approved apparatus is the Cell Search of the Veridex Company that is, however, very expensive (both in cost of the hardware and in cost of reagents i.e. antibodies) and can process only 8 samples per day. As a consequence, at present the only use of Cell Search is one analysis per patient carried out exclusively for prognostic value.
Using FACS analysis and fluorophore conjugated glucose molecules we have analyzed suspensions of mixed cancer cell lines and white blood cells and saw a different glucose uptake in the two populations. We hope to report at the meeting the first result of a study, presently in progress, in which we use a microfluidic circuit by which we count the CTCs, after their concentration from whole blood of cancer patients using glucose conjugated with magnetic nanoparticles. We should be able to analyze several tens of samples per day with reagent cost of less than 10 dollars per analysis. Moreover we shall have the added advantage that the counted populations will keep the cells alive, to be contrasted with antibodies' based methods, for example those that search for Cytokeratins used in the Cell Search device.

(*) This work has been conducted in collaboration with Daniela Cesselli , Fabio Del Ben, Matteo Turetta, Leonardo Venturelli. Funding for this work was made available by the ERC through a senior grant, made to G. Scoles.

ASSESSMENT OF COMPLEMENT ACTIVATION IN NANOMEDICINE

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On behalf of TECOmedical

Extensive growing of nanomedicine and drug delivery systems dictates new and strengh requirements for safety evaluation. Existing standards for biocompatibility evaluation are not well applicable in nanomedicine and there is a demand for confident and sensitive methods for evaluation of nanoparticles and drug delivery systems. The complement system is a front line defence against pathogens and artificial materials. Complement activation on artificial surface starts within a seconds after contact with material and depends on charge, nanostucture, and the site of contact. In vitro examination of complement activation is one of the most sensitive and reliable method to evaluate biocompatibility of nanoparticles and artificial devices. As traditional hemolytic assay is still in use for evaluation of functional activity of complement, EIA offers fast and stable determination of complement activity.
Initial evaluation of complement activation recommends to perform in solide phase, as it allows to detects even mild incompatibility. Further evaluation of liquid phase activation will give more detailed picture of material biocompatibility. Selection of assay is also important for evaluation. Assays based on polyclonal antibodies is less sensitive for in vitro activation of samples during handling and storage, but can detect both active and non-active complement molecules. In contrary monoclonal-based assays for detection of neoepitopes on complement molecules allows to measure activated complement molecules only. Selection of a test molecule should be also based on molecule half-life. C5a molecule with 1 min half-life can be difficult to detect in samples, and TCC with several hours half-life is the most reliable candidate to use for evaluation of biocompatibility. In addition to TCC, Bb molecule can be used as an indicatior of the alternative pathway activation by biomaterials, as well as C3c fragment as an indicator of the common pathway activation.
In conclusion, biocompatibility of nanomaterials is extremly important for patient safety and use of complement activation as an indicatior of incompatibility will allow not just improve safety of materials, but will lead to improved performance of nano-based devices and drug delivery systems.

LIGHT AS AN ACTIVATOR OF NANOPARTICLES AND CELLS

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Biointegration exerts stress on cells, in particular during first contacts, when the viability of the cells proximal to the biomaterial dramatically drops. Cell viability in vitro can be checked by quantitation of the ATP present, which reflects the presence of metabolically active cells. Izyumov et al.1 have shown that even a transient decrease in cellular ATP levels can commit cells to apoptosis. Thus, it is reasonable to search for routes to consolidate the performance of compromised cells in vivo. The only externally applicable physical method that is capable of replenishing ATP reservoirs in cells,

both in vitro and in vivo, is light, specifically red to NIR light applied at intensities and energy densities described by the "biostimulatory windows".2 While boosting ATP levels in the cells, the irradiation is instrumental in remodelling the ECM by stimulating fibroblasts to synthesize collagen.3 These advantages bring light automatically into the focus of regenerative medicine. Furthermore, irradiation with red to NIR light was shown to stimulate ROS production by mitochondria, a process accompanied by an increase in the permeability of blood vessels – a route for nanoparticles to overcome natural obstacles, including the blood-brain-barrier.4 Recently, we discovered further beneficial effects of the same light. The results of model experiments indicate potential applications ranging from the regeneration of the brain (elimination of cellular amyloid-b plaques) to the selective killing of hibernating cancer stem cells (believed to escape chemotherapy).5,6 Refuelling cellular energy reservoirs requires the presence of mitochondria and is virtually independent on the particular mode of irradiation (continuous or intermittent). The cellular killing program works in cells which present some permeability to water and are rich in nanoscopic interfacial water layers which can be modulated with intermittent irradiation, thereby activating powerful pumping processes forcing cancer cells to uptake drugs6 – presumably even against the action of ABC transporters. At present the use of light in nanomedicine is largely restricted to the activation of nanoparticles. The possibility to coincidently activate nanoparticles and cells opens totally new therapeutic perspectives, for instance, in combination with uptake processes in which the nanoparticles enter the cells by active processes requiring ATP.7 What is so exciting about the dual activation is the potential of the imaginable logical combinations and their applicability to almost each cell and almost all organs. Laser/LED based irradiation systems producing red to NIR light, including implantable components, could become the second pillar in clinical nanomedicine. Figure 1 is visual synopsis of the modalities to activate cells with light.

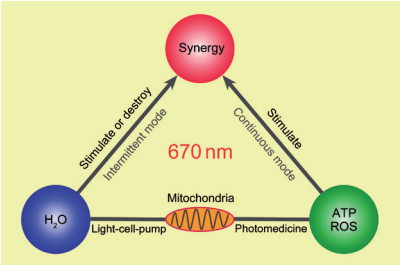

Figure 1 *Most cells proliferate in response to their irradiation with red to NIR light applied at biostimulatory intensities around 1·103 Wm-2 and energy densities in the range 1 – 4·104 Jm-2, whereby the mitochondria produce ATP and/or ROS. Complementary metabolic processes are induced in cells by intermittent irradiation (same irradiation parameters).*

REFERENCES

- Izyumov DS, Avetisyan AV, Pletjushkina OY, Sakharov DV, Wirtz KW, Chernyak BV, Skulachev VP. "Wages of fear": transient threefold decrease in intracellular ATP level imposes apoptosis. Biochim Biophys Acta. 1658, 141-147, 2004.
- Sommer AP, Pinheiro AL, Mester AR, Franke RP, Whelan HT. Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system. J Clin Laser Med Surg. 19, 29-33, 2001.
- Mester E, Mester AF, Mester A. The biomedical effects of laser application. Lasers Surg Med. 5, 31-39, 1985.
- Sommer AP. A novel approach for addressing Alzheimer's disease: the chemo-optical synergism. J Neurosci Res. 90, 1297-1298, 2012.
- Sommer AP, Bieschke J, Friedrich RP, Zhu D, Wanker EE, Fecht HJ, Mereles D, Hunstein W. 670nm laser light and EGCG complementarily reduce amyloid-β aggregates in human neuroblastoma cells: basis for treatment of Alzheimer's disease? Photomed Laser Surg. 30, 54-60, 2012.
- Sommer AP, Zhu D, Scharnweber T. Laser modulated transmembrane convection: Implementation in cancer chemotherapy. J Control Release. 148, 131-134, 2010.
- Canton I, Battaglia G. Endocytosis at the nanoscale. Chem Soc Rev. 41, 2718-2739, 2012.

NANOTOXICOLOGY AND PHARMACOLOGY

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Critical parameters that can influence the success of nanotechnology platforms include in vivo stability, tissue distribution and biocompatibility. This presentation will address the importance of selecting appropriate preclinical ADME and toxicity models, and the analytical challenges associated with monitoring the in vivo stability of nanotechnology platforms, using case studies to demonstrate the methodologies employed.

TARGETED DRUG DELIVERY IN INFLAMMATORY DISEASE – COMPARISON OF LIPOSOMES WITH OTHER NANOCARRIER SYSTEMS

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Previous studies have shown strong therapeutic activity of joint-targeted dexamethasone using arthtrotropic drug delivery systems in several models of rheumatoid arthritis (RA). Dexamethasone encapsulated in long-circulating liposomes, macromolecular polymeric dexamethasone prodrugs, and dexamethasone-loaded core-crosslinked polymeric micelles, are all highly effective in suppressing clinical signs of arthritis compared to the free drug. It is, however, unclear how these systems compare to each other regarding their therapeutic efficacy in RA. The current study deals with the comparison of the anti-arthritic efficacy of three dexamethasone-targeting systems in two preclinical models of arthritis. The selected systems were evaluated at three different single dose levels in the murine collagen antibody-induced arthritis (CAIA) model, and at one single dose level in the adjuvant-induced arthritis (AIA) model in rats. Whereas all systems are highly effective at the highest dose, at lower doses differences in therapeutic efficacy between the systems related to the differences in drug release profile. On the one hand, dexamethasone encapsulated in long-circulating liposomes was the only system that possessed significant therapeutic activity at the lowest dose, but the duration of its effect was not as long-lasting as compared to the other systems. On the other hand, the polymeric dexamethasone-prodrug showed no therapeutic efficacy at doses lower than 10 mg/kg, but its effect prolonged for a longer period of time than that of dexamethasone liposomes. In conclusion, these results demonstrate the value of comparative studies for assessing the differences in therapeutic potential between targeted drug delivery systems for the treatment of chronic inflammatory disorders.

BIOEQUIVALENCE CRITERIA FOR GENERIC LIPOSOME PRODUCTS

GERT STORM

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When the patent of a small molecule drug expires, generics may be introduced. In the case of oral dosage forms, they are considered therapeutically equivalent once pharmaceutical equivalence and bioequivalence (i.e. comparable pharmacokinetics) have been established in a cross-over volunteer study. However, this generic paradigm cannot be applied to complex drugs as biologics and a number of other therapeutic modalities (e.g., iron-carbohydrate drugs, low-molecular-weight heparins, glatiramoids). In contrast to these examples of 'complex drugs', arguments will be provided in this presentation to propose that the classical bioequivalence approach (i.e. evaluation of pharmacokinetics as surrogate for pharmacodynamics) used for the approval of oral dosage forms may also be applied for the approval of generic liposomal formulations (non-vaccine based), if properly adapted.

TRANSLATION OF AN ANTI-INFLAMMATORY NANOMEDICAL TREATMENT FOR ATHEROSCLEROSIS: FIRST EXPERIENCE IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Atherosclerosis is a progressive disease, which can be considered a chronic inflammatory disease of the large arteries. Since the introduction of statins 20 years ago, the development of novel therapeutic strategies for atherosclerosis has stagnated. Nanomedicine offers innovative treatment possibilities for local delivery. Although established in cancer, nanoparticle therapy for atherosclerotic disease is clinically unexplored, but may represent a new powerful treatment paradigm. Here we present an extensive translational program that is aimed at developing and translating a nanomedicine-based anti-inflammatory therapy. It involves the development of a GMP-grade anti-inflammatory nanoparticle therapy, extensive preclinical in vivo studies evaluating pharmacokinetics, toxicity and efficacy, as well as the first-in-human clinical trials in atherosclerosis patients. Preclinical evaluation of the nanotherapy, a PEGylated liposomal nanoparticle formulation of prednisolone phosphate (LN-PLP), accentuated its long-circulating properties and safety in rats, as well as local anti-inflammatory effects in atherosclerotic rabbits. Accumulation of LN-PLP into human plaque tissue was demonstrated in harvested atherosclerotic plaque specimens from patients with symptomatic atherosclerotic disease. Finally, after confirming successful delivery, a trial in atherosclerotic patients was performed using non-invasive multimodal imaging to evaluate therapeutic efficacy. This integrated program can serve as a guide for the development and imaging-assisted evaluation of future anti-atherosclerotic nanotherapy.

ATOMIC FORCE MICROSCOPY (AFM) FOR NANOMEDICINE

ROBERT SUM

Recently two AFM-related innovations attracted attention in the medical community: FluidFM®, which allows delivery or extraction of smallest quantities of liquid to or from a cell under exact force control through a hollow cantilever, and ARTIDIS®, which allows the investigation of the nanomechanical properties of tissues in order to better understand and diagnose diseases like cancer or ostheoarthritis.
FluidFM® uses microfabricated hollow cantilevers with a range of tip and aperture sizes for different applications. The FluidFM cantilevers have a small liquid reservoir, which is attached to a microfluidic control system that allows applying over- or underpressure in order to inject, deposit, or extract fluid, or to grab and release objects or cells. Recent experiments by Stiefel et al. showed the process of deposition of single virus particles onto a cell and the subsequent infection. Other experiments by Potthoff et al. measured cell-adhesion forces with an unrivalled speed, efficiency, and precision.
ARTIDIS® provides nanomechanical investigation of tissues, including stiffness and adhesion measurements and their statistical and quantitative analysis. When a patient is suspected of cancer, for example, time-consuming tissue analyses have thus far been necessary to obtain a reliable diagnosis. With ARTIDIS®, Plodinec et al. showed that lengthy waiting times can now be reduced from days to a matter of hours. Nanomechanical measurements on suspect tissues provide a characteristic "fingerprint", which allows classification into healthy tissue, benign material, or malignant tumor.

BIO-INSPIRED 3-DIMENSIONAL NANO-AND MICRO-STRUCTURED BIOMATERIALS FOR TISSUE ENGINEERING

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Tissue engineering (TE) nowadays focuses on the development of the tissue engineered scaffolds based on biodegradable natural or/and synthetic biomaterials able to support regeneration of the damaged region and undergo degradation within the regenerate tissue formation. There are many available techniques for scaffolds fabrication allowing for obtaining of constructs with different nano- and microsizes, shapes, porosity, degradability, physical and mechanical properties giving suitable environment for simulation of a new tissue formation depending on the specific requirements of the damaged region.
The aim of the presented study was to present high potential of bio-inspired synthetic and natural polymers for fabrication of novel nano- and microstructured scaffolds using different technique like blending, solid freeform fabrication and electrospinning. Several biomaterials including biodegradable polyesters like poly(ε-caprolactone) [PCL], poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [PHBV] and copolymers like poly(L-lactic acid)-co-poly(ε-caprolactone)[P(LLA-CL)], poly(lactide-co-glycoside) [PLGA] as well as composites composed of synthetic polymers and bioceramics and/or natural polymers (collagens, bovine serum albumin) were investigated for different applications in tissue regeneration. PCL, is a highly processible polymer and therefore of great interest for fabrication of scaffolds by means of Fused Deposition Modeling (FDM) technique. In order to accelerate the resorption of PCL-based scaffolds we prepared ternary composite materials which contained bioactive inorganic filler and different concentrations of fast-degrading PLGA in form of organic inclusions. The materials were used to fabricate fibers and porous scaffolds, which were subsequently studied (degradation experiment and tensile and compressive mechanical tests).

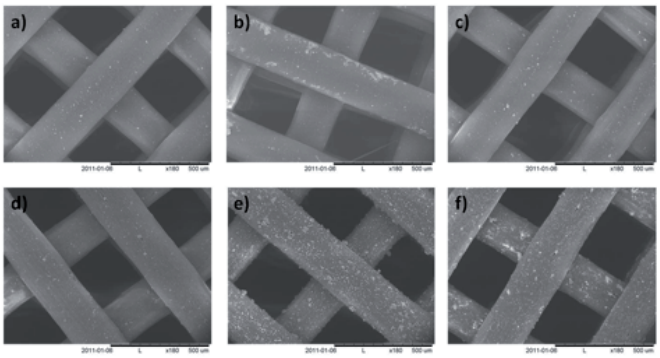

Figure 1 *SEM image of binary composite PCL95 and ternary composite PCL70 during degradation: a) PCL95 – Week 0, b) PCL95 – Week 5, c) PCL95 – Week 14, d) PCL70 – Week 0 e) PCL70 – Week 5 and PCL70 – Week 14. Scale bar 500 µm.*

Our results [Fig.1] showed that presence of inclusions of PLGA had a positive effect on tensile properties of the fibers [1], as well as it accelerated degradation of the scaffolds. Change of mechanical properties was correlated with amount of PLGA in a concentration dependent manner [2]. The scaffolds sustained mechanical stability during 20 weeks of incubation. This time should ensure time frame enabling formation of the neo-tissue. Ternary composites are promising materials for fabrication of hybrid scaffolds.
To improve cell adhesion and proliferation on synthetic materials, they can be modified by mixing synthetic polymers with natural proteins like collagen, which is, for instance, one of the major protein components of the native ECM of nerve and has a major effect on its integrity. Scaffolds composed of P(LLA-CL), collagen I and collagen III with different fiber orientations have been fabricated by electrospinning [Fig2]. Chemical, physical and mechanical properties of the constructs, along with the influence of their composition on the morphology, proliferation and differentiation of C17.2 nerve stem cells were studied. The influence of fiber topography on the differentiation of nerve stem cells was also investigated and the results of in vitro cell proliferation, cell-scaffold interaction and neurofilament protein expression studies demonstrated that the aligned P(LLA-CL)/collagen I/collagen III nanofibrous scaffolds mimic more closely the extracellular matrix of nerve and have great potential as a tissue engineering construct for accelerated regeneration of the nerve. [3]

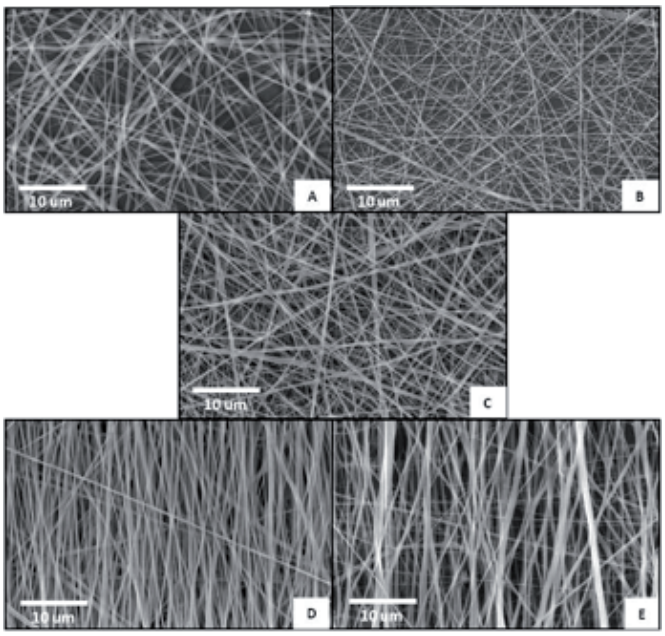

Figure 2*. SEM Images of random: A.P(LLA-CL) nanofibers, B.. P(LLA-CL)/collagen III nanofibers, C. P(LLA-CL)/collagen I/collagen III nanofibers and aligned: D. P(LLA-CL) nanofibers, E. P(LLA-CL)/collagen I/collagen III nanofibers*

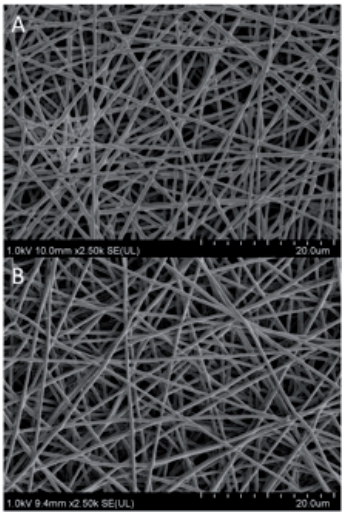

Figure 3 *SEM images of electrospun random (A) PHBV, (B) PHBV/BSA, nanofibers.*

PHBV are natural biodegradable polymers and it possesses good biocompatibility and degrades in vivo into d-3-hydroxybutyric acid which is a normal constituent of human blood. It has therefore been investigated for tissue engineering applications. The electrospinning method was utilized to create nanofibrous scaffolds from PHBV - biomass extract blended with bioactive protein – Bovine Serum Albumin (BSA) [Fig.3]. The morphology and topography of nanofibrous structures, their chemical and physical properties of the surface were investigated along with evaluation of release of bioactive agent. Conducted studies confirmed that PHBV electrospun nanofibrous structures are of the great potential for bone tissue engineering as a drug carriers.
Concluding the study shows that bio-inspired 3-dimensional nano- and micro-structured biomaterials are of the great potential to be utilized in tissue engineering of different tissues such as bones or nerves. However, designing and fabrication of the bioactive biomaterials with required properties over the time of exposure to implant conditions is still a big challenge.

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[1] J. Idaszek et al. European Cells and Materials; 2010, 20 (Suppl. 3): 118
[2] J. Idaszek et al.: Composite Theory and Practice; 2012, 12 (4): 228-231, [3] E.Kijeńska et al.: J Biomed Mater Res Part B 100B (2012) 1093-1102.

COMPLEMENT-MEDIATED HYPERSENSITIVITY REACTIONS TO NANOMEDICINES AND BIOLOGICALS: NEW TESTS AND MECHANISTIC INSIGHTS

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INTRODUCTION

Liposomes (e.g., Doxil), micellar drugs (e.g., Taxol and Taxotere), and monoclonal antibodies (mAbs), such as rituximab (Rituxan, MabThera) and trastuzumab (Herceptin) are known to induce hypersensitivity reactions (HSRs) in a relatively high percentage (10-80 %) of patients [1,2]. Another adverse immune effect caused by some nanomedicines and many mAbs is immunogenicity, i.e., the formation of anti-drug antibodies [1,2]. Considering that C activation can play a key role in both HSRs and immunogenicity, it has been proposed [2] that testing for C activation in vitro might provide a predictive laboratory test for C activation-related HSRs, which represents a non-IgE-mediated "pseudoallergy" [1,2]. Nevertheless, despite substantial indirect evidence for a causal role of C activation in HSRs [1-5], which are called C activation-related psuudoallergy (CARPA), the R&D and clinical validation of a C-based laboratory test that would enable the prediction of CARPA is still far from being realized.
The only clinical study to date supporting the potential us of a CAR-

PA test showed significant correlation between the clinical symptoms of HSRs of cancer patients treated with liposomal doxorubicin (Doxil) and strong C activation in patients in vivo (³ 5-10-fold rise of SC5b-9 over baseline in the plasma of reactive patients) [3]. The purpose of the present study was to extend the latter study and explore the relationship between C activation caused by reactogenic anticancer drugs in vitro, in pretreatment sera or cancer patients, and the HSRs they cause in vivo upon infusion. Among the tested drugs, Taxol, Taxotere and Rituxan are known to cause HSRs in a high (³ 10) % of patients, while Herceptin served as a rarely reactogenic negative control mAb [2]. Because Rituxan is known to bind to CD20+ B cells and activate C on the cell surface, a further aim of this study was to refine the in vitro measurement of C activation by this antibody to make it clinically more relevant.

MATERIALS AND METHODS

We enrolled in this pilot study 25 patients suffering from different cancer, who obtained standard infusion chemotherapy with the above listed drugs at the National Cancer Institute of Budapest. All patients were premedicated against HSRs with Solumedrol, Tavegil, Algopyrin or Oradexon, applied by standard protocols. Blood samples were taken before treatment (as part of pretreatment routine), and the serum (called "screening serum") was separated and then kept frozen (-20-80oC) until the in vitro incubations with the drugs were carried out (within days). The concentrations of drugs (in 80%-diluted) sera corresponded to their level in the infusion bag applied at chemotherapy. Following incubation for 30 min at 37oC, the levels of C3a, C5a and SC5b-9 were measured by ELISA (TECO-Medical, GE), as described earlier [2-5]. In addition, EDTA-anticoagulated plasma samples, taken from the patients before and at 5, 10, 20, 30 and 60 min post-infusion, were also subjected to the above C ELISAs. These measurements were approved by the Ethical Committee of the National Cancer Institute of Budapest.

Patients were observed during the infusion and all symptoms of HSRs were registered in order to grade the reactions between 0 and 4, as descried earlier [3]. The grades were then correlated with the rises of C3a, C5a and SC5b-9 after the 30 min incubation with the pre-treatment sera, expressed as ratio (x-fold increase) relative to baseline (PBS control). Statistical analyses of correlations and sensitivity-specificity relationships were done by computing Pearson's R and the significance of ROC (Receiver Operating Characteristic) plot.

In other experiments focusing on Rituxan, blood samples, taken from (Nonhodgkin) lymphoma patients before therapy, were anticoagulated with 50 mg/mL hirudin (lepirudin, Refludan, Celgene Europe) and incubated with 1,4 mg/mL Rituxan for 3 hours at 37oC. Cells were separated and the liberation of SC5b-9 in the plasma was measured by the SC5b-9 ELISA.

Yet in further in vitro experiments we assessed C activation by Rituxan via measuring the deposition of C3b and its derivatives (iC3b, C3d) on lymphocytes using flow cytometry (FACS). Ficoll-separated peripheral blood mononuclear cells (PBMCs) were suspended in undiluted (healthy) autologous serum, and the cells were incubated with Rituxan at 10 and 100 mg/mL for 30 min at 37oC. Deposition of C3b derivatives on T and B cells were measured by FACS on gated CD3+ (T cells) and CD19+ (B cells) using FITC-conjugated anti-C3/c3b/iC3b/C3d (1H8-FITC) antibody, generously provided by Dr. Ron Taylor, University of Virginia, Charlottesville, VA).

RESULTS

The Spearman and ROC analyses of C activatation-HSR data pairs in 25 patients indicated significant correlation between the rises of SC5b-9 in screening serum in vitro and the grades of HSRs in vivo only in the case of SC5b-9 (Fig. 1A,B).

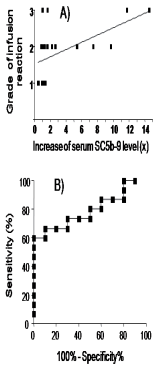

Figure 1: *Correlation between HSRs to different reactogenic anticancer drugs and C activation they cause in screening sera of patients in vitro, assessed by measuring SC5b-9 levels by ELISA. The number of patients treated with Rituxan, taxanes and trastuzumab were 17, 6 and 2, respectively. A: Spearman correlation: r=0,4837, p=0,0143; B) area = 0,793, p=0,01469, sensitivity: 73,3%, specificity: 70% criterion: >1,21*

The rises of C3a and C5a did not show correlation with clinical symptoms (not shown).

Table 1. provides a numeric summary of clinical and laboratory reactions and their coincidences for the case of SC5b-9 measurements. Among the data, the 60% overall incidence of HSRs is consistent with the literature, estimating the reaction rates to Taxol, Taxotere and Rituxan in the 10-80% range [2]. What is surprising is that this high reaction rate was observed despite thorough premedication of the patients. The 52% true positivity of serum test (laboratory positivity coinsides with clinical reaction), taken together with the ROC analyis, is encouraging, however, the high (28 %) of false negativity is still a major obstacle for the clinical utility of the test.

In an effort to explain the high false negativity of serum SC5b-9 test, it is important to consider that Rituxan exerts its therapeutic action partly via C activation on CD20+ B cells [6,7]. Cells, obviously, are not present in serum, therefore our next goal in the present study was to implement a whole blood assay for in vitro measurement of C activation by Rituxan. Fig. 2A shows our initial results in this effort, using hirudinized blood of 3 lymphoma patients. Incubation of these blood samples with Rituxan led to a slow, but significant rise of SC5b-9 in all 3 cases, reaching plateau between 2-3 hours at 2-5-fold higher values than baseline. This kinetics and extent of C activation during the first hour is remarkably similar to that seen in patients treated with Rituxan (Fig 2B).

Observations	n of patients		%
	+	all	+
presence of HSR	15	25	60
in vitro rise of SC5b-9 in serum	14	25	56
in vivo rise of SC5b-9 in plasma	11	23	48
rise of SC5b-9 in both tests	5	25	20
true positivity of serum test	13	25	52
false positivity of serum test	5	25	20
false negativity of serum test	7	25	28

Table 1: *Coincidence of clinical reactions and laboratory positivity for SC5b-9 analysis in screening sera of cancer patients treated with a variety of anticancer drugs.*

CONCLUSIONS

The relatively high (60%) incidence of HSRs following i.v. chemotherapy with taxanes and mAbs highlight the limited efficacy of currently applied premedication protocols. 2) Measurement of SC5b-9 in human serum or whole blood in vitro following incubation with reactogenic drugs may serve as a predictive test for HSRs, although the details and validation of the assays need to be elaborated for each drug. 3) In case of Rituxan and other drugs, whose therapeutic targets are blood cells, whole blood C assays may better mimic the in vivo C response than serum assays. 4) On the basis of the known role of C activation in the rise of specific immunity [8,9], in vitro C

tests might also be used to predict the immunogenicity of Rituxan and other protein therapeutics.

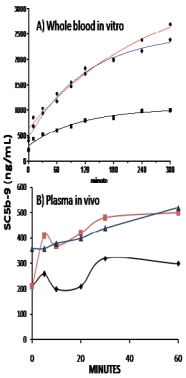

Figure 2: *Complement activation in cancer patients by Rituxan in vitro (A) and in vivo (B). Samples were analyzed for SC5b-9 as described in the Methods. The data shown represent 3 of 3 patients (A) and 3 typical SC5b-9 progress curves out of 11 patients in whom plasma SC5b-9 rose during Rituxan therapy (Table 1).*

REFERENCES

[1] Szebeni J, et al,, Adv. Drug. Deliv. Rev. 63, 1020, 2011
[2] Szebeni J, Eur. J. Nanomedicine, 1, 33, 2012
[3] Chanan-Khan A., et al., Ann. Oncol., 14, 1430, 2003
[4] Szebeni, J., et al., J. Natl. Cancer Inst., 90, 300,1998
[5] Weiszhár Zs, et al., Eur. J. Pharm. Pharmaceutics, 45, 492, 2012

THE GLOBAL MALARIA ACTION PLANFOR A MALARIA-FREE WORLD

THOMAS TEUSCHER

The malaria eradication research agenda (malERA) calls for 1) a single encounter radical cure and prophylaxis drug suitable for mass drug administration, 2) Vaccines that interrupt transmission, 3) new diagnostics for use with individuals, communities, mass drug administration, 4) surveillance as an intervention (case detection, case investigation, prevention of local transmission), 5) sustained vectorial capacity reduction tools (indoor and outoor), 6) Predictive modelling allowing strategic and operational (including costing) assessments of combining different control and elimination strategies, 7) minimal enabling framework for health system readiness. In support of the eradication agenda, a R&D backbone of enabling technologies is required to allow 1) the continuous culture of Plasmodium vivax, 2) the study of the biology of liver stages, 3) the establishment of genomic and proteomic platforms, 4) the identification of approaches and tools for measuring transmission, 5) the development of a framework and tools for the effectiveness decay analysis and health system integration, 6) in support of harmonization of data bases, model outputs and user interfaces of models, and 7) training. We will present requirements in product improvement of current control tools that would enhance value for money malaria control investments.

NANOTUBES IN (ORAL) DRUG DELIVERY?

CARSTEN TIMPE

Ph.D., Senior Principal Scientist, F. Hoffmann La Roche AG

Significant interest in pharmaceutical research has arisen in the last years in applying clay mineral based naturally occuring nanotubes (Halloysite nanotubes HNTs) with an inner diameter of 10 – 30 nm, outer diameters between 50 – 70 nm and a length of 0.5 – 1.5 µm for novel drug delivery systems.
These novel systems allow for delivering drugs in modified release patterns, enable taste masking in the fields of paediatric/geriatric applications for instance and could help to improve chemotherapy to reduce the risk of metastasis. In addition the biocompatible HNTs open interesting opportunities for bone tissue engineering and can be chemically modified/functionalized to improve drug loading.
The presentation also tries to give an industrial perspective what would be furthermore required to introduce these materials in a regular commercial drug development

ANALYSIS OF MICRO- AND NANO-SIZED PARTICLES FOR THE PRECISE ENGINEERING OF DRUG DELIVERY SYSTEMS FOR NANOMEDICINE APPLICATIONS

HANS VAN DER VOORN

Nanomedicine is predicted to be a very high growth area in medicine. So far most of the growth has been in the research phase and there are still only a few nanoparticle based therapeutics in clinical use. The regulatory approvals for nanomedicine products are somewhat of a grey area, with most of the traditional particle characterisation techniques deemed to be inadequate.
This information gap in particle characterisation of nanomedicines has now largely been closed by recent developments in nanopore based measurement, in particular Tunable Resistive Pulse Sensing (TRPS).
TRPS is a high resolution technique which enables accurate measurement of the size, charge, and concentration on a particle by particle basis. Accurate calibration is inherent in the technique so absolute values can be provided with confidence and these are independent of the user and the user assumptions on unknown parameters.
A recent advance has been the measurement of the individual particle charge (electrophoretic mobility), which can be converted to zeta potential. Many parties consider particle charge to be as important in predicting particle behaviour as size and therefore accurate charge distribution may be as important as an accurate size distribution.
All of these measurements can be done in physiological conditions and the recommendation is that particle aggregation be checked in higher molarity solutions than their expected use to provide a safety factor.
Lastly, there is an obvious need for analytical results to be transparent and able to be understood by the user and any subsequent reviewer of the information.

QUALITY REQUIREMENTS FOR PHOSPHOLIPID EXCIPIENTS IN LIPOSOMAL FORMULATIONS

PETER VAN HOOGEVEST

Lipoid GmbH, Ludwigshafen (D)

Since the US-FDA claims that the CMC of the lipid components of liposomes should be provided at the same level of detail expected for a drug substance, the development of liposomes for parenteral/intravenous administration requires the use of phospholipids with highly reproducible quality and composition. Although liposome products comprise synthetic phospholipids, as underscored by the phospholipid composition of the liposome products Ambisome, Doxil, Caelyx and Visudyne, phospholipids originating or derived from natural sources may also be used. For synthetic lipids, source and specifications for any starting material should be provided. The synthesis process and controls, positional specificity of fatty acids should be provided in detail. For natural lipids, the biological source, country of origin, supplier and specifications should be provided. Manufacturing procedures should ensure the removal of possible contaminants like proteins and viruses.
Quality control of the phospholipids should comprise validated methods, acceptance criteria and comparison with well documented reference standards. Stability should be assessed with stability indicating methods. Natural phospholipids should be defined by lipid composition including percentage of each lipid and fatty acid, positional specificity of acyl side chains and degree of fatty acid unsaturation.
The quality level of presently available phospholipid excipients complies with these requirements and poses a benchmark for other excipients to be used in alternative nano-carriers.

CRITICAL CONSIDERATIONS IN DEVELOPMENT, SCALE-UP AND MANUFACTURING OF INJECTABLE NANOMEDICINES

DANNY VAN HOORN

In the development of injectable nanomedicines many critical items need to be considered before the product can successfully

be testing in the different stage of clinical studies.
In the strongly emerging siRNA field, many companies are trying to develop targeted formulations aimed for instance to certain organs. The physicochemical properties of interest in such formulation should, after a first selection, be thoroughly investigated and tested in suitable ex-vivo and in-vivo safety and efficacy models.
While developing a suitable formulation, for instance liposomal, the loading of the active, the composition of excipients, the lipids and their ratios and overall the physicochemical interactions in the formulation are of importance to reach an effective and stable formulation that can be tested for its intended purpose; clinical studies.
However, the first screening and development of possible suitable nano-medicines formulations occurs at lab-scale. Already at the lab scale development one should consider that the process has to be scaled up and therefore should use techniques that a scalable for a later stage of development. The typical lab scale needs to up scaled up to approximately 10-40L or higher depending on the intended target, already considering GMP quality control, to enable a stable high yield first batch that can be used for non-clinical efficacy and toxicity studies. Important considerations amongst others are that the analytical methods at this stage should be fully developed to enable a good analysis of the batch leading to a future clinical batch. Once the non-clinical material is being tested further scale up to enable production clinical train manufacturing for phase I\II studies needs to be initiated. A process technology or technology transfer team needs to be in place consisting of at least the persons that developed the non-GMP process and the GMP team including QA and a regulatory person. This team composition enables a smooth transfer from development to a formal GMP quality controlled area.
In practice it works best to have one or more technology transfer batches, depending on the complexity of the process, performed at the intended scale with the same set of analytics, IPCs to allow a direct comparison between the GMP and non-GMP production scale and quality of manufacturing.
Involvement of regulatory affairs at an early stage of development, pre-clinical, is critical to consider the current regulation into the manufacturing process for early and late stage clinical trial material, the interactions, scientific or otherwise with the authorities, to enable registration of the production upon successful phase III results.

ALBUMINATED PLGA NANOPARTICLES FOR THE OPHTHALMIC DELIVERY OF BEVACIZUMAB INTENDED FOR RETINAL AND CHOROIDAL NEOVASCULARIZATION TREATMENT

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INTRODUCTION

Bevacizmab (Avastin®) is a humanized recombinant antibody against vascular endothelial growth factor (VEGF) which is clinically used to inhibit angiogenesis in malignant tissues as well as abnormal neovascularizations. The drug has been approved by FDA for the treatment of metastatic colorectal cancer and it has indicated positive effects in other cancers in clinical trials as well [1]. In recent years, bevacizumab has been successfully used by ophthalmologist as an off-label drug for treatment of retinal and choroidal neovascularization, age-related macular degeneration (AMD) and proliferative diabetic retinopathy [2]. Unfortunately due to its short half-life in the vitreous, repetitive intravitreal injections are required to maintain the drug efficiency [3]. Particulate polymeric carriers including micro and nanoparticles (NPs) have indicated immense potentials in the field of drug delivery. One of the most attractive biodegradable polymers in this area is poly(lactic-co-glycolic)acid (PLGA) which has a long clinical experience [4].
In this study we have prepared bevacizumab loaded PLGA NPs and evaluated their release profile after the rabbit intravitreal injections to achieve an efficient sustained release drug delivery system. In addition coumarin-6 encapsulated NPs were employed to investigate the penetration and persistence of the prepared NPs in the ocular tissues.

METHODS

Bevacizumab (Avastin®) was encapsulated in NPs using a modified double-emulsion solvent evaporation procedure. In order to reduce undesirable effects of emulsification process on the performance and integrity of the antibody, human serum albumin (HSA) was employed as a stabilizer. In addition coumarin-6 encapsulate NPs were formulated using the same procedure.
The VEGF165-binding affinity of bevacizumab was assayed by enzyme-linked immunosorbent assay (ELISA) which portrays the activity of the drug. The structural fracture and integrity (secondary and tertiary structure) of bevacizumab was evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and circular dichroism (CD) spectroscopy, respectively.
Surface morphology evaluation of NPs was performed by scanning electron microscopy (SEM) and the size, distribution and zeta potential were determined by dynamic light scattering (DLS).
To determine the entrapment efficiency and loading efficiency of NPs an indirect method was utilized. Therefore, after formation of NPs, the nano-suspension was centrifuged and the clear supernatant was collected and analyzed by ELISA method.
To investigate the in vivo release of bevacizumab determined amount of NP suspension, equivalent to 1 mg bevacizumab, was injected to the vitreous of 20 albino rabbits. Some other rabbits were received 1 mg of Avastin® intravitrealy, as controls.
Then at specific intervals (3, 7, 21, 42 and 56 days) rabbits were sacrificed and their vitreous and aqueous samples were taken and analyzed by ELISA test (animal killing procedures was conducted according to the guidelines of the Animal Care and Ethics Committee of Farabi Eye Hospital). The probable ocular toxicity of NPs was evaluated by histology tests and electroretinography (ERG).
To evaluate the distribution and persistence of the NPs in the ocular tissues, coumarin-6 loaded NPs were similarly injected, at given intervals eyes were enucleated and cryosections were prepared and observed under confocal laser scanning microscopy.

RESULTS

Results revealed that the proper concentration of albumin could successfully protect the activity and structural integrity of bevacizumab against the emulsion deleterious environment.
According to DLS results the average size of NPs was 190 nm and preparation of antibody loaded PLGA nanoparticles with albumin results in negatively charged particles (-24.5 in average). SEM images showed the NPs were spherical with smooth surfaces and had almost homogenous size distributions (Fig. 1). Entrapment efficiency and loading efficiency of NPs were 81.2 ± 5.8% and 6.9 ± 0.7%, respectively.

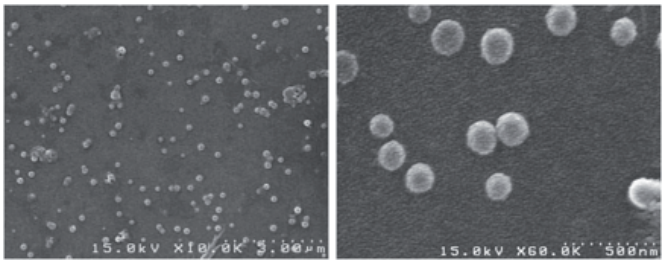

Fig.1. *Scanning electron microscopy images of bevacizumab loaded PLGA NPs.*

No significant ocular toxicity was observed during the 2 months after the intravitreal injection of the NPs in rabbits. Plotting the vitreous concentration of bevacizumab versus time indicated the

reduction of the drug clearance velocity after administration of the NPs in comparison with Avastin®. Also in the NPs release profile, 1.7 and 2 times increase in bevacizumab vitreous half-life and the areas under the drug concentration-time curve (AUC) were observed, respectively (Fig. 2). Accordingly, prepared NPs provided a sustained release bevacizumab for about 50 days and the drug vitreous concentration remained above the minimum concentration that completely blocks the VEGF-induced angiogenesis, for more than 6 weeks.

In figure 3 the green fluorescence derived from coumarin-6 loaded NPs in the confocal microscopic images shows the presence and distribution of the NPs in the retina and sclera. As can be observed the NP concentrations decreased with time and this trend was faster in retina.

CONCLUSION

According to our previous outcomes interfacial adsorption is the main protein destabilizing factor during double emulsion encapsulation technique and albumin demonstrated considerable antibody protective abilities due to successful occupation of the interface as well as shielding therapeutic protein and preventing its contact with hydrophobic phase. Our ex vivo studies showed a proper release profile of active drug from bevacizumab loaded albuminated-PLGA NPs in the extracted rabbit vitreous. Current results suggest that intravitreal injection of prepared NPs lead to an efficient sustained release of active drug with an appropriate bioavailability. Coumari-6 NP distribution in ocular tissues indicated the proper penetration of our NPs in retina and choroid. Finally, this formulation may offer improvements in treatment of eye posterior segment neovascularization and decrease the number of intravitreal injections which results in the reduction of associated concerns such as intravitreal hemorrhage, endophthalmitis, cataract, and retinal detachment. Moreover this systematic process may provide a basis for an innovative approach to design novel antibody delivery systems.

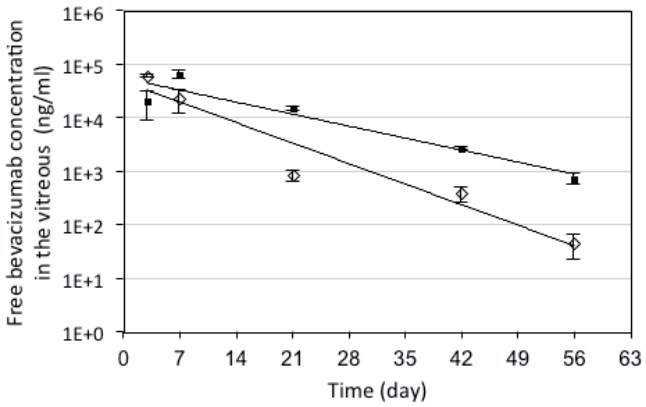

Fig. 2. *Semi-log plot of bevacizumab concentration versus time after the intravitreal injections of: bevacizumab loaded NPs (black markers) and Avastin® (white markers) into the rabbit eyes.*

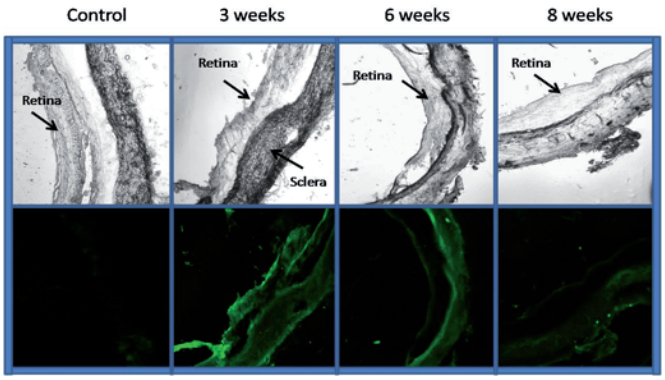

Fig. 3. *The images of rabbit retina and sclera sections, by light and confocal microscopy, 3, 6, and 8 weeks after the intravitreal injection of coumarin-6 encapsulated NPs. Control rabbit has received coumarin-6 free NPs.*

REFERENCES

1. Dorrell, M., Uusitalo-Jarvinen, H., Aguilar, E., Friedlander, M., 2007. Ocular Neovascularization: Basic Mechanisms and Therapeutic Advances. Surv. Ophthalmol. 52, S3-S19.
2. Spaide, R.F., Laud, K., Fine, H.F., Klancnik, J.M. Jr., Meyerle, C.B., Yannuzzi, L.A., Sorenson, J., Slakter, J., Fisher, Y.L., Cooney, M.J., 2006. Intravitreal bevacizumab treatment of choroidal neovascularization secondary to age-related macular degeneration. Retina. 26, 383-390.
3. Krohne, T.U., Eter, N., Holz, F.G., Meyer, C.H., 2008. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. Am. J. Ophthalmol. 146, 508–512
4. Danhier, F., Ansorena, E., Silva, J.M., Coco, R., Le Breton, A., Préat, V., 2012. PLGA-based nanoparticles: An overview of biomedical applications. J. Control. Release, 161, 505–522.

NANOMECHANICS – FUTURE TARGETS IN MOLECULAR MEDICINE?

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Topography and rigidity sensing are most fundamental processes that cells exploit to interact with their environments, either during development, wound healing or metastatic invasion. But how do cells sense their environments? To recognize the rigidity and topography of biological or synthetic materials, cells pull on the protein coated surfaces and try to deflect them. They exploit mechanical forces generated by their motors to pull on distal extracellular anchoring points. The mechanical forces are transmitted through the actin cytoskeleton to integrin junctions which finally make the adhesive connections to the extracellular matrix or to protein coated materials. As cells interact with their environments, all proteins that are part of these force-bearing junctions are mechanically stretched, and stretching can switch their structure-function relationships. Significantly though, only protein equilibrium protein structures have been actively targeted in the past in Molecular Medicine. Is it time to reconsider the approaches?

MULTIFUNCTIONAL NANOPARTICLE-BASED PROBES FOR CANCER CELLS AND BIOMARKERS DETECTION

YURI VOLKOV

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NAMDIATREAM (EU NMP4-LA-2010-246479) Project Coordinator

In spite of the major breakthroughs in nanotechnologies in Europe and worldwide, the applications of nanomedicine as an interdisciplinary area of science to patient benefit are still in their early stages. Major efforts integrating scientists, medical doctors and industry are urgently required to bring the nanotech advances into clinics, laboratories and patients' bedside. This is a key concern in relation to the most widespread and deadly diseases, such as cancer. Regardless of the very high quality level of medical care currently achieved in the European Union member states, cancer represents a major burden on the European population in terms of morbidity and mortality. Recent advances in nanotechnology enable to establish innovative approaches to cancer detection in non-invasive and miniaturized volume formats. We will present here some of the latest achievements from the interdisciplinary group of European scientists utilizing the expertise of 22 partners from 9 countries across Europe including leading academia, clinical centres and industry (project NAMDIATREAM) focusing their efforts towards the development of complementary in vitro diagnostic and imaging toolkits applicable for early stages of malignant disease onset and progression. The techniques implemented within the project are based on super-sensitive "lab-on-a-bead", "lab-on-a-chip" and "lab-on-a-wire" nanodevices. The developed innovative toolkits exploit photo-luminescent, plasmonic, magnetic and

unique optical properties of nanomaterials. This offers revolutionary ground-breaking advances over present technologies in terms of stability, sensitivity, time of analysis and probe multiplexing. The opportunities for cancer diagnostics offered through the implementation of advanced fluorescent nanomaterials technologies and magnetic nanowire barcodes for disease biomarker detection will be discussed in detail in the talk. On the other hand, rapidly expanding manufacture and use of nanomaterials emphasize the requirements for thorough assessment of possible impact on human health and environment associated with novel nanoparticles and nanodevices applications. From this prospective, the approaches to safety evaluation of new nano-enabled diagnostic devices will be also highlighted.

NAMDIATREAM project is a large-scale integrating project funded under the European Commission FP-7 Programme, (EU NMP4-LA-2010-246479), www.namdiatream.eu. NAMDIATREAM is a member of the "Targeted NanoPharmaceuticals and Early Diagnostics" EU projects cluster.

GMP PRODUCTION OF LIPOSOMES AND LNPS USING THE POLYMUM TECHNOLOGY

ANDREAS WAGNER

Over the past few years liposomal drug preparations have been increasingly used in clinical trials. Until now, several liposomal products have reached the market; many other formulations are still in the pipeline. For all these products, a simple, economic and GMP-conform production technique and facility is necessary.

Based on the ethanol injection technique, we have realised a scalable and sterile production technique. Continuous aseptic one-step operation permits the production of stable liposomes with a defined size distribution. The production plant is designed to meet several requirements, such as simplicity, robustness and easy handling of sterilisation procedures. Furthermore, the ethanol injection technique itself is distinguished by mild preparation conditions and the avoidance of hazardous solvents and forces, which may disrupt lipids as well as entrapped substances.

In the last years, we have guided many liposomal drug products from very early stage to clinical applications. Thereby many different hurdles have to be overcome. Liposomal oligonucleotide encapsulation processes with encapsulation rates up to 100 percent were developed and these products were guided from early formulation development into the clinics. Liposomal peptide formulations, to be used as vaccines were developed. These peptides were encapsulated into long term stable liposomes with loading rates up to 80 percent.

In conclusion, liposome manufacture with the crossflow technique is suitable for the production of liposomal vaccines and therapeutics. The main advantage of this technique is the feasibility of manufacturing batches of 50-100 millilitres, which is directly scalable to several litres. This is of particular importance when it comes to cost-intensive drugs that are to be manufactured by novel biotechnological procedures.

THE BOOSTING POWER OF PHOTODYNAMIC MEDICINE IN COMBINATION WITH NANOTECHNOLOGY OR ELECTROMAGNETIC FIELDS (EMF) ENHANCES BOTH DIAGNOSIS AND THERAPY

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Photodynamic medicine includes both Photodynamic Diagnosis (PD) and Photodynamic Therapy (PDT) and is a rather new alternative method for clinical detection and therapy of cancer and other ailments. The use of light in combination with a pharmacological substance (photosensitizer) that can be activated by light is an attractive combination and led to an impressive number of scientific studies up to now. We here present new data in order to further improve the power of PD and PDT.

Boost with nanocarriers:

Our Nanomed project is entitled Target-PDT and is designed to increase the effectiveness of PDT for treating cancer by developing a novel nanocarrier-based approach. The nanocarriers offer a high payload that will include antibodies targeting specific tumor biomarkers. PDT has already shown significant potential for improving cancer treatment because it offers a strictly focused application, biocompatibility with other forms of treatment, the option for repeated use, excellent cosmetic or functional outcomes and fast recovery. We expect that the nanocarrier-based approach will significantly improve delivery and targeting of the photosensitizer, enhancing concentrations at the tumor site even after systemic application.

Boost with electromagnetic fields (EMF):

EMF are invisible lines of force that surround any electrical device that is plugged in and turned on. Radiotherapy and EMF technology have been shown to be superior in combination against cancer. Would this be useful for an improvement of PDT? We started a series of in vitro experiments with cells of human squamous cell carcinoma, osteo sarcoma and prostate cancer. We could demonstrate that a combination of PDT with EMF significantly enhanced cell killing. Our results clearly show that EMF boosts PDT regardless to the cell type or the photosensitizer. In the future we expect similar improvements considering in vivo applications.

We hope that further developments will bring boosted PDT by both nanocarriers and EMF rapidly towards bedside.

THE REGULATORS' ROLE IN THE RESPONSIBLE DEVELOPMENT OF NANOTECHNOLOGY TO THE PATIENTS' BENEFIT

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Nanotechnology allows scientists to create, manipulate, explore the utility, and apply materials measured in nanometers (billionths of a meter) to improve a broad range of products and to address unmet needs by taking advantage of new and unique nanomaterial features. The U.S. Food and Drug Administration (FDA) regulates a wide range of products, including foods, cosmetics, drugs, devices, veterinary and tobacco products, some of which may contain nanomaterials or involve the application of nanotechnology. Such materials can have chemical, physical, and biological properties that differ from those of their larger scale counterparts. FDA has developed a regulatory science program in nanotechnology to foster the responsible development of FDA-regulated products that may contain nanomaterials or otherwise involve the application of nanotechnology. The FDA program establishes tools, methods, and data to assist in regulatory decision-making while providing in-house scientific expertise and capacity that is responsive to nanotechnology-related FDA-regulated products. The presentation will provide the latest information about current perspectives and advances in this emerging technology area and discuss necessities and opportunities for networking and scientific capacity building across stakeholders.

TOXICITY PROFILES OF GOLD NANOPARTICLES REGARDING DRUG DELIVERY

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Understanding the mechanism of toxicity of nanomaterials to be used in drug delivery remains a challenges to regulatory agencies and the scientific community. Here we show differential toxitiy profiles for t ultrasmall (1.4 nm9 AuNPs on the electrophysiology of HEK 293 cells expressing hERG, a standard benchmark for drug safety, depending on ligand composition. In patch clamp experiments phosphine-stabilized AuNPs irreversibly blocked hERG channels, whereas thiol-stabilized AuNPs of similar size had no effect in vitro, while neither particle blocks the channel in vivo. We conclude that safety regulations may need to be re-evaluated and adapted to reflect the fact that the binding modality of surface functional groups becomes a relevant parameter for the design of nanoscale bioactive compounds.

PICO- TO MICROLITER SPOTTING - THE TALE OF ARRAYS AND MULTIPLEXING

RON WOLBERT, MSc, Dr. Guido Bared
Scienion AG, Berlin, Germany

Laboratory analyses are shifting away from the lab towards the patient's bedside. Point-of-Care Tests (POCT) have evolved strongly and are becoming more and more convenient to use. Key factors are ease of use, rapidness, portability, implementation by the patient or at least: at the patient's bedside.
The global market for POCT was valued at $13.8 billion in 2011 and is expected to increase to $16.5 billion by 2016 (BCC Research). The tests are becoming more compact and increasingly demand high-accuracy high throughput machines. The tests can be separated into simple tests like blood glucose monitoring, rapid coagulation testing, rapid cardiac markers diagnostics and pregnancy testing and more complex tests like drugs of abuse screening, food pathogens screening, infectious disease testing and allergens. Multiplexing the latter will not only reduce production pricing but also save valuable patients' sample.
Scienion has dedicated its developments and contract manufacturing to enable both R&D-personnel and manufacturers to develop multiplex and produce state-of-the-art biosensors, arrays and lateral flow strips. This talk will focus on the various solutions offered.

RIBOSOMES, THE UNIVERSAL NANO-MACHINES, ARE TARGET TO MANY ANTIBIOTICS

ADA YONATH
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Ribosomes, the universal cellular nan-machines that translate the genetic code into proteins, are targeted by many antibiotics that paralyze them by binding to their functional sites. Antibiotics binding modes, inhibitory actions and synergism pathways have been determined for almost all ribosomal antibiotics. These indicated the principles of differentiation between patients and pathogens, suggested mechanisms leading to bacterial resistance and paved ways for improvement of existing antibiotics as well as for the design of advanced therapeutics capable of minimizing antibiotics resistance.

NANO: HOPE AND COEVOLUTION

ROLF ZINKERNAGEL

'Nano' has been 'applied' in the coevolution of viruses, bacteria and parasites with higher vertebrates' immune system. This includes multimeric binding to various receptors on and in cells to trigger distinct innate and specific immunity. B cells' antibody receptors binding to surface determinants on infectious agents define serotypically defined related infectious agents (e.g. Polio viruses I, II or III or, influenza, papilloma viruses) or protective determinants vary by mutation. Therefore, we can imitate coevolution but cannot do better than evolution when using the same coevolutionary tools. Interestingly, B cells are generally unresponsive (tolerant) against mono- or oligomeric self-antigens. This can be shown readily by multimerizing self-antigens and correlates with the finding that self-antigens, that are accessible to B cells in lymphatic tissues and blood, are not multimeric beyond 10 - 20 fold (e.g. IgM); multimeric self molecules are usually hidden from B cells in solid tissue (e.g. collagen or DNA). They however become accessible during chronic inflammatory processes leading to immune complex diseases such as Lupus or rheumatoid arthritis.

REFERENCES

1. Bachmann MF, Rohrer UH, Kuendig TM, Buerki K, Hengartner H and Zinkernagel RM (1993) The influence of antigen organization on B cell responsiveness. Science 262:1448-1451
2. Zinkernagel RM, Cooper S, Chambers J, Lazzarini RA, Hengartner H and Arnheiter H (1990) Virus-induced autoantibody response to a transgenic viral antigen. Nature 344:68-71
3. Zinkernagel RM and Hengartner H (2006) Infections, immunity and autoimmunity in the outer immune diseases. Elsevier 22: 289-295. Ed. by NR Rose and IR Mackay.
4. Fehr T, Bachmann MF, Bucher E, Kalinke U, Padova FED, Lang AB, Hengartner H and Zinkernagel RM (1997) Role of repetitive antigen patterns for induction of antibodies against antibodies. J.Exp.Med. 185:1785-1792

ABSTRACTS OF THE POSTERS

CLINICAL NANOMEDICINE & TARGETED MEDICINE

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Activation of mast cells and basophils by the clustering at least two high affinity receptors (FcεRI) by IgE bound to multivalent antigen induces signaling cascades leading to mediator release such as histamine [1]. The release of this mediator leads to inflammatory and allergic symptoms [2]. Here we describe the design of a nano-system as a potential drug carrier capable of targeting basophils and mast cells via FcεRI, which is restricted to these cell types. This will be performed via surface modification of model polystyrene nanoparticles (PS NPs) by attachment of two antibodies, IgE and IgG.

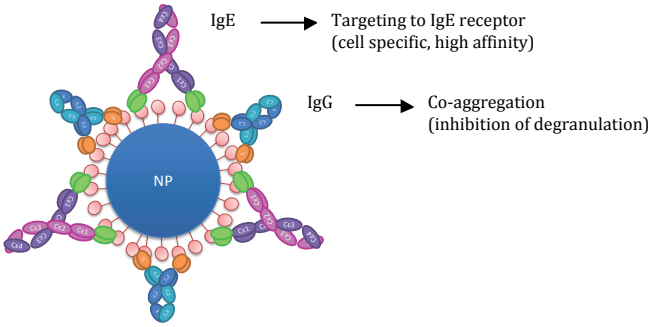


Figure 1 *Polystyrene nanoparticles coated with IgE and IgG*

The aim of the present work is to:

- Detect mediator release of rat basophilic leukemia (RBL-2H3) cells under various conditions in addition to defining the optimal condition for the degranulation assay which would suit both NPs colloidal stability as well as optimal mediator release.
- Establish a formulation procedure for surface modification of PS NPs with antibody (BSA used as a model) that achieves colloidal stability.
- Investigate basophils degranulation when PS NPs alone, NPs coated with IgE, DNP-BSA and NPs-DNP-BSA- IgE conjugate is applied to RBL-2H3 cells.

RESULT

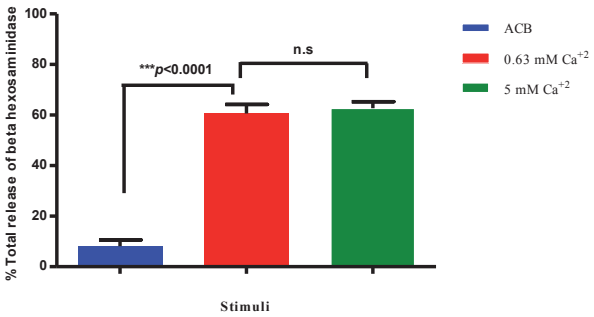


Figure 1 *Effect of increased Ca2+ concentration on RBL-2H3 cells degranulation in ACB-h (HBSS: D2O) (1:1 v/v). Cells were sensitized with 1µg/ml anti-DNP-IgE and stimulated*

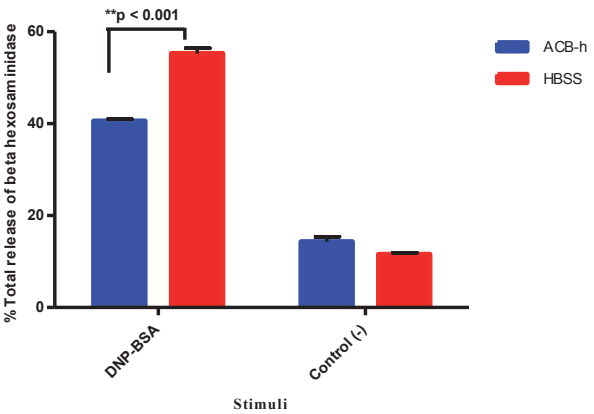


Figure 2 *Effect of using HBSS and ACB-h buffer on RBL-2H3 ß-hexosaminidase release. Cells were sensitized with 1µg/ml anti-DNP-IgE and stimulated by 1µg/ml DNP-BSA in HBSS and DNP-BSA in ACB-h buffer*

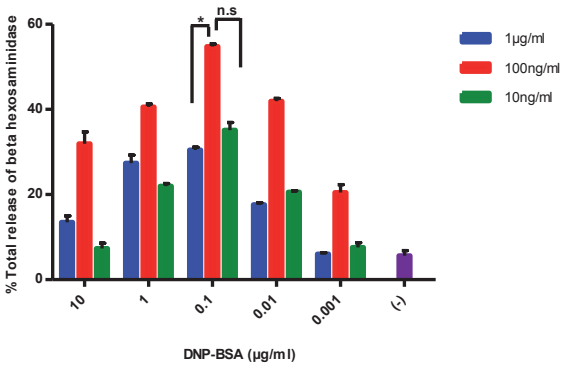


Figure 3 *Optimization of ß-hexosaminidase release of RBL-2H3 cells through antibody (anti-DNP-IgE) and ligand (DNP-BSA) concentration. Cells were sensitized with different anti-DNP-IgE concentrations and stimulated by serial DNP-BSA.*

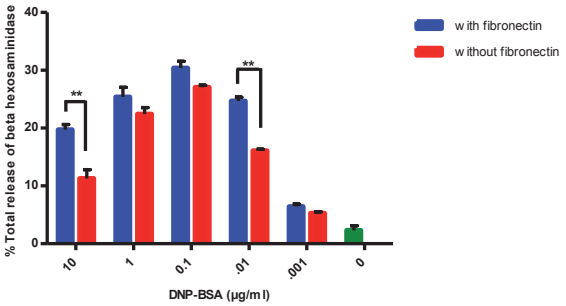
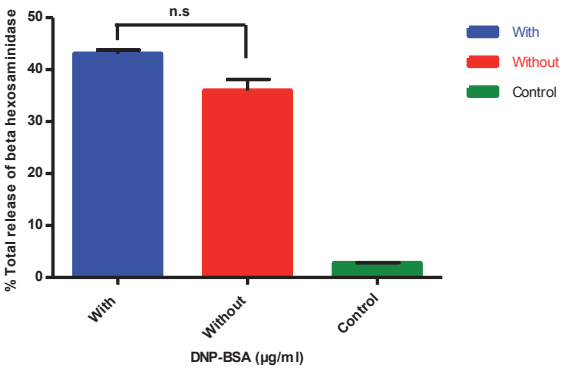


Figure 4 *Effect of fibronectin adherence on RBL cell degranulation. Cells were sensitized with 100ng/ml anti-DNP-BSA-IgE after the pre-incubation with 1µg fibronectin for 2h at 37oC and then washed four times with PBS. Cells*



Sample	DLS	Zeta potential
PS NPs surface modified with BSA	26nm	-31.4

Figure 5 Effect of poly ethylene glycol (PEG) presence on cell secretion. Cells were sensitized with 100ng anti-DNP-IgE

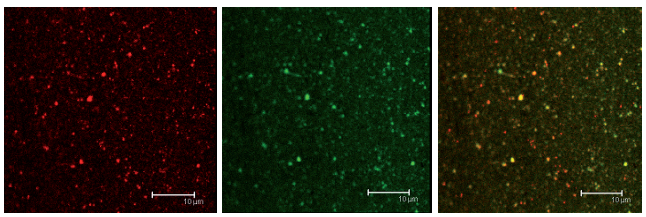


Figure 6 Confocal fluorescent microscopy images showing i) Red fluorescent PS NPs, ii) Green FITC-labelled BSA, iii) Overlay image of FITC-labelled BSA on the surface of red NPs.

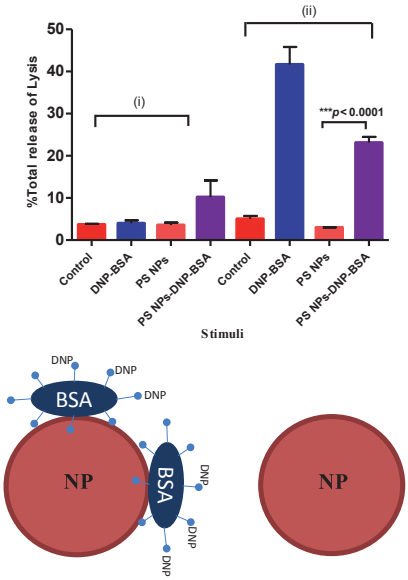


Figure7. *Release of β-hexosaminidase from RBL-2H3 cells stimulated by DNP-BSA, NPs and NPs-DNP-BSA. Cells were sensitized with 100ng/ml anti-DNP-BSA-IgE then stimulated by DNP-BSA of 100ng/ml, 250µg/ml PS NPs and PS NPS-DNP-BSA. The interactions between DNP ligand (ii) without and (i) with IgE sensitization*

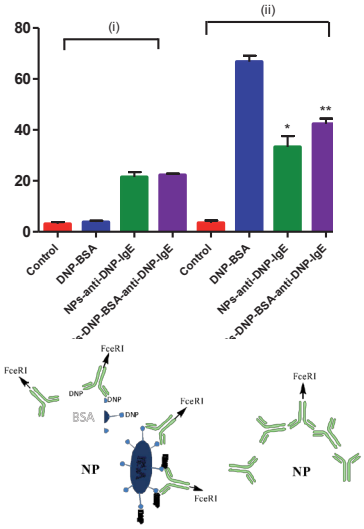


Figure 8. *Release of β-hexosaminidase from RBL-2H3 cells stimulated with DNP-BSA, NPs-IgE and NPs-DNP-BSA-IgE. Cells were sensitized with 100ng/ml anti-DNP-IgE then stimulated by 100ng/ml DNP-BSA, PS NPs-anti-DNP-IgE and PS NP-DNP-BSA-anti-DNP-IgE. The interactions between DNP ligand (ii) without and (i) with IgE sensitization.*

CONCLUSION

We have optimised the conditions of degranulation with respect to formation of stable PS NPs using RBL-2H3 cells. The maximal release was obtained when were sensitizing with 100ng/ml of anti-DNP-IgE followed by stimulation with 100ng/ml of DNP-BSA. The stable and monodispersed NPs were achieved when 3.7mg BSA was adsorbed on the surface of 0.025% w/v PS NPs, in addition to 0.1% w/v PEG in HBSS buffer. Data indicated that NP modified surfaces serve as an antigen for eliciting immune response leading to basophil degranulation compared to uncoated NPs which did not induce release.The next step will be to engage basophils via IgE without activating the cells. This will be achieved by co-adsorbing IgE and IgG1 on PS NPs, which engages the inhibitory IgG receptor FcγRIIb.

REFERENCES

• Beavil, R.L, Beavil, A.J & Reischi, I.G. (2006), ''IgE structure, receptors, and signaling". In Holgate, S.T., Church, M.K & Lichtenstein, L.M, Allergy , 3rd ed, Mosby Elsvier, China.

• Passante, E. & Frankish, N. (2009), "The RBL-2H3 cell line: its provenance and suitability as a model for the mast cell", Inflammation Research, vol. 58, pp. 737-7

VIRUS-RESEMBLING NANOPARTICLES FOR NEAR INFRARED IMAGING OF OVARIAN CANCER

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Recently, attention has been given to the use of biological materials, such as viruses, as platforms for the delivery of imaging and therapeutic agents.1-4 We recently provided the first report of the successful engineering of genome-depleted plant infecting brome mosaic virus (BMV) nano-constructs doped with the near infrared (NIR) chromophore, indocyanine green (ICG),5 and their utility in fluorescence and photoacoustic imaging.5,6 We refer to these virus-resembling nano-constructs as optical viral ghosts (OVGs) since the genomic content of the wild type BMV is eliminated and replaced with ICG, while the capsid protein (CP) subunits remain as the encapsulating shell.

One of our areas of interest for clinical applications of OVGs is in intraoperative NIR fluorescence imaging of ovarian cancer cells. Ovarian cancer ranks as the fifth most common cause of death due to cancer, and the leading lethal gynecologic malignancy in women. Clinical management of ovarian cancer is based on primary surgery for staging and cytoreduction with the goal of reducing the burden of the cancer to none or minimal residual tumor deposits. The degree of cytoreduction at surgery is one of the most important prognostic factors, with improved survival associated with complete resection of all visible cancer.7,8 However, a great challenge during cytoreduction is the detection of small (diameter <1 mm) peritoneal tumor deposits. The current surgical standard is removal of all visible deposits, on the order of 1-2 cm nodules. Smaller nodules are difficult to detect pre-operatively by CT, MRI, or visually at surgery, and if left behind, can result in recurrence, which is unfortunately all too common with this disease. Therefore, in individuals with advanced tumors, the ability to identify and destroy all ovarian peritoneal carcinomatosis will have a termendous impact on preventing turmor recurrence, treatment success, and patient survival.

Our approach in using OVGs to identify small tumors is based on targeted imaging of the human epidermal growth factor receptor-2 (HER2). HER2 and folate receptor-a are two key biomarkers over-expressed in ovarian cancer.9-13 HER2 over-expression is particularly associated with increased risk of cancer progression and death, especially among women with International Federation of Gynecology and Obstetrics (FIGO) stage I and II Ovarian carcinoma.[12]

The detailed methodology to fabricate OVGs is described in our previous publication.[5] A key advantage to using OVGs is that the abundance of naturally present amines on the CP shell provides addressable sites for covalent attachment of a variety of targeting moieties, including anti-HER2, using a simple and efficient biochemical method based on reductive amination.[14] We perform reductive amination using sodium dithionite to covalently bind double aldehyde-terminated polyethylene glycol (PEG) to the amines on the surface of OVGs on one end, and to the amines on anti-HER2 on the other end. Conjugation of the OVG with anti-HER-2 is verified using absorption spectroscopy and immunofluorescence staining.[15]

We use flow cytometry and fluorescence imaging to characterize the interaction between OVGs and ovarian cancer cells (SKOV3), which express high levels of HER2. For comparison, we use OVCAR3 cells, which have low expressions of the HER2 receptor. As determined by flow cytometry, the populations of SKOV3 cells incubated with no reagents (negative control), free ICG, or non-functionalized OVGs exhibited minimal fluorescence signals, indicating that these agents did not target the cells. However, upon incubation with anti-HER2-functionalized OVGs, a large population of SKOV3 cells exhibited fluorescence signals, indicating that the HER2 receptors on these cells were targeted by the functionalized OVGs (Fig. 1).

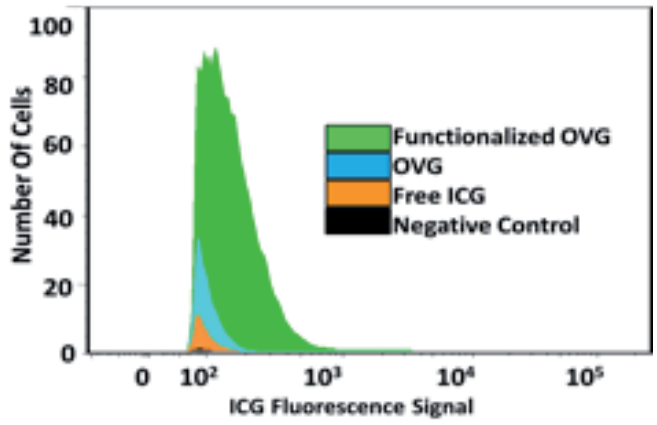

***Fig. 1**. Population distribution of SKOV3 cells emitting NIR fluorescence signal following incubation with various reagents for 45 min at 4 °C, as determined by flow cytometry.*

We also conducted experiments at physiological temperature (37 °C) where SKOV3 cells were incubated with free ICG, non-functionalized OVGs, or anti-HER2-functionalized OVGs for one hour. Images obtained by fluorescence microscopy confirm that anti-HER2-functionalized OVGs were effective in targeting the SKOV3 cells, and appeared to become internalized and subsequently localized to the periphery of the nucleus after one hour (Fig. 2). These preliminary results indicate that OVGs can be used to target ovarian cancer cells in-vitro. We are currently pursuing the use of OVGs functionalized with HER-2 for targeted in-vivo imaging of ovarian cancer cells in mice.

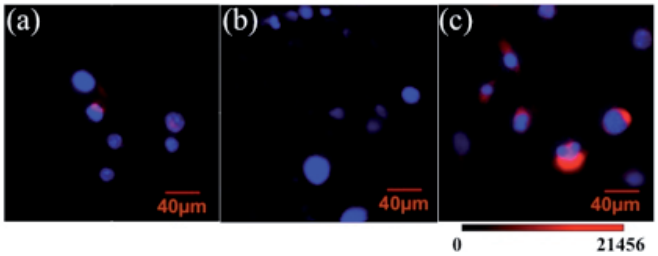

***Fig. 2**. Fluorescent images of SKOV3 cells following one hour of incubation at 37 °C with (a) free ICG, (b) non-functionalized OVG, and (c) anti-HER2 functionalized OVGs. Blue channel represents nucleus staining by DAPI. Red channel represents NIR emission.*

REFERENCES

1. Yildiz, I.; Shukla, S.; Steinmetz, N. F., Applications of viral nanoparticles in medicine. Curr. Opin. Biotechnol. 2011, 22, 901-908.
2. Manchester, M.; Singh, P., Virus-based nanoparticles (VNPs): platform technologies for diagnostic imaging. Adv. Drug Deliv. Rev. 2006, 58, 1505-1522.
3. Singh, R.; Kostarelos, K., Designer adenoviruses for nanomedicine and nanodiagnostics. Trends Biotechnol. 2009, 27 (4), 220-229.
4. Young, M.; Willits, D.; Uchida, M.; Douglas, T., Plant viruses as biotemplates for materials and their use in nanotechnology. Annu. Rev. Phytopathol. 2008, 46, 361-384.
5. Jung, B.; Rao, A. L.; Anvari, B., Optical nano-constructs composed of genome-depleted brome mosaic virus doped with a near infrared chromophore for potential biomedical applications. ACS Nano 2011, 22, 1243-1252.
6. Gupta, S.; Chatni, M. R.; Rao, A. L. N.; Vullev, V. I.; Wang, L. V.; Anvari, B., Virus-mimicking nano-constructs as a contrast agent for near infrared photoacoustic imaging. Nanoscale 2013, 5, 1772-1776.
7. Zivanovic, O.; Aldini, A.; Carlson, J. W.; Chi, D. S., Advanced cytoreductive surger: American perspective. Gynecol. Oncol. 2009, 114 (2 Suppl), S3-S9.
8. Vergote, I.; van Gorp, T.; Amant, F.; Leunen, K.; Neven, P.; Berteloot, P., Timing of debulking surgery in advanced ovarian cancer. Int. J. Gynecol. Cancer 2008, 18 (Suppl 1), 11-10.
9. Steffensen, K. D.; Waldstrom, M.; Andersen, R. F.; Olsen, D. A.; Jeppesen, U.; Knudsen, H. J.; Brandslund, I.; Jackobsen, A., Protein levels and gene expressions of the epidermal growth factor receptors, HER1, HER2, HER3 and HER4 in benign and malignant ovarian tumors. Int. J. Oncol. 2008, 33, 195-204.
10. Crane, L. M.; van Oosten, M.; Pleijhuis, R. G.; Motekallemi, A.; Dowdy, S. C.; Cliby, W. A.; van der Zee, A. G.; van Dam, G. M., Intraoperative imaging in ovarian cancer: fact or fiction. Mol. Imaging 2011, 10 (14), 248-257.
11. van Dam, G. M.; Themelis, G.; Crane, L. M.; Harlaar, N. J.; Pleijhuis, R. G.; Kelder, W.; Sarantopoulos, A.; de Jong, J. S.; Arts, H. J.; van der Zee, A. G.; Bart, J.; Low, P. S.; Ntziachristos, V., Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results. Nat. Med. 2011, 17 (10), 1315-1319.
12. Verri, E.; Guglielimini, P.; Puntoni, M.; Predelli, L.; Papadia, A.; Lorenz, P.; Rubagotti, A.; Ragni, N.; Boccardo, F., HER2/neu oncoprotein overexpression in epithelial ovarian cancer: evaluation of its prevalence and prognostic significance. Clinical study. Oncology 2005, 68, 154-161.
13. Pils, D.; Pinter, A.; Reibenwein, J.; Alfanz, A.; Horak, P.; Schmd, B. C.; Helfer, L.; Horvat, R.; Reinthaller, A.; Zeillinger, R.; Krainer, M., In ovarian cancer the prognostic influence of HER2/neu is not dependent on the CXCR4/SDF-1 signalling pathway. Br. J. Cancer 2007, 12 (96), 485-491.
14. Bahmani, B.; Lytle, C. Y.; Walker, A. M.; Gupta, S.; Vullev, V.; Anvari, B., Effects of nano-encapsulation and PEGylation on biodistribution of indocyanine green in healthy mice: quantitative fluorescence imaging and analysis of organs. Int. J. Nanomed. 2013, In press.
15. Guerrero, Y. A.; Bahmani, B.; Jung, B.; Vullev, V. I.; Kundra, V.; Anvari, B. In Targeted imaging of ovarian cancer cells using viral nanoparticles doped with indocyanine green, BiOS SPIE Photonics West, Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXII, San Francisco, CA, Kessel, D. H.; Hasan, T., Eds. SPIE: San Francisco, CA, 2013; p doi: 10.1117/12.2006278.

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2B3-201, GLUTATHIONE PEGYLATED LIPOSOMAL METHYLPREDNISOLONE, ENHANCES THE BRAIN DELIVERY OF METHYLPREDNISOLONE AND REDUCES NEUROINFLAMMATION

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BACKGROUND

Chemotherapy-induced peripheral neuropathy is a dose-limiting debilitating symptom that affects 30 to 40% of patients treated with anticancer treatments [1]. It is the most common reason that patients stop their chemotherapy treatment early. The neuropathic pain is thought to be a peripheral symptom, however, central sensitization has recently been suggested as a possible causal mechanism for chronic pain conditions; nociceptive neurons in the dorsal horns of the spinal cord become sensitized by a neuroinflammatory response through chronic peripheral tissue damage or inflammation [2-4].

Methylprednisolone belongs to the glucocorticoid class of steroid hormones that has potent anti-inflammatory effects and has been shown to act on central sensitization in nociceptive neurons. It is commonly prescribed for the treatment of various autoimmune disorders. However, treatment with methylprednisolone for neuropathic pain is limited due to its short half-life and relatively poor transport across the blood-brain barrier to brain and spinal cord. This requires either frequent and lengthy intravenous infusions of high doses, or highly invasive local delivery by intracerebroventricular, intrathecal or epidural injection, leading to multiple acute and chronic side-effects from treatment or potentially fatal side-effects from administration. To effectively target central sensitization and neuroinflammation in neuropathic pain, higher and sustained

levels of methylprednisolone through a safe administration route would allow for a disease-modifying therapy. Systemic administrations of encapsulated methylprednisolone in glutathione PEGylated liposomes (2B3-201) have recently resulted in superior efficacy and reduced side effects compared to the free methylprednisolone in rodent models with neuroinflammation [5]. Therefore, 2B3-201 was hypothesized to have therapeutic value in preventing chemo-induced neuropathic pain as well.

2B3-201 PRODUCTION

Glutathione pegylated (GSH-PEG) liposomal methylprednisolone was prepared using active loading of methylprednisolone hemisuccinate against a calcium acetate gradient as was developed by the group of Prof. Dr. Barenholz [6,7]. This hemisuccinate prodrug was used as it forms a relatively stable poorly soluble calcium salt inside the liposomes, which, after release, is converted into the active drug methylprednisolone.

PHARMACOKINETICS AND BIODISTRIBUTION

2B3-201 was initially investigated in a pharmacokinetic and biodistribution study in healthy rats. Compared to free methylprednisolone, 2B3-201 showed an enhanced plasma circulation with a half-life of ~7 hours vs. several minutes for free methylprednisolone. In addition, higher sustained levels of 2B3-201 in brain were measured.

2B3-201 HAS AN IMPROVED SAFETY PROFILE AFTER CHRONIC ADMINISTRATION

A repeat-dose safety study was carried out with 6 weekly intravenous injections at 3 dose levels of 2B3-201 (3, 10 or 30 mg/kg/week) and daily intraperitoneal injections of free methylprednisolone (0.4, 1.42, or 4.29 mg/kg/day) in rats. 2B3-201 induced a dose-dependent pharmacological effect on white blood cell counts, while methylprednisolone at the equivalent total weekly dose did not. In contrast, dose-limiting side effects on urine retention were observed at the higher free methylprednisolone doses, while equivalent doses of 2B3-201 did not lead to these side-effects.

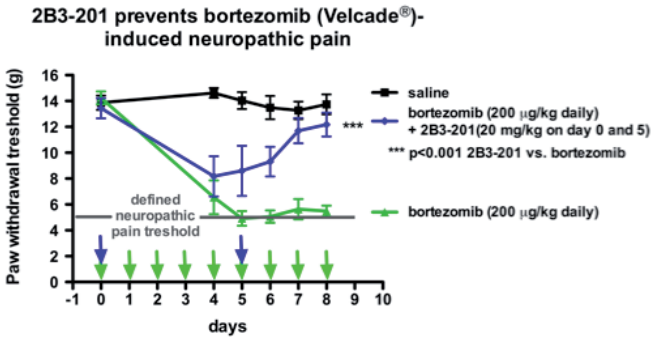
2B3-201 DID NOT CAUSE ACUTE CNS SIDE EFFECTS

A behavioral study in rats showed that administration of a therapeutically effective dose of 2B3-201 did not result in acute CNS side effects, such as psychotic-like and other behavioral effects as were caused by an effective dose of free methylprednisolone.

REDUCING CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

The efficacy of 2B3-201 was investigated at WuXi AppTec (Shanghai, China) in two chemo-induced neuropathic pain models with demonstrated spinal cord pathology as a consequence of repeated bortezomib or paclitaxel administrations. Rats received paclitaxel (1 mg/kg/day, 8x) or bortezomib (0.2 mg/kg/day, 8x), and the effect of 2B3-201 on mechanical allodynia was evaluated at three dose levels (10, 20, or 30 mg/kg). Rats were monitored daily and allodynic measurements were taken at baseline and from days 4 to 8 after treatment start.

The body weight gain of the rats was slightly inhibited by paclitaxel and bortezomib, and co-therapy with 2B3-201 showed a dose-dependent larger inhibition of weight gain. Despite the increased signs of chemotherapeutic side effects on body weight, 2B3-201 dose-dependently relieved neuropathic pain in both models; 20 mg/kg 2B3-201 showed the optimal preventative effect (Figure 1). The lower dose of 10 mg/kg showed a moderate effect and the positive treatment effect at 30 mg/kg 2B3-201 was decreased again, possibly through drug-disease interactions.



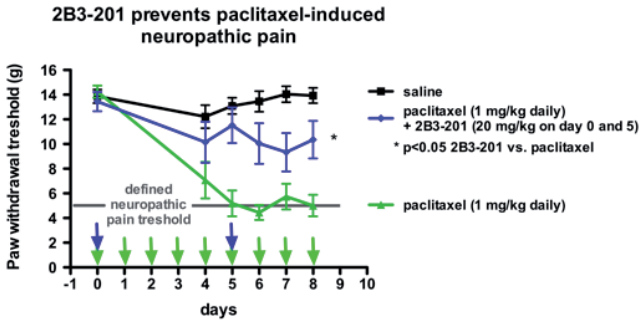


***Figure 1**: 2B3-201 prevents both bortezomib-induced and paclitaxel-induced neuropathic pain*

CONCLUSION

The positive treatment effect of 2B3-201 against chemotherapy-induced neuropathic pain, in combination with a good PK, biodistribution, and safety profile, warrant the further development of 2B3-201 for this indication.

ACKNOWLEDGMENTS

The authors would like to thank the collaborating investigators Dr. Qiang Lu, Dr. Xiao Hong Yu and Mr. Zhijing Hu of Wuxi Apptec for the animal model and data (Contact: yu_xiaohong@wuxiapptec.com). In addition, the authors would like to thank Delta Phenomics BV (Schaijk, the Netherlands) and NOTOX (currently WIL Research, 's Hertogenbosch, the Netherlands) for their expertise in behavioural and toxicity studies, respectively.

REFERENCES

1.http://www.cancer.gov/aboutnci/ncicancerbulletin/archive/2010/022310/page6
2. Zhuo M (2011) Molecular Brain 4:31, doi:10.1186/1756-6606-4-31
3. Kiguchi N (2008) Neurosci Lett 445(2):140-143
4. Takeda K (2004) Anesthesiology 100(5):1249-1257
5. Gaillard PJ (2012) J Control Release 164(3): 364-369
6. Clerc S (1995) Biochim Biophys Acta 1240(2): 257–265
7. Avnir Y (2008) Arthritis Rheum 58(1): 119-129

POLYCATIONIC NANOLIPOSOMES AS DNA NANOCARRIERS WITH LOW TOXICITY: A PROMISING GENE TRANSFER SYSTEM FOR FURTHER IN VIVO STUDY

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OBJECTIVES

Gene therapy is based on the development of efficient and safe carrier system able to transfer DNA in to cells. In this study, we attempted to develop an effective carrier by the combination of the advantages of both cationic liposomes and cationic polymers. We prepared nanoliposomes modified with hydrophobic polyethylenimine (PEI) to enhance the transfection efficiency.

METHODS

10 kiloDalton (kD) PEI polymer was modified via the substitution of two percentages of its primary amines (10%-50%) with short (6 carbons) , moderate (10 carbons) and long (18 carbons) alkyl chains, by reaction with bromoalkane and acrylate. Cationic liposomes composed of modified PEI and three different phospholipids such as 1, 2-dioleonyl-sn-glycero-3-phosphoethanolamine (DOPE), 1, 2-diole

oyl-3-trimethylammonium-propane (DOTAP) and 1, 2-dipalmitoyl-sn-glycero-3phosphoethanolamine (DPPE) were prepared using film hydration method. These polycationic nanoliposomes (PCLs) were complexed with plasmid DNA at different C/P ratios and the resulting nanoparticles were characterized by particle size analyzer (Malvern Zetasizer, UK) and ethidium bromide test to determine particle size, zeta potential and complex formation ability. Transfection efficiency was also tested in cultured Neuro2a cell line using the plasmid pRL-CMV as reporter gene. One-way ANOVA statistical test and multiple comparison Turkey test were used to compare the means of different groups.

RESULTS

We examined the effect of types of hydrophobic chains, types of phospholipids and length chain on lipopolyplex-mediated gene transfer.
Each lipid might have its own mechanism for enhancing transfection efficiency. For example DOPE is a nonbilayer phospholipid that forms hexagonal II phase at low pH and induces fusion or destabilization of the endosomal membrane. DOTAP has positively charged head group with the mechanism of destabilization of the endosomal membrane by cationic head group.
DNA binding of modified polymers (PEI 6C-10%, PEI 6C-50%, PEI 10C with bromoalkane) measured by fluorescence quenching when ethidium bromide is prevented from intercalation in plasmid DNA by polycation binding in HBG buffer. All of PCLs could condense DNA at C/P ratio of 1.

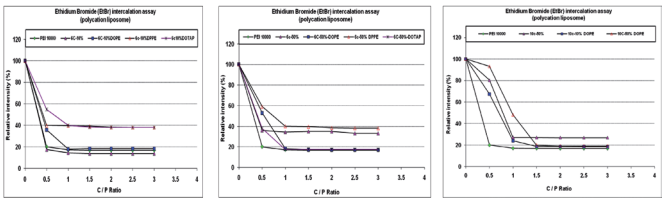

***Figure 1**: Binding affinity of PLCs to plasmid DNA by ethidium bromide exclusion assay*

Particle size measurement of polyplexes showed that bromoalkane and acrylacte derivatives form particles in the size range of (91-213) nm with the positive ZP and (42.4-474) nm in the ZP range of (-15.4-30.3) mv, respectively.

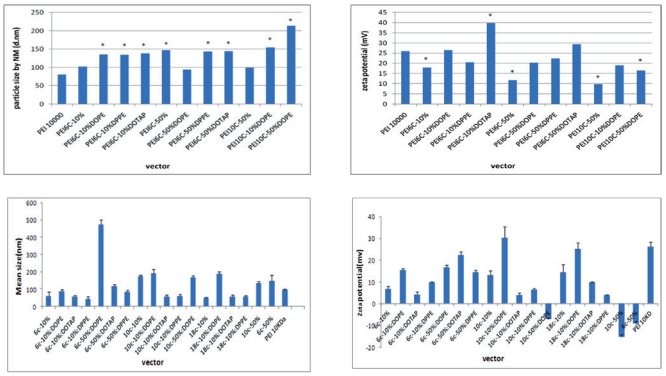

***Figure 2**: particle size and zeta potential of the PCLs constructed from PEI modified with bromoalkane (up) and acrylate (bottom) derivatives compare to unmodified PEI10kD (P<0.05*).*

It was observed that using of DOTAP by derivatives with length chain of 6 carbons, significantly increase the transfection activity (p < 0.05) in both hydrophobic chains. Almost in all cases, transfection increased by increasing the ratio of cationic lipid to DNA. The fact that 6C-10%-DOTAP was more effective at delivery of DNA, could be the result of the interaction between several factors, including: condensation ability, hydrophobic–hydrophilic balance, surface charge, particle size and unpacking ability.

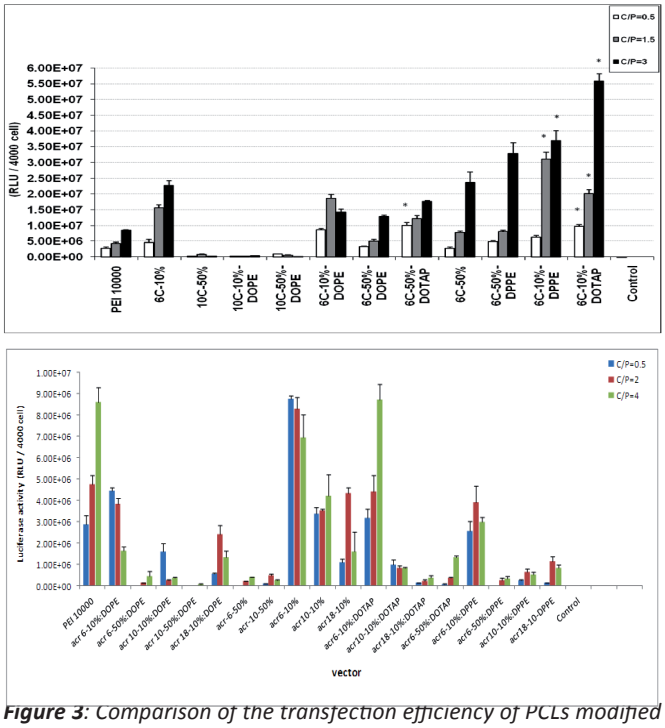

***Figure 3**: Comparison of the transfection efficiency of PCLs modified with acrylate (up) and bromoalkane (bottom) derivatives compare to unmodified PEI10kD (P<0.05*). It was observed that using of DOTAP by derivatives with length chain of 6 carbons; significantly increase the transfection activity (p < 0.05) in both hydrophobic chains.*

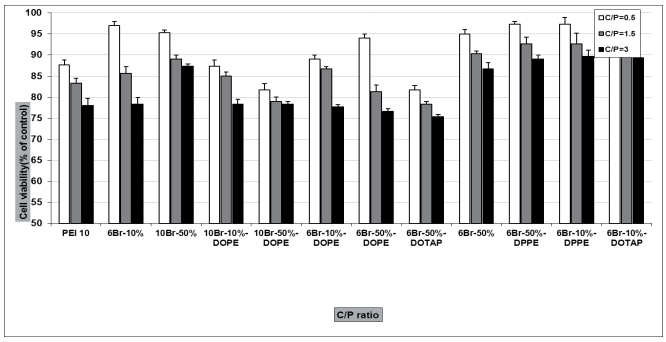

***Figure 4**: Comparison of cytotoxicity of PCLs constructed from PEI modified with bromoalkane derivatives with unmodified PEI10kD. Almost All formulations showed no significant toxicities in Neuro2A cells by MTT assay.*

CONCLUSION

In conclusion, we have prepared a small library of 10kD PEI derivatives and PCLs, nanoliposomes modified with hydrophobic PEI, to enhance the transfection efficiency by the combination of advantages of both cationic polymer and cationic lipid. Results indicate that the PCLs described in this study, preferentially with bromoalkane derivatives, have significant transfection efficiency with low toxicity so would be a promising candidate for further in vivo study.

MULTIFUNCTIONAL MAGNETIC NANO-ASSEMBLIES FOR THE TARGETED DELIVERY OF CISPLATIN TO TUMORS: IN VITRO EVALUATION AND CELL STUDIES

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INTRODUCTION

Cisplatin is a potent anticacer agent. However, it exhibits serious side effects [1] due to lack of selectivity. Therefore, a more selec-

tive cisplatin delivery to tumors is pursued by incorporating cisplatin in targetable nanocarriers. In this work, hybrid magnetic nanoasssemblies loaded with ciplatin were prepared and evaluated in vitro. The nano-asssemblies were synthesized through grafting of poly(methacrylic acid)-graft-poly(ethyleneglycol methacrylate) (p(MAA-g-EGMA) on magnetite nanocrystallites. Poly(ethylene glycol) chains should confer to the nanocarriers bio-repellent properties. The formation of a distinct second inner polymeric corona with an abundance of carboxylate groups provides the binding sites for cisplatin molecules.

METHODS

Synthesis of Polymers

Different types of p(MAA-g-EGMA) copolymers with varying length and density of PEG chains were synthesized as described previously [2].

Preparation and characterization of nano-assemblies

Core-shell magnetic nano-assemblies were prepared by hydrolytic alkaline precipitation from a single iron molecular precursor in the presence of the above p(MAA-g-EGMA) copolymers [3]. The nanoassemlies were loaded with cisplatin by incubating them with cisplatin solutions of different cisplatin concentration. Non-entrapped cisplatin was removed by centrifugation and redispersion of the nano-assemblies in cisplatin free aqueous medium. The loading of the prepared magnetic nanoparticles with cisplatin was determined using atomic absorption spectroscopy and UV-Vis spectrophotometry with appropriate color developing reagents. The nanoassemblies were characterized with regard to their size and zeta potential at different salt concentrations using dynamic light scattering (DLS). The effect of pH on cisplatin loading was investigated. Drug release studies were performed in phosphate buffered saline (pH 7.4) at 37°C. The influence of applying an AC magnetic field on the release profile of cisplatin was also investigated using a homemade AC (400 kHz) magnetic field generator 5 mT.

Cell studies

The cytotoxicity of blank and cisplatin-loaded nano-assemblies against A549 human lung cancer cell line was assessed by flow cytometric measurement of cellular fluorescence after staining with propidium iodide (PI). The cellular uptake of the magnetic nano-assemblies was quantificated, as well. For this purpose, the magnetic nanoparticles were surface loaded with propidium iodide.

RESULTS

The chemical structure of the p(MAA-g-EGMA) copolymers synthesized is shown in Figure 1 and their structural properties are presented in Table 1.

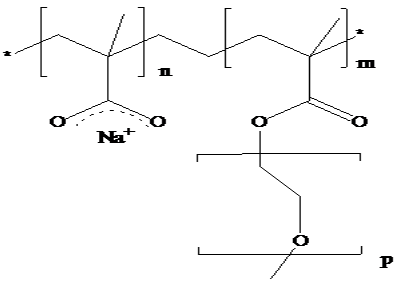

Figure 1. *Chemical structure of p(MAA-g-EGMA) copolymers*

Table 1. *Structural characteristics of p(MAA-g-EGMA) copolymers used to prepare the nanoasemblies.*

Polymer	Length of chain p	Density of chains m:n	Mw (g/mol)	n	m	%n	Mol anionic sites
102-6	102	1:6	67000	76.8	12.8	12.4	$1.1\ 10^{-3}$
23-6	23	1:6	18900	64.3	10.7	36.7	$3.4\ 10^{-3}$
10-1	10	1:1	47000	86	71	19.74	$1.8\ 10^{-3}$
102-2	102	1:2	78000	32.4	16.2	4.48	$4.2\ 10^{-4}$

The hydrodynamic diameter of the nanoasssemblies was in the range of 100–150 nm depending on the length and density of the polyether chains. All nanoparticles types exhibited high colloidal stability and did not aggregate even in media of high ionic strength (2M NaCl). Relatively high cisplatin loading (8%) was obtained at pH 10 with the 23-6 nanocarriers having the highest carboxylate molar content (Figure 2 left panel). Sustained drug release from the nanoassemblies was observed, which could be triggered by AC magnetic field (Figure 2 right panel) indicating the magnetic responsiveness of the nanoassemblies. The blank magnetic nanoparticles did not show cytotoxicity. In the contrary, the cisplatin-loaded nano-assemblies exhibited comparable to free cisplatin cytotoxicity against A549 cancer cells , which was correlated to the degree of uptake of the different types of nano-assemblies by the cells and cisplatin release (Figure 3).

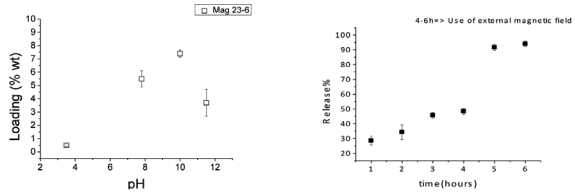

Figure 2. *Effect of pH on cisplatin loading in 23-6 nanocarriers (left panel) and magnetically triggered cisplatin release from 23-6 nanocarriers (after the first 4 hours, an AC magnetic field (4 kA/m, 400 kHz) was applied for the last 2 hours).*

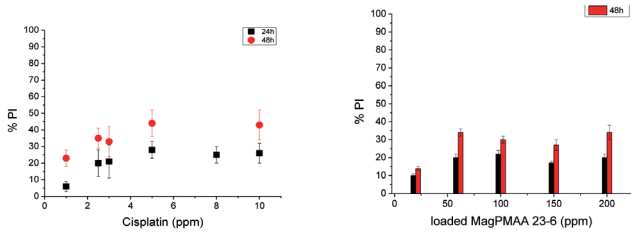

Figure 3. *Cytotoxicity (%PI) of cisplatin after 24 and 48 hours incubation (left panel) and cytotoxicity of cisplatin-loaded 23-6 nano-assemblies at the same concentrations on a cisplatin basis (right panel).*

CONCLUSIONS

The multifunctional p(MAA-g-EGMA) nanocarriers of cisplatin exhibit high colloidal stability, satisfactory drug-loading effficiency, magnetcally-triggered drug release and in vitro anticancer activity against A549 cancer cells comparable to free cisplatin. Thus, these nanocarriers appear to be promising candidates for magnetically-targeted cisplatin delivery to tumors and deserve further in vitro and in vivo evaluation.

REFERENCES

C. A. Rabik and M. E. Dolan, Cancer Treatment Reviews 2007, 33, 1.
F. Winnefeld , S. Becker , J. Pakush , T. Gotz , Cement Concrete Comp.2007 , 29 , 251.
A. Bakandritsos , A. Papagiannopoulos , E. N. Anagnostou ,K. Avgoustakis, et al. Small 2012 8,15.

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QUALITY-BY-DESIGN IN NANOMEDICINE

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MOTIVATIONS & CHALLENGES

The development of multifunctional nanoparticles for medical applications (drug delivery, targeted therapies, diagnostics or imaging [1,2,3,4]) is an emergent and very promising field in pharmaceutical drug development. Face to the multiplicity of factors in the design and manufacturing of medical nano-products, the elaboration of ef-

ficient and safe nanoparticles can be prohibitively time, labor and cost intensive. Controlling the quality of multifunctional nanoparticles while reducing the cost and risk associated with their preclinical development is an interdisciplinary and multifactorial challenge that requires a rational and robust methodology of development [5].

RESULTS

Inspired from the Quality-by-Design (QbD) guideline recommended in Pharmaceutical development by ICH1 Q8(R2) [6,7], our project aims at developing a model-based engineering approach devoted to the preclinical and clinical studies in nanomedicine. The goal is to surely optimize the quality (efficiency and safety) of the medical nanoparticles while minimizing risks and costs during their development. The nano-engineering cycle is described in Fig.1 and is decomposed into six main steps, in which not only the nanoparticle design but also its manufacturing process are analyzed in terms of quality.

1. In a first step, the quality target profile of the desired nanoparticle is defined. This profile may account for therapeutic efficiency, targeting ability, desired imaging contrast enhancement, expected pharmacokinetics, production scalability, stability, purity, sterility, bioavailability, etc.
2. The second step aims at identifying the critical quality attributes of the nanosystem, i.e. measurable physical, chemical, biological or microbiological properties or characteristics of the nanoparticle that should be within an appropriate limit, range or distribution to ensure the desired quality.
3. It is then necessary to determine the critical process parameters in a third phase, i.e. defining the manufacturing factors, which have a significant effect on quality.
4. In a fourth step, multidimensional relationships between the quality criteria (step 1) and the input variables (steps 2 and 3) are established. The latter model is then used to define design space, i.e. a region inside the multidimensional space of the input variables in which quality assurance is obtained.
5. The fifth step concerns the definition and integration of technological solutions to measure online critical quality attributes of the nano-product and critical process parameters.
6. Finally, the last factor is to design and implement a control strategy of the manufacturing process, which allows maintaining in real-time critical quality attributes and critical process parameters within the design space defined in step 4. This condition is the assurance that the quality obtained meets stakeholders' requirements. Such a method requires strong collaborations between clinicians, biologists, statisticians and system control engineers. To facilitate those interactions, we have modeled the whole nano-product engineering cycle and implemented it into an executable environment to perform traceability along with the preclinical studies, facilitate the project management, monitor the analyses in real-time and improve communication between the partners.

REFERENCES

[1] G. Le Duc, I. Miladi, C. Alric, P. Mowat, E. Braüer-Krisch, A. Bouchet, E. Khalil, C. Billotey, M. Janier, F. Lux, T. Epicier, P. Perriat, S. Roux, and O. Tillement. Toward an image-guided microbeam radiation therapy using gadolinium-based nanoparticles. ACS Nano, 5(12):9566–9574, 2011.

[2] B. G. Prajapati. Nanoparticles as platforms for targeted drug delivery system in cancer therapy. The Internet Journal of Nanotechnology, 3(1), 2009.

[3] L. Zhang, F. X. Gu, J. M. Chan, A. Z. Wang, R. S. Langer, and O. C. Farokhzad. Nanoparticles in medicine: Therapeutic applications and developments. Clin. Pharmacol. Ther., 83:761–769, 2008.

[4] D. Bechet, P. Couleaud, R. Vanderesse, C. Frochot, A.-C. Faure, S. Mordon, S. Roux, O. Tillement, F. Guillemin, M. Barberi-Heyob, and F. Auger. Nanoparticles for MRI and vascular photodynamic treatment of brain tumor. In XXVèmes Journées scientifiques du GTRV (Groupement thématique de Recherche sur la Vectorisation), Toulouse, France, Dec. 2010.

[5] H. Benachour, T. Bastogne, M. Toussaint, Y. Chemli, A. S`eve, C. Frochot, F. Lux, O. Tillement, R. Van- deresse, and M. Barberi-Heyob. Real-time monitoring of photocytotoxicity in nanoparticles-based pho- todynamic therapy: A model-based approach. PLoS ONE, 7(11):e48617, 2012.

[6] A. S. Rathore. Roadmap for implementation of quality by design (QbD) for biotechnology products. Trends in Biotechnology, 27(9):546 – 553, 2009.

[7] L. Yu. Pharmaceutical quality by design: Product and process development, understanding, and control. Pharmaceutical Research, 25:781–791, 2008.

***Figure 1**: Model of the nano-system engineering process management.*

SYSTEMATIC IDENTIFICATION OF POTENTIAL BREAST CANCER STEM CELL MODULATORS

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Cancer stem cells (CSC) have been documented in a variety of cancer types and are a specialized and rare subpopulation, comprising only few percent of the tumor cells. They are thought to give rise to recurrence of therapy-resistant and metastatic tumors after treatment, leading to patient death due to incurable cancer. Targeting of cancer stem cells is therefore thought to lead to a more effective treatment for cancer. In recent years, the in vitro mammosphere formation assay has become a routine assay for measuring the self-renewal of breast cancer stem cells and for determining the number of breast cancer stem cells present in a given population of cancer cells. Accordingly sphere cells isolated or cultured from the human breast cancer cell line MCF7 were reported to enrich breast cancer stem-like cells, which possess the stem cell-like gene signature, express stem cell markers and have higher tumorigenicity. Thus, it can be used as a model for studies of breast cancer stem cells. Within this project, we selected a set of 43 candidate genes that could play a potential role in the cancer stem cell phenotype. Our aim is to systematically test the effect of the candidate genes on the breast cancer stem cell phenotype in MCF7 cells. Consecutively, we will systematically explore, which genes/miRNAs have the greatest potential to induce a shift to a CSC-like phenotype via mammosphere assays. Here, we report on the first results from the candidate gene set as well as expression level analysis for selected candidate genes.

COLLOIDAL CLUSTERS OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES STABILIZED WITH BIOACTIVE CATECHOLS FOR MAGNETIC PARTICLE IMAGING

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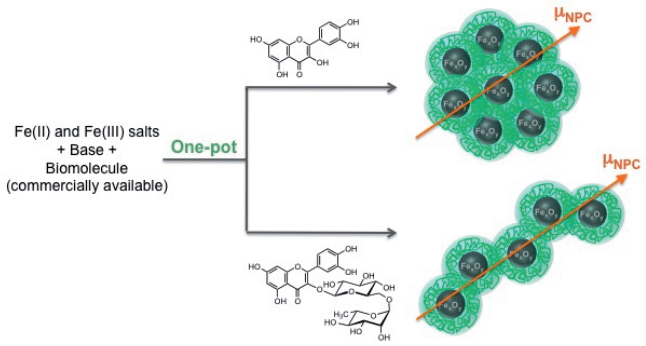
Nanoparticles hold immense promises for significantly improving existing diagnosis and cancer therapy.[1,2] First, nanoparticles can reach cancer cells by benefiting from the Enhanced Permeability and Retention (EPR) effect, which takes into account the defective tumour vasculature, as well as the low lymphatic drainage of the tumour tissue;[3] moreover, they are passively uptaken by cells via endocytosis, paving the way to intracellular diagnostic and drug delivery.[4] Second, if these nanoparticles are made stealth with the help of a biocompatible coating, their stay in the blood pool can be prolonged, leading to a more favorable in vivo biodistribution, thus improving the efficiency of the nanoparticles and limiting their potential side effects. More recently, clusters of nanoparticles have emerged as a further option for biomedical applications, provided that their sizes stay in the nanometer range.[5] Indeed, in the frame of cancer diagnosis and therapy, stable suspensions of superparamagnetic nanoparticles also called Ultrasmall Superparamagnetic Iron Oxides (USPIOs, consisting generally of maghemite γ-Fe2O3 or magnetite Fe3O4) and their clusters find applications as contrast agents for Magnetic Resonance Imaging (MRI)[6] or more recently for Magnetic Particle Imaging (MPI)[7,8] and as drug delivery vehicles (they can be positioned by applying a magnetic field to the site of interest, e.g. a tumor) or in hyperthermia.[9] For diagnostic applications, it is expected that MPI will play an important role in future medical application areas. On the contrary to MRI, which detects protons and therefore the magnetic particles only indirectly, MPI performs a direct measurement of superparamagnetic iron oxide nanoparticles: the magnetic nanoparticles (MNP) can be "seen" directly with high sensitivity and spatial and temporal resolution. Herein the quality of the particles are of tremendous importance, and attempts to define optimal particle properties for MPI are underway and recently of great debate. Commercialized Resovist® particles show good performances in terms of the measured MPI signal amplitude but the structure of Resovist® particles is very complex and not clearly defined as revealed by the finding of a bimodal size distribution of its primary particles.[10] Nevertheless, the presence of stable aggregates in form of clusters of primary particles seems to be a characteristic of Resovist® and is very likely of key importance for particles suitable for MPI.[11] Moreover, for biomedical applications, nanoparticles dispersions have to be stable under physiological conditions. Thus, in order to render the nanoparticles stable in biological media, to limit their aggregation state (of prime importance when nanoparticles are administered intravenously) and to make them biocompatible, it is necessary to coat them with stabilizing molecules or polymers.

In this contribution we report on a simple one-pot synthesis of stable clusters of USPIOs for MPI. Stabilization was performed using biologically relevant molecules containing catechol moieties (known for their ability to bind iron) such as the flavonoids quercetin and rutin, or dopamine. Radii of the primary iron oxide nanoparticles were around 5 nm, while the clusters' radii of gyration a well as hydrodynamic radii were less than 50 nm as determined from small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS) respectively. Detailed analysis of the SAXS data reveal a mass-fractal structure for quercetinand a wormlike chain structure for rutin-stabilized clusters (see Scheme 1).



Scheme 1. *Schematic illustration of synthesis and improved magnetic properties of nanoparticles clusters (NPC)*

We also compare the peformance of these samples in Magnetic Particle Spectroscopy (MPS) with two clinically approved MNP samples (Resovist®, Feraheme®). Clusters of nanoparticles such as those coated with quercetin and dopamine generate up to one order of magnitude higher MPS signal amplitudes than the non-clustered single cores of Feraheme®. That implies that clustering of small nanoparticles potentially increases MPS signal amplitudes. In the case of rutin coated clusters, the MPS signal amplitudes are smaller compared to Feraheme®, which can be attributed to the chainlike clustering determined by SAXS measurements. The subsequent higher shape anisotropy of the clusters may decrease the mobility of particle moments and their ability to respond to the oscillating magnetic excitation field.

Finally, given that the clusters are less than 100 nm in radius (of importance for intravenous injections and reaching cancer cells), stable under physiological conditions and biocompatible (no cellular toxicity to various relevant cell lines at relevant concentrations: lymphocytes and hepatocytes), these flavonoid-coated clusters present a real potential for in vivo applications. Indeed, assessment of cellular toxicity is of prime importance when dealing with nanomaterials for biomedical applications, especially since the new EU directives in the frame of REACH, the European Community Regulation on chemicals and their safe use.

[1] Nanoparticles: A boon to drug delivery, therapeutics, diagnostics and imaging. S. Parveen, R. Misra, S. K. Sahoo, Nanomedicine: NBM, 2012, 8, 147-166.
[2] Nanoparticle technologies for cancer therapy. F. Alexis, E. M. Pridgen, R. Langer, O. C. Farokhzad in Handbook of Experimental Pharmacology: Drug Delivery (Ed: M. Schäfer-Korting, Springer-Verlag Berlin Heidelberg), 2010, 197, 55-86.
[3] The hallmarks of cancer. D. Hanahan, R. A. Weinberg, Cell, 2000, 100, 57-70.
[4] Regulated portals of entry into the cell. S. D. Conner, S. L. Schmid, Nature, 2003, 422, 37-44.
[5] Superparamagnetic colloidal nanocrystal clusters coated with polyethylene glycol fumarate: A possible novel theranostic agent. H. Amiri, M. Mahmoudi, A. Lascialfari, Nanoscale, 2011, 3, 1022-1030.
[6] Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. Y. X. J. Wang, S. M. Hussain, G. P. Krestin, Eur. Radiol., 2001, 11, 2319-2331.
[7] X-Space MPI: Magnetic nanoparticles for safe medical imaging. P. W. Goodwill, E. U. Saritas, L. R. Croft , T. N. Kim, K. M. Krishnan , D. V. Schaffer, S. M. Conolly, Adv. Mater., 2012, 24, 3870-3877.
[8] Fundamentals and applications of magnetic particle imaging. J. Borgert, J. D. Schmidt, I. Schmale, J. Rahmer, C. Bontus, B. Gleich, B. David, R. Eckart, O. Woywode, J. Weizenecker, J. Schnorr, M. Taupitz, J. Haegele, F. M.Vogt, J. Barkhausen, J. Cardiovasc. Comput. Tomogr., 2012, 6, 149-53.
[9] Progress in applications of magnetic nanoparticles in biomedicine. Q. A. Pankhurst, N. T. Thanh, S. K. Jones, J. Dobson, J. Phys. D-Appl. Phys., 2009, 42, 224001.

[10] In situ analysis of a bimodal size distribution of superparamagnetic nanoparticles. A. F. Thunemann, S. Rolf, P. Knappe, S. Weidner, Anal. Chem., 2009, 81, 296-301.
[11] How the size distribution of magnetic nanoparticles determines their magnetic particle imaging performance. D. Eberbeck, F. Wiekhorst, S. Wagner, L. Trahms, Appl. Phys. Lett., 2011, 98, 182502.

NANOMEDICINE AND ITS GOVERNANCE IN INDIA

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Nanotechnology finds applications in various areas ranging from energy sector to healthcare. Nanomedicine is a progressive field of nanotechnology based research and it has exhibited accelerated commercialization. In the year 2009 the global nanomedicine market was valued at $53 billion and has been forecasted to increase at a compound annual growth rate (CAGR) of 13.5% to reach more than $100 billion in 2014. However, acceptability of nanomedicine is dependent on the kind of impact it may have on the environment as well as human health. Monitoring at every level of the lifecycle of the nanomedicinal product right from research to the release of the product is critical (Fig.1). The major safety challenges pertaining to nanomedicine are nanotoxicity, immunological reactions, biocompatibility, biodistribution and lack of evaluation systems. The commercialization and utilization of these products is also dependent on the regulatory regimes that ensure the safe use of nanomedicine. As the exact mechanism of interaction of these nanomedicinal products (owing to their size) with the biological system is yet not clear[1] hence raising biosafety issues. The emerging research field of nanotoxicology by its analytical protocols may be able to address such issues. Like many countries, India also has budget allocated towards nanosafety programs and nanotoxicity research.

***Figure 1**: Lifecycle of a nanomedicinal product*

Research in novel life sciences technologies such as nanomedicine and synthetic biology are progressing at an unprecedented pace. Although, such technologies could contribute towards the bio-based economy of India, however, such technologies need to overcome the hurdles for public support and acceptability. Addressing the concerns is essential at the right stage, else nanomedicine will encounter the same fate as genetically modified crops, wherein large amount of investments have been made, however, the crops and products based on such crops are not readily accepted by the end user. The approved field trials for genetically modified crops in India have been opposed and the matter is currently under Supreme Court of India's review. The expert committee formed by the court has advised to stop such trials till appropriate mechanisms for monitoring trials and biosafety data evaluations are developed[2]. Similarly, stem cell research has faced resistance from all the stakeholders; however, draft guidelines have been developed to direct the research[3]. Every emerging technology suffers from fear of misuse and nanomedicine is not an exception, such as development of nanodevices using synthetic biology tools[4] as well as free availability of synthetic biology based nanoparts library have raised concerns of misuse. Hence risk assessment and impact assessment of such technologies become very pertinent in the contemporary scenario. Various aspects of nanotechnology in India such as risk assessment, user acceptability have been analyzed[5-8] and existing laws pertaining to health care application of nanomedicine in India have been assessed [9-10]. Although, all these studies emphasise on the need for risk assessment and generation of data, however, these studies fail to provide solutions for effective enforcement to enhance growth of nanomedicine in India. Information on impact of nanomedicine on environment can be generated using a mechanism as exemplified by Genome Canada11. The present study proposes development of a field specific framework involving various stakeholders in India, which would play a significant role in sustainable growth of this emerging application of nanotechnology to promote safe research as well as use of the products.

REFERENCES

- Maynard AD, Warheit DB, Philbert M (2011). The new toxicology of sophisticated materials: nanotoxicology and beyond. Toxicological Sciences 120(S1):S109–S129.
- Jayaraman. KS Science panel urges halt to GM field trials in India. Available from: http://blogs.nature.com/news/2012/10/science-panel-urges-halt-to-gm-field-trials-in-india.html. (Accessed on March 14 2013)
- Mukhopadhya CS, Tokas J, Mathur PD (2011). Prospects and Ethical Concerns of Embryonic Stem Cells Research-A Review. Veterinary World 4(6): 281-286.
- Yun Y , Conforti L, Muganda P and Sankar J (2011). Nanomedicine-based Synthetic Biology. Journal Nanomedicine Biotherapeutic Discovery 1:1.
- Sarma. SD (2011). How Resilient is India to Nanotechnology Risks? Examining Current Developments, Capacities and an Approach for Effective Risk Governance and Regulation. European Journal of Law and Technology 2(3).
- Sahoo. S, Sarma. SD. Nanotechnology, Scientists and Perceptions: A View from the Field, Survey Report of Capability, Governance and Nanotechnology Developments: A Focus on India. 2010
- Sahoo S. Socio-ethical Issues and Nanotechnology Development: Perspectives from India. IEEE NANO. 2011: 1205-1210, DOI:10.1109/NANO.2010.5697887.
- Purushotham. H. Knowledge Management and Regulatory Issues- Key for Sustainable Development of Nanoscience and Technology in India. International Conference on Nanoscience, Engineering and Technology (ICONSET) 2011 November 28-30.
- Srivastava N, Chowdhury N (2008). Regulation of Health related Nano Applications in India: Exploring the limitations of the Current Regulatory Design. International Conference: Mapping the uncertainty of nanotechnology. Challenges to law, ethics and Policy making. May 2008, Available from http://www.nanotechregulation.org/TeriNano01.pdf
- Vivekanandan J (2009). Nano Applications, Mega Challenges: The Case of the Health Sector in India. Studies in Ethics, Law and Technology 3(3).
- Genome Alberta 2013. Available from http://genomealberta.ca/default.aspx (Accessed on March 14 2013)

MASS SPECTROMETRY AND HIGH THROUGHPUT CELLULAR ASSAYS ARE CRUCIAL TOOLS FOR ADVANCED NANOMEDICINE CHARACTERIZATION

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Life and medical sciences have advanced dramatically over the last couple of decades. This has – to a large extent – been facilitated by technological progress, both by immensely increased sample throughput (High Throughput / High Content Screening) and by the introduction of entirely new analytical principles. One key enabling technology in life science has been the widespread adaptation of mass spectrometry (MS). The '3S' combination of sensitivity, selectivity and specificity for a very wide range of molecular species has made MS the method of choice e.g. for pharmacological analyses in traditional medicine.
SINTEF is a private, non-profit contract research organization (CRO), with more than 2000 employees working in a number of research areas; the unified objective of SINTEF is 'Technology for a better society'.
Nanomedicine – although novel in its targeted approach and formulations – is faced with many of the same analytical challenges as traditional medicine. This applies both to the study of drugs/carriers, and to the investigation of their clinical effects. Nanomedicine is a prioritized research area within SINTEF, with a dedicated group working on the synthesis of multifunctional nanocarriers for drug delivery and diagnostic purposes.
Here, we present the SINTEF MS/HTS analytical facility. In a nanomedicine context, MS analyses and robotized, high throughput biological assays form a powerful combination, which is further supplemented with complementary analytical techniques such as GC, LC, optical spectroscopy and light scattering. The full set of available MS techniques is presented, including imaging mass spectrometry (IMS) for label-free imaging of drugs and metabolites in tissue, and ultrahigh-resolution (FT-ICR) MS for very complex samples. Nanomedicine examples from ongoing research projects are presented, including polysaccharide heterooligomers with structure-dependent antimicrobial activity, and hybrid organic-inorganic nanoparticles that serve as flexible scaffolds for modification with functional groups delivering biological activity.

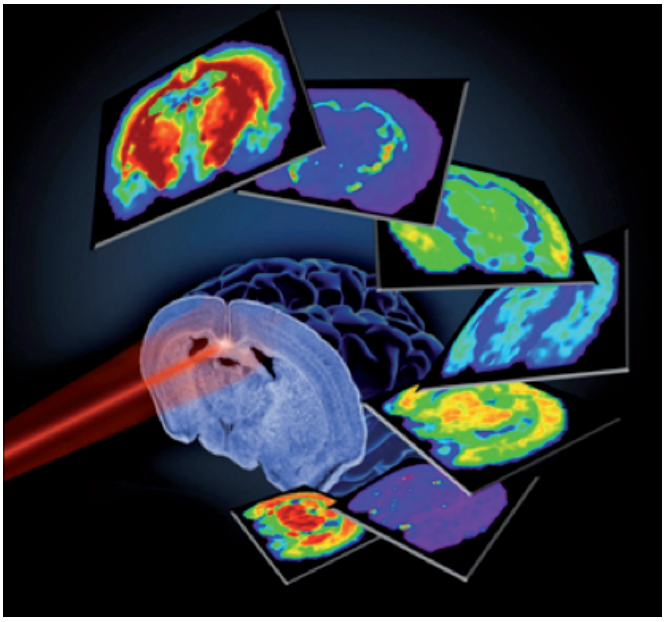

Figure 1. *Schematic of mass spectrometry (MALDI) imaging*

SURFACTANT'S PHASE BEHAVIOR AS POTENTIAL TECHNOLOGICAL PLATFORM TO DEVELOP NANOSTRUCTURED BIOADHESIVE DRUG DELIVERY SYSTEMS

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INTRODUCTION

Exploiting new routes of administration can be a strategy to modify the drug's action or absorption profile, since alternatives routes may avoid problems related to the physiological barriers of the oral route[1]. The mucous tissue of the vaginal, rectal, periodontal and buccal routes have been explored for drugs' administration, aiming a target action; other mucous tissue such as the nasal route is widely studied for systemic action and bioavailability's rise, as well as for nervous central system targeting[2]. The success of drug delivery systems designed for mucosal routes is related to their capacity of enhancing the retention and localizing them to specific sites in the body, as result of their bioadhesion[3]. Mucous membranes are the main administration sites for bioadhesive systems, but new bioadhesive formulations for dermal application are also needed; where prolonged cutaneous action is desired. A prolonged effect after the dermal administration of creams, solutions, and lotions is not normally expected, since such preparations can easily be removed from the skin by moisture, temperature, and physical movements[4].
Surfactant systems such as microemulsions (ME) and liquid crystals (LC) have great potential as topical vehicles. Surfactants are able to stabilize mixtures of components with different polarities, creating additional regions for hydrophilic and lipophilic drugs' solubilization. The drug can be protected against the dissolution medium due to the interfacial membrane composed of the surfactant layer, which the drug must overpass to be released5. In this manner, surfactant systems also confer reservoir properties. The phase behavior of surfactants able to form ME and LC is extremely attractive for the nanotechnology, since their structures are organized in nanometric scale. Besides its stability, their production can be easily scaled up. Varying the composition and proportions of surfactant, oil and aqueous phase, it is possible to control the viscosity and texture of the systems above, which could lead to the immobilization or localization of the formulation in specific sites in the body and routes of administration. In view of that, surfactant systems have great potential as topical nanostructured bioadhesive drug delivery systems[5].
Therefore, the objective of this study was to present the current developments of our research group concerning the application of the phase behavior studies of polyoxypropylene (5) polyoxyethylene (20) cetyl alcohol (PPG-5-CETETH-20) based surfactant systems in combination with different oil phases and drugs, designated for several routes of administration such as the skin and some mucous tissues.

METODOLOGY

Phase diagrams were constructed for the surfactant and each of the oily phases (oleic acid, mineral oil, isopropyl myristate, copaiba oil). The systems were structurally characterized by polarized light microscopy (PLM), small-angle X-ray scattering (SAXS) and rheological measurements. The texture and bioadhesive force were measured using a texture analyzer. It was tested the incorporation of fluconazol, propolis, dexamethasone and zidovudine. The transport and drug absorption across the skin or mucous tissues were studied by in vitro techniques employing a diffusion chamber and excised porcine skin or mucous tissues from slaughterhouses. The efficacy some samples was assessed in vivo in rats.

RESULTS

The construction of the ternary phase diagrams for each oil phase (Figure 1) showed that oleic acid, copaiba oil and isopropyl myristate resulted in diagrams with similar profiles. A wide range of liquid, transparent and thermodynamically stable systems (LTTSS) were formed with low water content. The increase of water changed the systems with higher amounts of surfactant to lamellar and hexago

nal phases. The systems with lower amounts of PPG-5-CETETH-20 changed to opalescent systems or phase separation. The biggest region of liquid crystals was achieved for oleic acid and copaiba oil, since both present similar molecular structures. Mineral oil leaded to the formation of a large area of phase separation, due to its great lipophilicity. Conversely, mineral oil promoted the formation of the cubic phases7. LTTSS samples showed Newtonian behavior; the LCs behaved as plastic and pseudoplastic systems with thixotropy, which facilitates the extrusion of the formulation from a container or syringe or their application on the skin or mucosa, besides the recover of their original structure after shear. The results of the texture and biodhesive force measurements showed that the cubic phases were more adhesive than the hexagonal ones, which were more adhesive than the lamellar phases. So, higher degrees of stiffness make the systems more adhesive, and these properties can be exploited in the development of bioadhesive formulations[6].

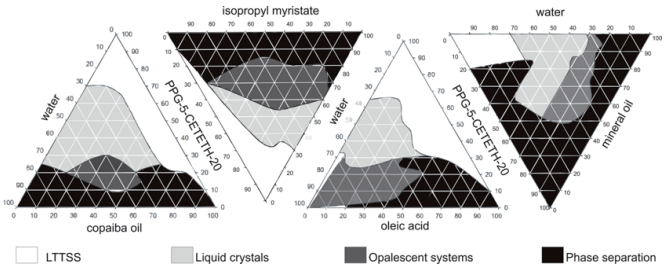

***Figure 1**: Ternary phase diagrams of PPG-5-CETETH-20 surfactant and water combined with copaiba oil, isopropyl myristate, oleic acid and mineral oil as oil phases. Adapted from Carvalho5, Rocha7 and Urban8.*

Systems composed of PPG-5-CETETH-20/water/oleic acid increased the solubility of fluconazol (6-fold) and the transport across everted gut sac (5-fold) compared to aqueous solution7. Systems composed of PPG-5-CETETH-20/water/copaiba oil increased the fluconazol antifungal activity against Candida albicans. Moreover, permeation studies using porcine ear skin showed that these systems promoted the retention of the drug on the skin, which is ideal for dermatomycoses treatment. Histological microscopy of both ternary systems showed no dermatological toxicity followed by in vivo administration on rats' skin8. Hexagonal phases composed of PPG-5-CETETH-20/water/ isopropyl myristate promoted the dermic retention of dexamethasone studied by Franz's diffusion cell using porcine ear's skin. A conventional dexamethasone cream did not exhibit such reservoir effect9. Propolis microparticles loaded LTTSS has designed as syringeable precursor systems of liquid crystalline phase for application to the periodontal pocket. They presented easy insertion into the periodontal pocket and subsequent stable retention therein. Furthermore, the release profile studies showed that the propolis can be released for a prolonged period of time (more than 7 days). These properties indicate a possible advantageous role in the treatment of periodontal disease10. Zidovudine (AZT) loaded LTTSS samples were tested for nasal administration. The phase transition of AZT-LTTSS to a lamellar phase, when coming into contact with artificial nasal mucus, was accompanied by a stiffness change, which may result in biodhesion (Figure 2)11. The drug transport across the nasal mucosa employing a diffusion chamber and an excised porcine nasal mucosa tissue showed a promising permeation enhancer property. The in vivo results of nasal administration of AZT-LTTSS in rats revealed a fast absorption (Cmax= 6.67 min) compared to commercial oral formulations (Cmax= 0.5–1.5 h)11.

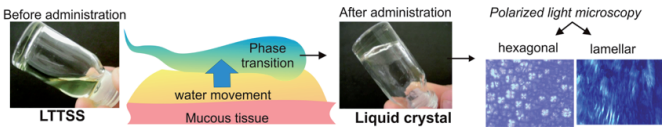

***Figure 2**: Schematic illustration of surfactants' phase behavior explored in this work as technological platform to design new topical nanostructured bioadhesive drug delivery systems.*

CONCLUSIONS

The technological platform presented here provided a wide variety of systems with different rheological, mechanical and adhesive properties by changing only the proportions of their components; therefore, it has great potential for engineering new nanostructured topical bioadhesive drug delivery systems.

REFERENCES

- Ojewole et al., Eur J Pharm Biopharm 70(3): 697-710, 2008.
- Mainardes et al., Curr Drug Delivery 3: 275-285, 2006.
- Andrews et al. Eur J Pharm Biopharm 71(3): 505-518, 2008.
- Carvalho et al., Drug Dev Ind Pharm 2012.
- Carvalho et al., J Biomed Nanotechnol 8(2):280-9, 2012.
- Klein, T., UNESP Master thesis, 2007.
- Rocha, H.S., UNESP PhD thesis, 2011.
- Urban, M. C. C., UNESP Master thesis, 2004.
- Carvalho et al., J Pharm Pharmacol 62: 430- 439, 2010.
- Bruschi et al, Drug Develop Ind Pharm 34: 267–278, 2008.
- Carvalho et al., Eur J Pharm Biopharm, 2012.

ENGINEERED SILVER NANOPARTICLES: PROTEIN CORONA FORMATION AND TOXIC EFFECTS ON BRAIN CELLS

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Silver nanoparticles (Ag-NPs) have gained much popularity because of their enhanced antiseptic activity. A wide range of applications of Ag-NPs has recently emerged in consumer products, ranging from food packaging and odour resistant textiles to medical devices and household appliances.[1] As a consequence of such an extensive usage, the potential for adverse health effects in humans and environment is very high.[2] However, little is known about the health impact of the prolonged exposure to Ag-NPs at various concentrations. In particular, since Ag-NPs can penetrate the blood-brain barrier (BBB) and gain access to the brain, there is the need for a quantitative risk assessment in brain cells.[3]

In order to evaluate the toxicity of Ag-NPs, it is worthy to note that proteins in biological fluids associate with nanoparticles and lead to a protein corona, which defines the biological identity of the particle.[4] Therefore, this work was aimed at studying the protein corona formation and the potential toxicity of Ag-NPs on astrocytes as a function of nanoparticle properties (coating, size, concentration).

METHODS

In this study, commercial Ag-NPs of 10, 40 and 100 nm in size were employed, both nude and coated with polyvinylpyrrolidone (PVP). To study the protein corona formation, Ag-NPs were incubated in a cell-free medium at two different concentrations (12.5 and 50.0 µg/mL), and their hydrodynamic diameter was measured by Dynamic Light Scattering (DLS, Nano ZS90, Malvern, UK) at fixed time points (24 h - 168 h). To further characterize the protein corona, samples were fixed in 2.5% gluteraldehyde solution and analysed by transmission electron microscopy (TEM, FEI Tecnai G2, Eindhoven).

In vitro cell viability of primary astrocytes was evaluated in real time by xCELLigence apparatus (Roche). Astrocytes were maintained in culture in the presence/absence of increasing amount (from 3 µg/mL up to 50 µg/mL) of Ag-NPs for up to 20 days. Intracellular uptake was determined by confocal microscopy and IMARIS software analysis.

RESULTS AND DISCUSSION

The Ag-NPs/proteins complex was observed by TEM after incubation with the cell medium (Fig. 1). The DLS data suggested that Ag-NPs concentration did not affect significantly the protein corona, which in turn was highly dependent on both coating and size of Ag-NPs. Indeed, the lower the size, the higher was the increase of

Ag-NPs diameter. In particular 10 nm-sized particles underwent a huge increase in diameter, which could be related to the formation of macro-aggregates Ag-NPs/proteins. Finally, the PVP coating seemed to increase the protein corona, especially after 168 h of exposure at the lower concentration (12.5 µg/mL, Table 1).
Concerning the in vitro experiments, Ag-NPs of 100 nm size showed the highest level of toxicity. The PVP coating seemed to reduce the toxicity of Ag-NPs, especially using 40 nm-sized particles at lower concentrations (from 10 µg/mL down to 3 µg/mL). According to confocal microscopy analysis, Ag-NPs were internalized into astrocytes after an overnight exposition. After 6 days of incubation, they formed aggregates inside the cells, whose morphology was clearly altered compared to cells not exposed to Ag-NPs.
Overall, it was found that the greater the Ag-NPs diameter, the smaller the protein corona, the higher the toxicity on astrocytes. Therefore, these results suggested that the protein corona could represent an effective tool to reduce the Ag-NPs toxicity.

REFERENCES

[1] S.W.P. Wijnhoven et al., Nano-silver: a review of available data and knowledge gaps in human and environmental risk assessment, Nanotoxicology, 2009; 3, 109-138.
[2] Ahamed M., AlSalhi M.S., Siddiqui M.K.J., Silver nanoparticle applications and human health, Clinica Chimica Acta 2010, 411, 1841–1848.
[3] Tang J., Xiong L., Zhou G., Wang S., Wang J., Liu L., Li J., Yuan F., Lu S., Wan Z. Et al: Silver nanoparticles crossing through and distribution in the blood-brain barrier in vitro. J. Nanosci. Nanotechnol. 2010, 10, 6313-6317.
[4] Mahmoudi M., Lynch I., Ejtehadi M.R., Monopoli M.P., Baldelli Bombelli, Laurent S. Protein-Nanoparticle Interactions: Opportunities and Challenges, Chem. Rev. 2011, 111, 5610–5637.

FIGURES

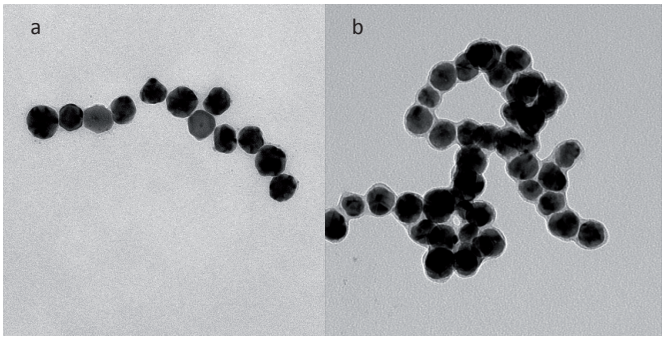

***Fig. 1**: TEM images of nude 40 nm sized Ag-NPs before (a) and after (b) the protein corona formation. The scale bar is 50 nm.*

Sample – Ag NPs	12,5 µg/mL		50 µg/mL	
	peak (nm, 24 h)	peak (nm, 168 h)	peak (nm, 24 h)	peak (nm, 168 h)
10 nm - citrate	70 ,0	83,9	72,1	75,1
10 nm - PVP	110,8	127,9	96,0	102,0
40 nm - citrate	101,4	100,9	107,7	100,1
40 nm - PVP	123,4	136,4	107,2	107,2
100 nm - citrate	170,2	161,7	154,9	151,2
100 nm - PVP	170,2	180,8	166,8	168,5

***Table 1**: DLS measurements of Ag-NPs size after 24 h and 168 h incubation with cell-free medium.*

INTELLI NANO DOTS-AN APPLICATION OF NANOTECHNOLOGY IN MEDICINE

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In today's era we feel an urgent need to face unsolved problems in medicine and the healthcare industry. Increasing global population and deadly diseases, only contribute to it. The field of nanomedicine has shown great hope in this regard and commercial efforts worth billions have been made and are seen on an upward trend.

Table 1 Commercial efforts in nanomedicine[a]

Healthcare sector	Number of products	Sales ($ billions)	Product pipeline: Total	Product pipeline: Advanced stages[b]	Product pipeline: Companies
Drug delivery	23	5.4	98	9	113
Biomaterials	9	0.07	9	6	32
In vivo imaging	3	0.02	8	2	13
In vitro diagnostics	2	0.78	30	4	35
Active implants	1	0.65	5	1	7
Drugs & therapy	0	0	7	1	7
Total	38	6.8	157	23	207

Wagner et al.Nature Biotechnology,2006(USD 6.8B in 2004; USD 12B in 2012

Intelli nano Dots are nano entities capable of sensing, signalling, information processing and thus enabling ultra-specified local drug delivery and defence against hostile particles. These may prove vital and find potential use in changing our approach in the treatment of Malaria, HIV, diabetes and even vaccine development.
The concept talks about creating the ability to steer right upto the lesion and deliver treatment to the target. A roadmap includes the journey from identifying exact nano sensors—testing them for specific pathogens—intelligence programming and control analysis—in lab testing and further on.
Probably now is the time we need to take a step back, look upon the clues that nature provides us in even the possibly smallest living particles, and add to our approach. A humble effort needs to be put forward to change fundamentals and conventional ways of making decisions for innovations in this ever emerging field of nanomedicine.
It should be noted that the concept needs further research and analysis under the collaborative efforts of different wings of nanotechnology to provide an integrative result. There may be no immediate return on investment but this may be a sustainable innovation in the long term to reduce the burden of global healthcare. The least this paper can do is to trigger a novel thought process in the most able minds or throw open a new ray of light to those stuck onto similar objectives!

DEVELOPMENT OF ALL CHOLESTEROL-BASED NANOVECTOR FOR HIGH EFFICIENT DELIVERY OF NUCLEIC ACIDS INTO CELLS

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Nowadays, gene delivery vehicles are applied broadly in experimental studies, but their clinical applications are still to be evaluated. Researchers are working hard for developing an efficient and safe delivery system for gene therapy. Since viral vectors have serious problems in safety and production concerns, scientists are focusing on the improvement of nonviral vectors to optimize their future clinical uses. The cationic lipid vectors generally consist of two major classes. One is based on hydrocarbon chain as backbone, the other is the use of cholesterol as backbone. Beside of cationic lipids, some helper lipids (cholesterol and phospholipid DOPE) are needed in the lipid nanoparticle formulation. Guanidinium-based lipids did not receive much attention until recently. With a favorable pKa of 13.5 and the ability to form zwitterionic hydrogen bonds with the DNA phosphate anions, together with its propensity to develop hydrogen bonding with nucleic bases such as guanine, the guanidinium group offers several desirable features that could be exploited to create efficient non-viral vectors. Thus, we have designed and synthesized a guanidinium-containing cholesterol derivative, 3-beta-[N-(2-guanidinoethyl)- carbamoyl]-cholesterol (GEC-Chol). Subsequently, GEC-Chol was mixed with cholesterol or DOPE at different molar ratio to produce lipid nanoparticles. We have studied the physical parameters, e.g. morphology, size, surface charge, of the lipid nanoparticles. Due to serum-compatibility, high transfection efficiency as well as low cytotoxicity, the micellar formulation of GEC-Chol/Chol was most frequently used in our laboratory for designing nucleic acid therapeutics. It also outperforms most of the transfection reagents in market for in vivo studies. Therefore, we hope to develop this lipid nanocarrier for a wide variety of biomedical applications including manipulating genes for human therapeutics.

ROS-INDUCED STRONG OXIDIZING PROPERTY OF COPPER NANOPARTICLES (NPS) STABILIZED BY DOPAMINE DERIVATIVES

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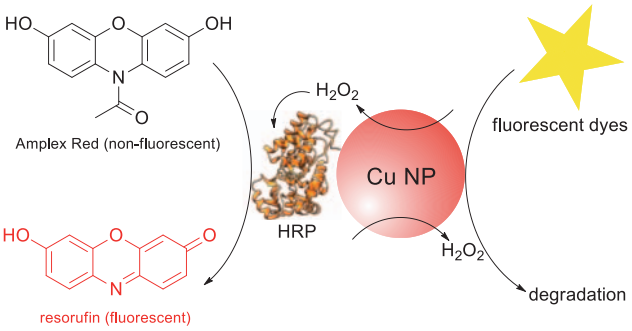
In the past decade NPs have attracted lots of attention as potential enzyme mimetics due to their stability under different reaction conditions, large number of functional groups on the surface and inherent catalytic properties.[1] Particularly interesting is their use in bioinspired catalytic processes where they could act either as enzyme replacing catalytic species or as enzyme activators. For example, Fe3O4 magnetic NPs were found to possess an intrinsic enzyme-mimetic activity similar to that of natural peroxidases.[2] CdS quantum dots and TiO2 NPs alone were used as photosensitive activators of P450 and peroxidase enzymes enabling temporal and spatial control over enzymatic activity.[3, 4]

One class of NPs that has a huge and still not fully tapped potential are Cu NPs, which have interesting properties particularly useful for catalysis or applications in nanomedicine.[5, 6] However, they are not widely used due to the ease of surface oxidation, which affects their properties and makes the synthetic routes difficult to optimise. In search for stable Cu NPs, we have employed L-ascorbic acid, dopamine, and a series of new dopamine-based linkers as capping agents, and the as-synthesized Cu NPs were stable in aqueous solutions over months of storage. Peroxidase activity assays show that Cu NPs could generate reactive oxygen species (ROS) and have excellent oxidizing activity towards dye pollutants. Due to their good ROS production ability, Cu NPs might also have some apoptosis effects on cells and can be used for nanomedical applications, which will be further investigated in our ongoing studies.



Scheme 1. *Cu NPs-generated ROS could activate peroxidase enzyme and oxidize fluorescent dyes*

REFERENCES

1. J. X. Xie, X. D. Zhang, H. Wang, H. Z. Zheng and Y. M. Huang, Trac-Trend Anal. Chem., 2012, 39, 114-129.
2. L. Z. Gao, J. Zhuang, L. Nie, J. B. Zhang, Y. Zhang, N. Gu, T. H. Wang, J. Feng, D. L. Yang, S. Perrett and X. Yan, Nat. Nanotechnol., 2007, 2, 577-583.
3. B. I. Ipe and C. M. Niemeyer, Angew. Chem. Int. Ed., 2006, 45, 504-507.
4. L. Fruk, V. Rajendran, M. Spengler and C. M. Niemeyer, ChemBioChem, 2007, 8, 2195-2198.
5. F. Alonso, Y. Moglie, G. Radivoy and M. Yus, Tetrahedron Lett., 2009, 50, 2358-2362.
6. G. P. Jose, S. Santra, S. K. Mandal and T. K. Sengupta, J. Nanobiotechnol., 2011, 9.

CHITOSAN OLIGOSACCHARIDE-MODIFIED GOLD NANORODS FOR IN VIVO TARGETED DELIVERY

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Gold nanorods have recently been identified as potential candidates for in vivo imaging because of their robustness and unique optical properties in the NIR region. In principle, gold nanorods have two plasmon resonance peaks in their absorption spectra, the longitudinal surface plasmon peak (SPR) and the transverse surface plasmon peak. This longitudinal SPR peak can be tuned to the NIR region by changing the aspect ratio and diameter of the nanorods. When these nanorods are excited at NIR wavelengths, they can simultaneously behave as optical contrast agents and photothermal transducers. In addition, the simple synthesis, tunability, good biocompatibility and relative ease of conjugation with antibodies and other targeting moieties of gold nanorods make them excellent candidates for non-invasive imaging and image-guided therapies based on localized hyperthermia. However, in vivo targeted delivery of gold nanorods is the key obstacle in obtaining high contrast images and more efficient photothermal treatment. To achieve targeted delivery using gold nanorods, many surface modification schemes have been used to replace CTAB, but they often suffer from poor biocompatibility, increased particle size and a low capacity for loading of additional moieties to enable multifunctional applications. Most of these modified particles have only improved targeting and photothermal effect in in vitro cancer cells. Among these modifications, PEGylated gold nanorods have shown the best performance in terms of toxicity and photothermal effect both in vitro and in vivo, but they do suffer from rapid excretion (half life ~1 hours). To solve this rapid excretion problem, chitosan has been introduced recently to extend the circulation time. Chitosan is biodegradable, biocompatible and water-soluble. The presence of primary amine groups also enables bioconjugation with a variety of molecules and permits the introduction of other functional groups such as carboxyl or acetylene groups. Furthermore, it maintains its structure in a neutral environment, although it becomes soluble and degrades in an acidic environment, which indicates that chitosan can act as an acid-responsive drug delivery carrier by degrading and releasing the drug in the desired environment. However, the high molecular weight of chitosan limits its performance in biological applications due to limited water solubility and particle–particle crosslinking. Therefore, low molecular weight chitosan or chitosan oligosaccharide (~5000 Da) is a better choice for in vivo applications. Most of the methods reported for the in vitro/in vivo application of chitosan have used photo polymerization of acrylated chitosan or thiol and oleic anhydride modification of chitosan to replace the CTAB on the surface of the gold nanorods. However, these reported methods require the attachment of functionalized chitosan directly onto the surface of the gold nanorods, which may lead to aggregation and limit its performance for in vivo applications. Therefore, covalent conjugation of chitosan to the gold nanorods is highly desired to provide additional stability for in vivo applications. Hence, our aim is to develop a multifunctional probe, which utilizes the unique properties endowed by gold nanorods as photothermal transducers when excited at near infrared wavelength and its covalent linkage with chitosan oligosaccharide for a successful acid-responsive delivery of the nanorods. The synthesized probes will be further decorated with cancer–specific antibody to enhance the localization of the probe which was subsequently used for specific targeting and non-invasive imaging based on localized hyperthermia after NIR irradiation.

We report the systematic functionalization of CTAB-capped gold nanorods to construct the multifunctional probe. First step involves the simple replacement of CTAB with MUA (11-mercaptoundecanoic acid) on the nanorod surface with PEG(polyethylene glycol) as an intermediate layer for stability. Next step involves the covalent linkage of MUA-coated gold nanorods with low molecular weight chitosan oligosaccharide (~5000) using EDC chemistry to produce CO-GNRs (chitosan oligosaccharide-modified gold nanorods). EDC facilitates the formation of a covalent amide bond

between the amine groups present on chitosan oligosaccharide and the carboxyl groups on the nanorod surface generated from the MUA treatment. Final step deals with the conjugation of CO-GNRs with monoclonal antibody against EGFR (epidermal growth factor receptor) to achieve tumor targeting functionality due to over-expression of EGFR in CAL 27 cells. Before in vivo tumor targeting application, we have also evaluated the toxicity of the CO-GNRs both in vitro and in vivo and quantified the cellular uptake by ICP-MS (inductively coupled plasma mass spectrometer). To test the potential of multifunctional gold nanorods-based probe, two-photon luminescence imaging was performed over CAL 27 cultured cells. Later on, in vivo studies performed via NIR laser irradiation at tumor sites in CAL 27 xenografts (CAL27 cells injected in living mice to obtain tumor) on BALB/c nude mice as the model, further confirm the localized targeting as observed by elevated temperature at the tumor site. These results have confirmed that these functionalized gold nanorods can serve as a biocompatible, non-toxic and cancer-specific probe to be utilized in targeted delivery and can be helpful in photothermal-related therapies.

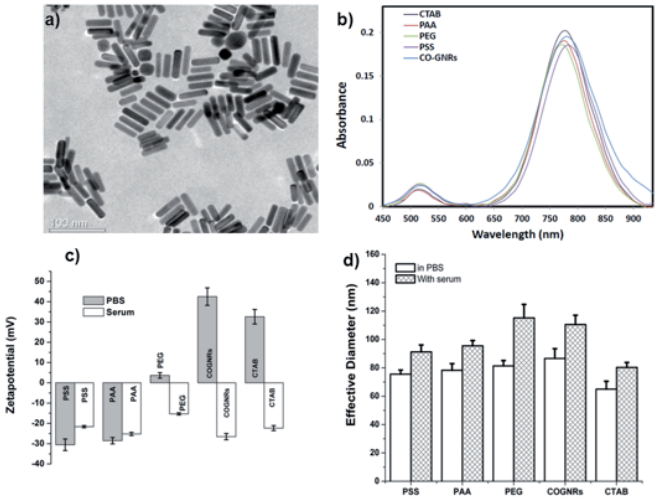

Figure 1. *a) TEM image of 780 nm gold nanorods, b) the UV-vis-NIR extinction spectra of surface modified and CTAB capped gold nanorods with a 780 nm peak, c) Zeta potential measurements of various surface-modified gold nanorods before (in PBS) and after treatment with serum d) particle size measurement by DLS before (in PBS) and after treatment with serum for 8 hrs.; n=3 for DLS and zeta potential measurements.*

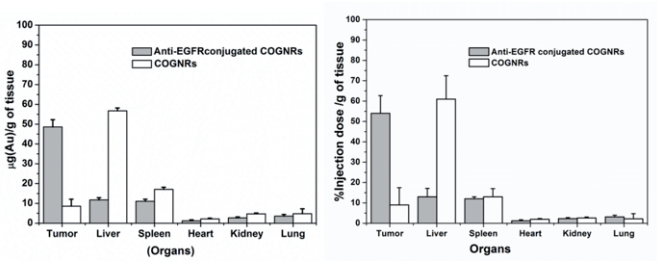

Figure 2. *Biodistribution of gold nanorods in nude BALB/c mice at 24 h after intravenous (tail vein) injection. The gold amounts in tissue samples were measured by ICP-MS (n = 3)*

PROTAMINE NANOCAPSULES FOR COMBINATORY ANTICANCER THERAPIES

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INTRODUCTION

Despite the enormous amount of research efforts devoted to the development of new oncological therapies, the advances made are still limited. Poor patient survival is directly attributable to widespread metastasis, drug resistance and the lack of effective treatment strategies[1]. Therefore, the development of new strategies aimed to overcome the mechanisms of cancer resistance and dissemination is urgently needed. Multiple anti-cancer therapies are the key to most cancer treatments, since they offer the potential for tackling multiple targets and pathways simultaneously to more effectively kill cancer cells and prevent drug resistance[2].

The field of nanotechnology applied to the delivery of bioactive molecules is having increasing impact in the development of new oncological therapies[3]. Nanocarriers can be prepared out of a wide array of materials and be specifically designed for effective association a variety of molecules and biomolecules. Moreover, nanocarriers can improve the penetration of drugs in the tumour-surrounding environment and accumulate in the tumour. Core-shell nanocapsules have the potential to entrap hydrophobic anticancer molecules in the inner compartment, and associate hydrophilic macromolecules to the outer layer, hence constituting versatile systems for the development of novel nano-oncologicals[4,5].

OBJECTIVE

The aim of this work was to evaluate the potential of protamine-nanocapsules to develop a nanocarrier-based combination therapy. As a first approach, we selected curcumin, a hydrophobic compound with antitumoral properties, and nucleic acids (pDNA and siRNA), for a targeted gene therapy[8,9]. Experiments were conducted to optimize the physicochemical properties of these prototypes and to evaluate their behaviour in vitro.

EXPERIMENTAL METHODS

Nanocapsules of protamine with an oily core of α-tocopherol were prepared by the solvent displacement technique. The inclusion of other excipients, as the surfactants Tween 80 (T80), PEG stearate (PEG-st) or d-alpha tocopherol succinate (TPGS), was also explored. The size and zeta potential were measured in a Nanosizer ZS (Malvern Instruments, UK), and the morphology studied by Transmission Electron Microscopy (TEM, CM 12 Philips, Eindhoven, The Netherlands). The efficacy of encapsulation of curcumin was explored by spectrophotometry (λ= 535 nm, Mithras LB 940-Berthold Technologies, Germany), and the efficacy of association of nucleic acids by agarose gel electrophoresis (Nanodrop®, Thermo Scientific, USA). The ability of the nanocarriers to interact with cancer cells, and deliver the associated drugs, was explored by Confocal Laser Scanning Microscopy (CLSM, Leica TCS SP2, Spain). Gene transfection was studied in a panel of cancer cells lines using for that purpose pGFP and siRNA-Luciferase.

RESULTS AND DISCUSSION

The physicochemical characterization of the core-shell protamine nanocapsules reveals that we were able to obtain nanocarriers with a small particle size (<300nm), a narrow size distribution (polydispersity index 0.1-0.2) and a positive superficial charge (>+25 mV). These nanocapsules are highly stable, regarding their colloidal stability, either during storage or in presence of simulated biological fluids, and show a good biocompatibility profile in vitro (cells culturing and haemolysis studies) and in vivo, after intravenous injection to mice.

Nanocapsules prepared with the surfactant T80 presented the highest capacity to associate pDNA and siRNA. Indeed, results manifested the importance of carefully modulate the surface properties of the nanocarriers, since the association of nucleic acids was strongly influenced by type of surfactant, a fact attributed to the length of the PEG moieties (T80 < TPGS <PEG-St). With respect to the encapsulation of curcumin, it was efficiently incorporated into the oily core, achieving final concentrations higher than 1mg/mL.

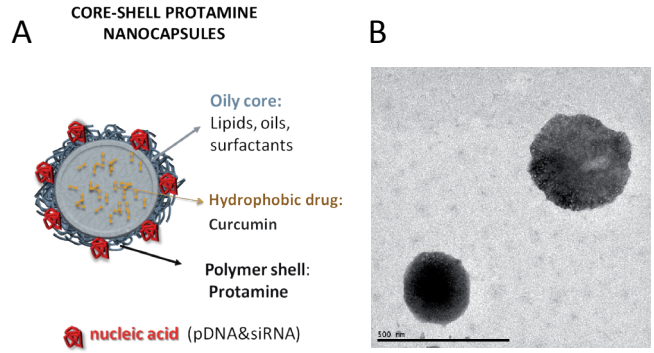

***Figure 1**. A) Schematic representation of the proposed nanocarrier-mediated combinatory therapies. B) TEM micrograph.*

The associated biomolecules were successfully delivered to cancer cells in their active forms, as confirmed with cell proliferation and protein expression assays. Experiments in cell culture show as protamine nanocapsules are efficiently internalized, an expected fact on view of the reported ability of protamine for translocation through cellular and nuclear membranes6,7. Our results indicate that protamine nanocapsules efficiently transfect cancer cells. For example, the expression of the reported protein GFP was observed in several cancer cells lines.

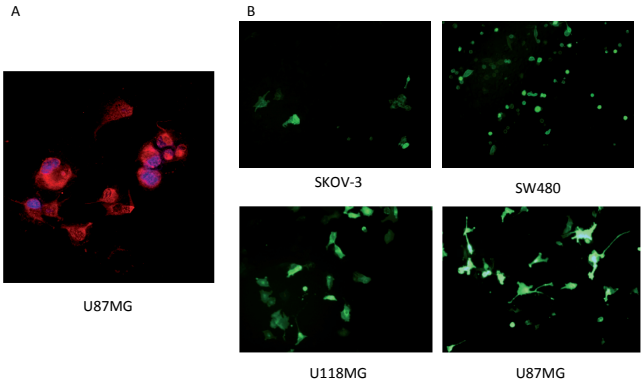

Figure 2. A) Cell uptake of TAMRA-labelled protamine nanocapsules, evaluated by CLSM. B) GFP expression evaluated by fluorescence microscopy in cancer cells transfected with pGFP-loaded Protamine nanocapsules.

CONCLUSIONS

In conclusion, we successfully developed and characterized a versatile nanocarrier with a high ability to associate hydrophobic antitumoral compounds and nucleic acids. This nanoparticulate prototype mediates a strong interaction with cancer cells, leading to the desired biological effect, therefore holding a great promise for the development of combined anticancer therapies. Subsequent studies will be carried out to explore their activity in tumour-bearing animal models.

REFERENCES

1. Jemal, A. et al. CA: a cancer journal for clinicians 61, 69-90 (2011).
2. Lane, D. Nature biotechnology 24, 163-164 (2006).
3. Zamboni, W.C. et al. Clinical cancer research 18, 3229-3241 (2012).
4. Gonzalo, T. et al. Journal of controlled release, In Press.
5. Torrecilla, D. et al. European journal of pharmaceutics and biopharmaceutics 83, 330-337 (2013).
6. Choi, Y.S. et al. Biomaterials 31, 1429-1443 (2010).
7. Hoare, M. et al. The journal of gene medicine 12, 207-218 (2010).
8. Liu, J. et al. Current pharmaceutical design 19, 1974-1993 (2013).
9. Kay, M.A. Nature reviews. Genetics 12, 316-328 (2011).

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BONE TARGETED MULTIVALENT PBLG-PGLU NANOPARTICLES FOR BONE CANCER APPLICATIONS

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ABSTRACT SUMMARY

Novel nanoparticles PBLG-PGlu with the dual functionality of bone targeting and cisplatin complexing have been designed. In vitro bone binding properties have been confirmed by fluorescence and electrochemical techniques using hydroxyapatite as an in vitro model. PBLG-PGlu nanoparticles can incorporate cisplatin and can release it in a surprisingly sustained manner, showing cytotoxicity towards three different prostate cancer cell lines that have the potential to metastasize to bone.

INTRODUCTION

In the recent years, much effort has been devoted to the development of site specific drug delivery systems. Site specific drug delivery would allow to attain therapeutic concentrations locally, and therefore reducing side effects and the dose administered. Nanomedicine plays a major role in tissue specific drug delivering. Several diseases affect the skeleton, such as osteoporosis, Paget's disease, osteoarthritis, osteomyelitis or bone cancers . The skeleton is the preferred site for breast and prostate cancers, which constitutes the second leading causes of cancer death in women and in men respectively [1].

Cisplatin is used for the treatment of different types of cancers. It is also used in combined chemotherapy of advanced prostate cancer. However, its use is limited due to several side effects, the most threatening ones being neurotoxicity, nephrotoxicity and ototoxicity [2, 3]. Here, we report the preparation of novel bone targeted PBLG-Pglu nanoparticles that are able to associate cisplatin via a coordinate bond and to release it in a very sustained manner so as to induce a cytotoxic effect on prostate cancer cell lines that constitute cellular models for bony metastases.

EXPERIMENTAL METHODS

PBLG-PGlu copolymer and the fluorescent derivate PBLG-dansyl were synthesized by ring opening polymerization (ROP) and characterized. PBLG-PGlu nanoparticles were prepared by a novel nanoprecipitation method. The in vitro affinity of PBLG-PGlu nanoparticles for bone was assayed using hydroxyapatite(HAP) as a model. Multifunctional nanoparticles PBLG-PGlu/PBLG-dansyl 0.1% Pluronic® F68 were incubated with hydroxyapatite in PBS at a pH of 7.4 during 24 hours. The suspensions were centrifuged at 5000 rpm for 5 minutes, washed and vortexed three times with poloxamer 1% in PBS 7.4 and supernatant fluorescence quantified by a Perkin Elmer Luminescence spectrometer LS 50B at room temperature (λ excitation =340 nm, λ emission = 472 nm).

In vitro release of cisplatin from the PBLG-PGlu nanoparticles was studied by the dialysis method in a 0.01 M phosphate buffer saline (PBS pH 7.4) with 0.138 M of NaCl and in distilled MilliQ water at 37 °C. Briefly, 0.950 mL of nanoparticles containing 0.1% of Pluronic® F 68 were introduced in 1 mL microdialyzers and dialyzed against 15 mL of medium using dialysis membranes with a molecular weight cut off of 3500 Da. The solution outside the microdialyzers was sampled at defined periods and replaced with fresh medium. The release of cisplatin was measured by AFF. Experiments were carried out by triplicate, from three different batches of nanoparticles . Release experiments with cisplatin solution in the conditions described above were also carried out.

The in vitro cytotoxic effect of free cisplatin, PBLG-PGlu nanoparticles and cisplatin-incorporating PBLG-PGlu nanoparticles was studied in three different prostate cancer cell lines, PC-3, DU-145 and LNCaP by two different methods: trypan blue exclusion assay and the MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium, inner salt) cell viability assay 48 and 72 hours after exposure. PC-3, DU-145; LNCaP were grown in RPMI 1640 (BE 12–702 F, Lonza) supplemented with 10%

(v/v) heat-inactivated fetal bovine serum (Lonza), penicillin (100 UI/mL), and streptomycin (100 µg/mL). Cells were maintained in a 95% humidified atmosphere of 5% CO2 at 37 °C.

RESULTS AND DISCUSSION

The preparation of novel PBLG-PGlu nanoparticles was achieved by a new nanoprecipitation method, modified from a method previously described [4]. PBLG-PGlu nanoparticles present a hydrodynamic diameter of about 50 nm, and a negative zeta potential, resulted from the ionised carboxylate groups, which enhance the stability of the nanoparticles. PBLG-PGlu nanoparticles have a core-shell structure, where the inner hydrophobic core is formed by the α-helix of PBLG and the outer hydrophilic shell by the PGlu chains. The multivalent interaction of PBLG-PGlu nanoparticles with hydroxyapatite (HAP) was studied by means of fluorescence. The in vitro HAP binding assay showed the property of glutamic acid oligopeptide to bind to HAP, as it has been previously described for acidic oligopeptides [5-8]. However, this is the first time such affinity is displayed by glutamic acid oligopeptide attached to polymeric nanoparticles.

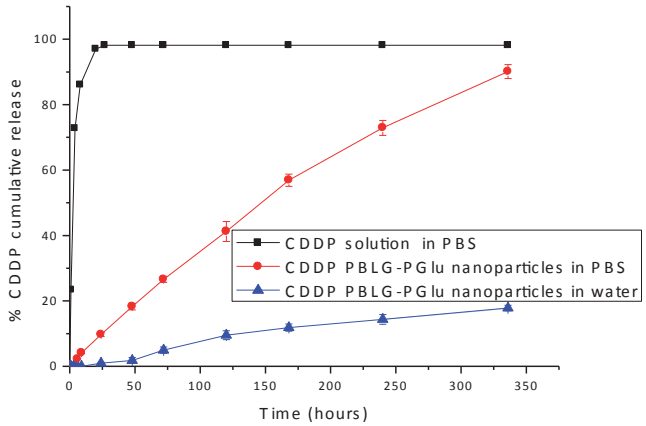

***Figure 1**: Degree of binding expressed in percentage of nanoparticles bound to HAP for PBLG-dansyl nanoparticles and for PBLG-PGlu/PBLG-dansyl 2/1 nanoparticles.*
The cisplatin-loaded nanoparticles released cisplatin in a sustained manner with absence of initial burst under physiological conditions, as shown by the linear gradual profile in the accumulative release.

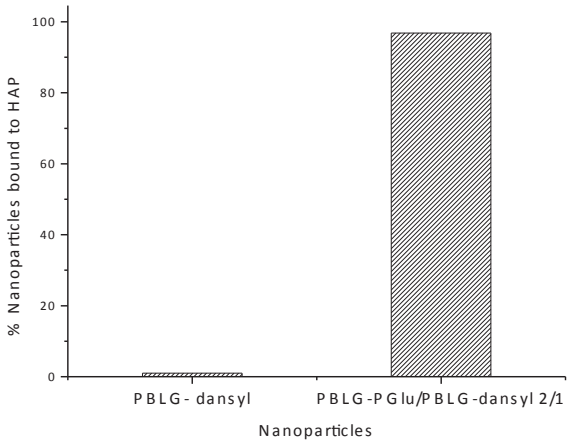

***Figure 2**: In vitro release of cisplatin from a cisplatin solution in PBS and from cisplatin-loaded nanoparticles in 0.01 M PBS (pH 7.4) containing 0.138 M of NaCl and in water.*

Cisplatin was completely released from the nanoparticles, more than 90 % being released after 14 days. The release rate of cisplatin from nanoparticles was slow; less than 10 % of cisplatin is released after 24 hours and 7 days are required so that 50 % of cisplatin is released. This is of great importance as it would mean than once nanoparticles administered intravenously into the organism, the percentage of released cisplatin before nanoparticles attain their target would be very low, minimising side effects and increasing the therapeutic efficacy at the target site. The absence of a burst effect suggest that cisplatin was mainly associated to nanoparticles through complexation to the carboxylate groups and not physically entrapped.

CONCLUSION

The preparation of novel bone targeted nanoparticles that are able to release cisplatin in a very sustained manner have been reported for the first time. PBLG-PGlu-cisplatin nanoparticles were shown to be toxic in three different prostate cancer cell lines that have the potential to metastasize to bone. The absence of toxicity of PBLG-PGlu nanoparticles not containing cisplatin have also been shown. Such nanoparticles have a great potential in bone cancer applications.

REFERENCES

1. Siegel, R.; Naishadham, D.; Jemal, A. Cancer statistics, 2012. CA: A Cancer Journal for Clinicians 62, (1), 10-29.
2. Harmers, F. P.; Gispen, W. H.; Neijt, J. P. Neurotoxic side-effects of cisplatin. Eur J Cancer 1991, 27, (3), 372-6.
3. Pinzani, V.; Bressolle, F.; Johanne Haug, I.; Galtier, M.; Blayac, J. P.; Balmès, P. Cisplatin-induced renal toxicity and toxicity-modulating strategies: a review. Cancer Chemotherapy and Pharmacology 1994, 35, (1), 1-9.
4. Barbosa, M. E. M.; Montembault, V.; Cammas-Marion, S.; Ponchel, G.; Fontaine, L. Synthesis and characterization of novel poly(γ-benzyl-L-glutamate) derivatives tailored for the preparation of nanoparticles of pharmaceutical interest. Polym. Int. 2007, 56, (3), 317-324.
5. Yokogawa, K.; Miya, K.; Sekido, T.; Higashi, Y.; Nomura, M.; Fujisawa, R.; Morito, K.; Masamune, Y.; Waki, Y.; Kasugai, S.; Miyamoto, K. Selective delivery of estradiol to bone by aspartic acid oligopeptide and its effects on ovariectomized mice. Endocrinology 2001, 142, (3), 1228-33.
6. Ishizaki, J.; Waki, Y.; Takahashi-Nishioka, T.; Yokogawa, K.; Miyamoto, K.-i. Selective drug delivery to bone using acidic oligopeptides. J. Bone Miner. Metab. 2009, 27, (1), 1-8.
7. Wang, D.; Miller, S. C.; Shlyakhtenko, L. S.; Portillo, A. M.; Liu, X. M.; Papangkorn, K.; Kopeckova, P.; Lyubchenko, Y.; Higuchi, W. I.; Kopecek, J. Osteotropic Peptide that differentiates functional domains of the skeleton. Bioconjug. Chem. 2007, 18, (5), 1375-8.
8. Ross, R. D.; Roeder, R. K. Binding affinity of surface functionalized gold nanoparticles to hydroxyapatite. Journal of Biomedical Materials Research Part A 99A, (1), 58-66.

NOVEL THERAPEUTIC NANOPARTICLES FOR IN VIVO DELIVERY OF LOW DOSE SIRNA IN LIVER CELLS AND FOR THE TREATMENT OF LIVER FIBROSIS ASSOCIATED NONALCOHOLIC STEATOHEPATITIS

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siRNA is poised to be the next therapeutic drug. Potent siRNA can silence any gene, including non-druggable genes, at picomolar concentration. As a result, there is now a great deal of interest in using siRNA in vivo to better understand diseases but also to be use as a therapeutic molecule. The goal of this study was to develop new in vivo delivery nanoparticles to deliver siRNA in liver cells by screening a library of lipid based formulations. Methods : An siRNA targeting FactorVII (FVII) was complexed with each formulation and injected intravenously at a 1mg/kg to 0.0125 mg/kg doses. Bid 3nd protein silencing was evaluated 48 hours after injection. The nanoparticles resulting in initial FVII knockdown were further optimized by design of experiment (mixture DOE) and evaluated for their ability to deliver other type of RNAi molecules. For NASH study, plasma and liver tissue were collected for determination of NASH features by histopathology, cell death assessment including TUNEL assay and immunoblotting using mitochondrial fractions. Hepatic stellate cell (HSC) activation was determined by real time PCR and liver fibrosis quantitated by image analysis of Sirius-red stained sections. Results: After a single intravenous injection of FVII siRNAs (0.05mg/kg) complexed with this new reagent (formulation 401), we observed more than 90% mRNA and protein level reduction in liver cells for more than 2 weeks and this silencing was dose dependent with an ED50 < 0.02mg/kg. We also observed a reduction of Choles

terol and LDL after silencing the APOB gene with this reagent. In addition, by mixing the siRNAs together, we were able to knockdown at least 4 genes at the same time after a single injection.

Finally we showed that we can use these formulations to deliver an siRNA against a key pro-apoptotic gene (Bid 3) for treatment of fibrosis in NASH mice model. C57BL/6 mice were placed on choline-deficient L-amino acid defined (CDAA) diet for NASH mice model. After 19 weeks of CDAA diet, mice with severe fibrotic-NASH, were injected with Bid 3 siRNA/Formulation 401, weekly for three weeks at1.5 mg/kg (week1) and 0.15 mg/kg (week 2 and 3). At the end of the treatment, Bid 3 mRNA was suppressed to 50% ($p<0.003$) and Bid 3 protein was reduced to 10% ($p<0.002$). In Mice treated with Bid 3 siRNA, liver fibrosis was improved as assessed by Sirius red quantitation and mRNA expression of fibrosis genes such as TIMP-1 ($p<0.03$) or CTGF ($p<0.05$). These changes were associated with marked reduction on TUNEL-positive cells and reduction on mitochondrial BAX.

Conclusion: We have identified novel therapeutic lipid nanoparticles for the delivery of siRNA with an ED50 of less than 0.02mg/kg. This study also demonstrates that these formulations with a pro-apoptotic gene (Bid 3) siRNA can be used to improve liver fibrosis associated with experimental NASH Gene. These findings are consistent with evidence that apoptosis triggers HSC activation and liver fibrosis and suggest that Bid 3 inhibition may be useful as an antifibrotic NASH therapy.

TOOLS FOR STUDYING CELLULAR UPTAKE AND TRAFFICKING OF ARCHAEOSOMES: A FIRST APPROACH

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INTRODUCTION

Nanotechnology has shown new and promising strategies for the prevention, diagnosis and treatment of human diseases. One of the major challenges is understanding the interaction that occurs between nano-objects and living cells and in which way they are internalized. The archaeolipids (lipids extracted from Archaebacteria) are non saponificable molecules that form self sealed mono or bilayer vesicles named archaeosomes (ARC). Unlike liposomes (L) made from conventional glycerophospholipids, the ARC possess a higher structural resistance to physicochemical and enzymatic degradation and surface hydrophobicity. [1] In this research, techniques for studying the endocytic uptake pathway(s) and the intracellular trafficking were described. In particular, inhibitors of clathrin and caveolae mediated endocytosis, macropinocytosis and phagocytosis were used to determine the mechanism(s) of ARC uptake by J774 macrophage cell line.

MATERIALS AND METHODS

Materials: Hydrogenated phosphatidylcholine from soybean (HSPC) was obtained from Northern Lipids (Vancouver, Canada), Modified Eagle's medium with non-essential aminoacids (MEM-NEAA), was purchased from Gibco (Buenos Aires, Argentina). Heat-inactivated fetal bovine serum (FBS), glutamine (200 mM), antibiotic-antimycotic solution (10,000 units/ml penicillin G sodium, 10,000 mg/ml streptomycin sulphate and 25 mg/ml amphotericin B), pyruvate, and trypsin/ ethylenediamine tetra-acetic acid were purchased from PAA Laboratories GmbH (Pasching, Austria). Phosphatidylethanolamine-C14-Lissamine™ rhodamine B (RhPE), 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), Chloroquine diphosphate salt (CQ), cytochalasin D (Cyt D) from Zygosporium mansonii, genistein (Genis), 5-(N-Ethyl-N-isopropyl) amiloride (Amil), wortmannin (Wort) and 3-(4,5-dimethythiazole-2-yl)-2,5- diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). Nocodazole (Noc) and methyl-β-cyclodextrin (MβCD) were acquired from Fluka (Buenos Aires, Argentina) and chlorpromazine (CPZ) was a gift from Laboratorios Ceballos, Argentina. Transferrin-Alexa Fluor 633 (TFR); BODIPY-LacCer (LacCer) and Dextran-Alexa Fluor 647(DEX) were purchased from Life Technologies, (Buenos Aires, Argentina).

Culture growth and characterization: Halorubrum tebenquichense archaeabacteria, were isolated from soil samples from Salina Chica, Península de Valdés, Chubut, Argentina, as described by Gonzalez et al. [1]

ARC and L preparation and physicochemical characterization: ARC were prepared according to the thin film hydration method. TPL (20 mg) were mixed in chloroform:methanol (1:1; v/v) and dried with a rotary evaporator at 40°C in a round bottom flask until organic solvent elimination. The thin lipid film was flushed with N2 and hydrated at 40°C with 1 ml of 10 mM Tris-HCl buffer plus 0.9% w/v NaCl, pH 7.4 (Tris/NaCl buffer). The resulting suspensions were sonicated (1 h) in a bath sonicator at 80 W. Multilamellar archaeosomes (ARC M), so formed, were forced through polycarbonate membrane of a 100 nm defined pore size using a Miniextruder ® (Avanti Polar Lipids, Alabaster, Alabama, U.S.A.). L made of HSPC were prepared in the same way. Phospholipids were quantified by Bötcher method [2]. The Z-average size and zeta potential of ARC and L were determined using a Zetasizer NanoZS instrument (Malvern) at 25 ◦C in Tris/NaCl buffer. For flow citometry studies, Rh-PE (lipids/Rh-PE ratio 200:1 w/w) was added to prepare the films and ARC-RhPE or L-RhPE were obtained. For fluorescence microscopy studies, HPTS (35 mM) was added to the buffer and then the free fraction HPTS was separated with molecular exclusion column method (Sephadex G-50), and ARC- HPTS or L-HPTS were obtained.

Kinetics of cellular uptake: Cell uptake was further investigated using flow cell cytometry (Becton Dickinson FACSCalibur, San Jose, CA). J774 cells were seeded at a density of 2 × 105 cells/well, in six-well plates and allowed to attach 24 h. Subsequently, the medium was replaced with fresh supplemented MEM-NEAA without FBS containing ARC-RhPE (0,5 mM, final concentrations) and the cells were incubated for 0,5; 1; 3 and 5 hours at 37°C in 5% CO2. After each incubation period, the medium was removed, the cells were washed three times with phosphate-buffered saline (PBS, pH 7.4), and harvested by trypsinization. After trypsin inactivation and washing with PBS, the cells were fixed with 1% formaldehyde in PBS and stored at 4ºC until analysis. A total of 10,000 cells were analyzed by flow cytometry. In all cases fluorescence was quantified by the number of cells that emitted above the level of autofluorescence in control cells. Cell debris was excluded based on the forward-and side-scatter characteristics of the cell populations Data were analyzed using WinMDI 2.9 software. L-RhPE were used as control.

Toxicity of endocytosis inhibitor treatments: To evaluate the effect of inhibitor treatments on ARC uptake by cells, the non-toxic concentration of inhibitors was chosen. For this, different inhibitor doses were pre-incubated during 1 h and then ARC (0,5 mM, final concentration) were added and incubation was extended 3 h. Citoxicity was evaluated with MTT assay and LDH leakage. Non-toxic concentrations are shown in Table 1.

Uptake in presence of endocytosis inhibitors: J774 were seeded in six-well plates as stated previously. The medium was then replaced with fresh supplemented MEM-NEAA without FBS containing endocytic inhibitors at a nontoxic concentration (Table 1). The cells were incubated for 1 hour at 37°C, ARC-RhPE (0,5 mM, final concentration) were added and the cells were incubated for another 3 hours. The medium was then removed, the cells were washed twice with PBS and harvested by trypsinization, then processed for flow cytometry as stated before. The percentage of cellular internalization was calculated on the basis of the geometric mean (Gm), as follows: (Gmobs/Gmcontrol) × 100, where Gmobs is the Gm of cells incubated with inhibitors and ARC-RhPE or L-RhPE and Gmcontrol is the Gm of cells incubated with ARC-RhPE or L-RhPE, respectively.

Inhibitor	Concentration	Affected mechanism
CPZ	2 µg/ml	Clathrin mediated endocytosis (CME)
Genis	75 µM	Caveolae mediated endocytosis (CvME)
MβCD	15 µM	Endocytosis
CQ	100 µM	Lysosome pH
Noc	30 µM	Lysosome-vesicle fusion
Cyt D	2 µM	Fagocytosis/Macropinocytosis
Amil	50 µM	Macropinocytosis
Wort	0,001 nM	Macropinocytosis

***Table 1**. Inhibitor concentrations and affected internalization mechanisms*

Endocytosis markers:
To evaluate the selected concentration of inhibitors allowing sufficient inhibition of specific endocytic pathways, endocytosis markers were analyzed. Positive controls for CME, CvME and phagocytosis/macropinocytosis were TFR; LacCer and DEX, respectively. Cells were incubated 1 h with inhibitors prior to the addition of the markers, TFR (15 min; 25 µg/ml); LacCer (15 min; 0.81 µM) and DEX (2h; 250 µg/ml). Each marker was removed with a special procedure and harvested by trypsinization, then processed for flow cytometry as stated before. Confocal microscopy: For confocal microscopy cells were cultured on glass coverslips. After treatment with ARC-HPTS or L-HPTS during 1h, 3h and 5h and washes, the cells were fixed in 4% paraformaldehyde and mounted onto slides in mounting medium. Samples were observed using the FluoView FV300 Microscope (Olympus)

RESULTS

Physicochemical characterization: Particle size (ZAve and PdI) and Z potential of ARC and L are shown in Table 2.

Nano-object	ARC	L
ZAve (nm)	117±4	111±4
PdI	0.191±0.009	0.078±0.018
ZPot (mV)	37.9±0.8	4.2±0.5

***Table 2.** Particle size and Z Potential of nano-objects*

Kinetics of cellular uptake: ARC were internalized by J774 with an exponential kinetics. Curvilinear cellular uptake kinetics, due to saturation of membrane binding sites, would be characteristic of adsorptive endocytosis [3]. In contrast, L uptake was not detectable by this technique (Fig. 1).

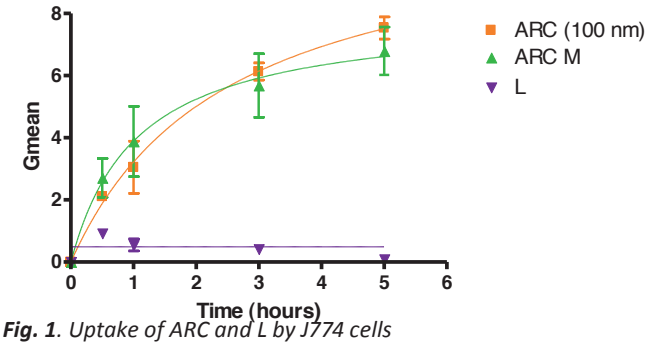

***Fig. 1**. Uptake of ARC and L by J774 cells*

On the basis of the kinetic data, we defined 3 hours as the incubation time, ensuring sufficient uptake to allow further quantification.
Uptake in presence of endocytosis inhibitors: As shown in Fig. 2, both CZ and Genis treatments led to a significant reduction in ARC uptake implying a possible role for CME and CvME. MβCD treatment was used here as a general endocytosis inhibitor. Cyt D treatment resulted in a significant drop in the level of ARC uptake (Fig. 2). This indicates that phagocytosis or macropinocytosis were involved in the uptake of ARC by J774 cells. Amil did not result in any significant decrease in the level of ARC uptake. Wort is very toxic for J774 cells and non-toxic concentration probably was not sufficient for inhibition. ARC uptake was significantly reduced by CQ treatment (Fig. 2), so the internalization was affected through the altered intracellular trafficking due to the pH increase in endosomes and lysosomes.

Noc treatment had a great impact on ARC uptake (Fig. 2) indicating microtubule involvement in the intracellular trafficking of these nano-objects that would affect their internalization.
In short, ARC can employ multiple pathways for cellular entry, by means CME, CvME and phagocytosis in J774 cells (Fig. 2). These mechanisms of endocytosis were evaluated with TFR, LacCer and DEX, respectively. Results were summarized in Table 3.

	CZ	Genis	MßCD	CQ	Noc	Cit D	Amil	Wort
ARC	(p<0,01)	(p<0,01)	(p<0,05)	(p<0,01)	(p<0,01)	(p<0,01)	NO	NO
TFR	(p<0,05)	-	NO	(p<0,01)	NO	-	-	-
LacCer	-	(p<0,05)	NO	-	NO	(p<0,01)	-	-
DEX	-	-	-	-	-	(p<0,01)	(p<0,01)	NO

Table 3. ARC and markers uptake in presence of inhibitors ↓: decrease; ↑ increase; NO: Not change

Inhibitor Effect on ARC Uptake

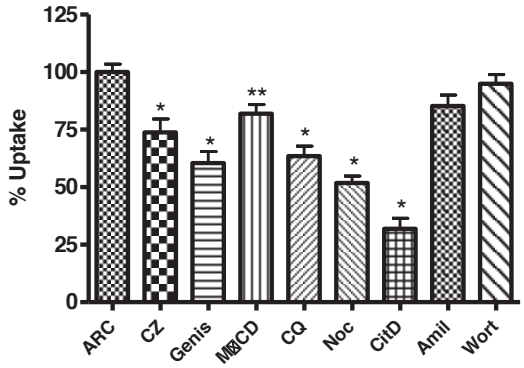

***Fig. 2.** Uptake of ARC and L by J774 cells*
Notes: Bars represent means ± standard deviations (n = 6)
**p<0,01 **p<0,05 techniques.*

Confocal microscopy: ARC uptake was increased with incubation time, the longer the time the higher fluorescence intensity in J774 cells (Fig. 3). Whereas L were not taken by these cells. These findings were coincidental with flow cytometry results (Fig. 1).

CONCLUSION

Our results have proven that ARC uptake by J774 was mediated by clathrin, caveolae and phagocytosis. The techniques used in this study are tools that shed light on the complex mechanisms of cellular uptake of nano-objects in a modern and simple way. Further studies are required to understand the uptake of these nano-objects, such as colocalization techniques.
As inhibitory effects are highly cell line dependent, it is important to apply these techniques to other cell lines [4]. This simple experimental setting has shown two remarkable properties of the interaction of ARC with phagocytes. By labeling the inner compartment with the hydrophilic and fluorescent dye pyranine, we firstly determined that ARC are taken up by phagocytic cells at a much higher rate than conventional liposomes made of phosphatidylcholine. In the second place, we found that ARC can enter the phagocytic cells by pynocitic pathways apart from phagocytosis. Such unexpected findings suggest, in other words, that ARC and not conventional liposomes could provide with a fast and massive delivery of hydrophilic molecules to phagocytic cells. This conclusion is of paramount importance in the field of therapeutic delivery of drugs to macrophages, key cells involved in vital processes such as triggering and resolution of inflammation, tissue remodeling and wound healing.

REFERENCES

[1] Gonzalez, R. O.; Higa, L. H.; Cutrullis, R. A.; Bilen, M.; Morelli, I.; Roncaglia, D. I.; Corral, R. S.; Morilla, M. J.; Petray, P. B; Romero, E. L. BMC Biotech. 2009, 9, 1-12.
[2] Bötcher, C. J. F.; van Gent, C. M.; Pries, C. Anal. Chim. Acta, 1961, 24, 203-204.
[3] Huang M., Ma Z., Khor E., Lim L.Y. Pharm Res. 2002; 19 (10):1488–1494
[4] Vercauteren, D.; Vandenbroucke, R.E.; Arwyn T Jones, A. T.;

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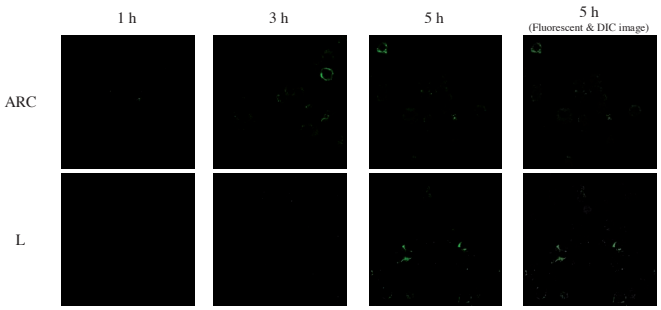

Fig. 3. *Confocal Microscopy. ARC and L uptake by J774. Clusters of fluorescent vesicles were observed outside the cells incubated with L.*

A METABOLISM-BASED METHOD FOR CIRCULATING TUMOR CELLS DETECTION

FABIO DEL BEN, M. Turetta, D. Cesselli, G. Scoles

Counting Circulating Tumor Cells (CTCs) have been recently widely accepted as a diagnostic tool with prognostic values and for therapy monitoring. Most of techniques today use antibody labeling or physical features to detect CTCs in blood. We focused on metabolism as the discriminating criterium between cancer and normal cells. Using fluorescence microscopy and Fluorescence-activated cell sorter (FACS) analysis we analyzed mixed suspensions of cancer cells and white blood cells and we saw a significant difference in glucose uptake by using a fluorescent-conjugated glucose (2-NBDG).

ALGINATE-COATED CHITOSAN NANOGEL CAPACITY TO MODULATE THE EFFECT OF TLR LIGANDS ON BLOOD DENDRITIC CELLS

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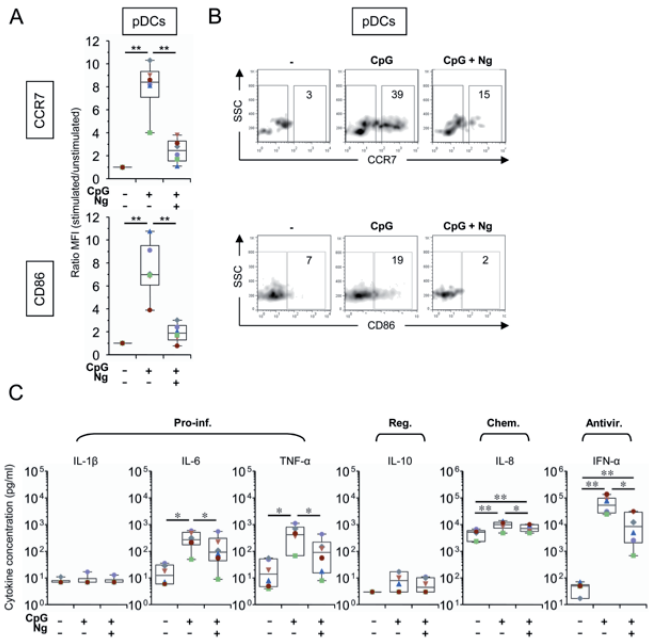
While biodegradable nanoparticles have been assessed for vaccine delivery, there is little information on their modulation of immune responses, a particularly important characteristic considering the value of adjuvants in a vaccine formulation. Using alginate-coated chitosan-nanogels (Ng), we analysed the immunomodulatory role played by individual Toll-like receptor (TLR) ligands – Pam3Cys-SK4 (P3C) and both class-A and class B CpG-oligodeoxynucleotides (CpG) – when admixed or incorporated into the Ng. These were assessed on both porcine and human blood dendritic cell (DC) subpopulations. Admixing Ng with P3C did not appear to impair the immunomodulatory activities or maturation of the DCs, although IL-1β was enhanced with the admixed formulation. Using labelled P3C, it appeared difficult to associate this with the Ng, other than through admixing. In contrast, the CpG could be both admixed with and incorporated into the Ng. With the admixed formulation, the Ng impaired CpG-induced IFN-a, IL-6 and TNFa, as well as the up-regulation of CD86 and CCR7, and cell migration, indicative of DC maturation (Figure 1).

Figure 1. *Nanogel influence on TLR2 triggering of human cDCs. Cells from six individuals were stimulated for 16h with 10µg/ml of Pam-3Cys-SK4 in the absence or presence of 10% (final culture volume) Ng. A. Ng influence on CCR7 and CD86 upregulation on cDCs – mean fluorescence intensity (MFI), separate symbols per individual – following Pam3Cys-SK4 (P3C) stimulation. * p<0.05. B. Representative density plot of gated blood cDC expression of CCR7 and CD86, stimulated as in A. C. Cytokine profiles produced by blood DCs stimulated as in A. The cytokines are grouped as "pro-inflammatory" (Pro-inf.), "regulatory" (Reg.), "chemotactic" (Chem.), "antiviral" (Antivir.). From Nanomedicine: NBM 2013; In press*



Then, we formulated Ng by encapsulating class-A CpG-ODN-2216 and class-B CpG-ODN-2006 (Ng-[2216] and Ng-[2006], respectively). The influence of the Ng was divergent dependent on the class of CpG employed. Compared to the CpG alone, uptake was inhibited by Ng-[2216] – seen with plasmacytoid DCs (pDCs), conventional DCs (cDCs) and B cells. In contrast, Ng-[2006] uptake was increased over the CpG alone – seen with pDCs, cDCs and monocytes. For Ng-[2216], the inhibition of delivery was associated with a strong inhibition of IFN-α production.
Altogether, our results demonstrate that formulation of biodegradable vaccine delivery vehicle with adjuvant can have opposing effects, which are critically influenced by the choice of adjuvant. Moreover, delivery of adjuvants restricted to one subpopulation of DCs can be expanded to different subpopulations when Ng-based delivery is employed. This would have considerable influence on the innate immune profile induced, and therefore on any subsequent adaptive immune defence development.

ORAL ADMINISTRATION OF MESOPOROUS SILICA NANOPARTICLES FOR TARGETING INTESTINAL EPITHELIAL CELLS

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Mesoporous materials have been put forward as suitable drug delivery carriers for poorly soluble drugs via oral administration. The major obstacles for oral delivery of many drugs are poor stability in the gastric environment, poor aqueous solubility due to insufficient dissolution throughout the GI tract as well as poor penetration through mucosal barriers resulting in poor oral bioavailability. [i] Increased solubility of the drugs can consequently be attained by incorporating them into such a porous matrix. Moreover, the inorganic matrix may protect sensitive compounds from the harsh conditions in the GI tract. Both these advantages lead to increased bioavailability of the active compound. Optimally designed nanoparticle-based targeting for oral treatment of disease of the lower GI tract such as colon cancer, ulcerative colitis and Crohn's disease, could, thus, overcome the challenges caused by digestion and low intestinal pH. As we have previously shown that oral administration

of drug-loaded mesoporous silica nanoparticles (MSNs) exerted controlled activity in intestinal stem cells,[ii] we wanted to investigate whether the MSNs could be delivered to and internalized by intestinal cancer cells in vitro and in vivo, to enable more effective future lower GI tract cancer treatment.

Mesoporous hybrid silica nanoparticles were prepared by surface hyperbranching polymerization of poly(ethylene imine), PEI, to introduce positive charge to the MSNs [,] and the performance of these were compared to that of MSNs with neutral net charge. To potentially enhance the mucosal penetration of the MSNs, as the mucosal barrier otherwise could constitute a significant obstacle to mucosal delivery,[v] some MSNs were further modified by introducing a poly(ethylene glycol), PEG, layer. PEG coatings have been suggested to improve the penetrability of nanoparticles through mucosal barriers.[vi] Due to high abundance of folate receptors in many cancer cells, folic acid was used as the targeting ligand for specific delivery.[vii]

Improving the oral bioavailability of drug requires reduction in direct elimination of the drug-loaded nanoparticles in the GI tract, and thus, strong interactions between the nanoparticles and the GI tract mucus/epithelial cells are essential to extend the residence time and hence the absorption of the drug-loaded nanoparticles. Caco-2 and HT-29 cells are both derived from human colon carcinomas, representing the most abundant absorptive intestinal cells. The cellular uptake of differently surface-modified MSNs in terms of different combinations of PEI, PEG, and FA, was evaluated by using Caco-2 and HT-29 cells as an in vitro model with the aim to apply the nanoparticles for oral therapy. Caco-2 is an epithelial cell line derived from human colon adenocarcinoma that differentiate in culture, and exhibit structure and functional differentiation patterns characteristic of mature enterocyte.[viii]. HT-29 cells form the mucus-secreting goblet cells upon in vitro culture, which represent the second major type of intestinal cells.[x,ix] Emphasis was laid on possible effects induced by the charge and surface functionalization of the particles on cell viability and cellular uptake of the MSNs. Surface-functionalization induced differences was clearly observed, the effect of which varied between the cell lines. Caco-2 cell monolayers have been suggested to possess attributes that make it a suitable in vitro model system for the investigation of transport across the small intestinal epithelium to predict absorption of orally administered drugs. [,] Permeability of drug-loaded MSNs was accessed across Caco-2 cell monolayers.

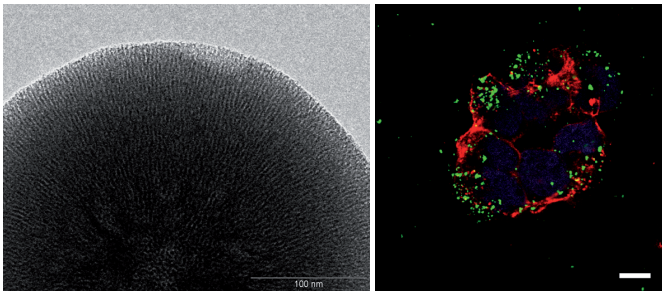

Fig. *a) TEM image of the produced mesoporous silica particles. b) Confocal microscopy image of the mesoporous silica particles (green) incubated with Caco-2 cells (Scale bar 10µm).*

The aim of the present work was to investigate the effect of MSNs as an oral drug delivery system and to study whether orally delivered MSNs were taken up by intestinal cells in vivo. After in vitro screening of cell viability, the cellular uptake in cancerous (Caco-2 and HT-29) and normal (HEK293) cell lines and intracellular distribution patterns were investigated by fluorescence-assisted cell sorting (FACS) and confocal laser scanning microscopy (CLSM). For in vivo studies, animals were fed with a few selected MSNs by oral gavage. The assessment and quantification of the biodistribution of delivered MSNs in the cells of the GI tract was determined by CLSM, ICP and fluorimetric analysis, whereby surface-modification induced differences could also be discerned.

The results showed that the MSNs are not cytotoxic even at high concentrations and were internalized by different cancer cells to different extents depending on the surface functionalization, and the particles show specific uptake in colon cancer cell lines. Neither pure PEG alone nor a positive particle surface charge had a significant impact on cellular uptake in Caco2 cells, whereas the combination of the two provided for efficient particle uptake both in vitro and in vivo. In addition, we have shown that selected MSNs also improve the drug permeability across differentiated Caco-2 cell monolayers. Confocal microscopy shows that the silica nanoparticles were localized in epithelial cells of the colon and small intestine of mice, thus making them suitable as drug delivery carriers to colon cancer cells. This work reveals the significant potential for the use of MSNs as oral drug delivery systems which provides safe cell interactions and efficient delivery of drugs through epithelial barriers, provided the right surface functionalization design is employed.

DEVELOPMENT OF A MULTIFUNCTIONAL LIPID CARRIER FOR TARGETING MMP-12 IN ATHEROSCLEROTIC VULNERABLE PLAQUES

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Atherosclerosis is a chronic systemic inflammatory disease in which the arterial wall thickens as a result of the accumulation of fatty material within the vessel wall. Although considerable advances have been made in the treatment of this pathology, atherosclerosis remains the major cause of morbidity and mortality in the field of cardiovascular disease. The most severe complications arise from ruptured atherosclerotic plaques in coronary arteries, which account for the majority of fatal acute myocardial infarction, and plaque rupture in carotid arteries that causes ischaemic stroke. Within atherosclerotic lesions, Matrix MetalloProtease 12 (MMP-12) also called macrophage metalloelastase is overexpressed and is directly involved in the progression and destabilization of atherosclerotic plaques (Johnson et al. 2005, Liang et al. 2006, Halpert et al. 1996, Jormsjo et al. 2000 and Morgan et al. 2004). Moreover, in a mice model of atherosclerosis with established plaques, the RXP470, a highly potent and selective matrix MMP-12 inhibitor (Devel et al. 2006) was recently shown to fully block further plaque growth and prevent plaque rupture (Johnson et al. 2011). In this context, MMP-12 represents a valuable target 1) to specifically image atherosclerotic vulnerable plaques, an unresolved issue in human atherosclerosis management, 2) to deliver molecules to treat atherosclerosis but also, as discussed below, a therapeutic target on its own.

Although still in its infancy, nano-medicine is likely to have a substantial role in the management of atherosclerosis (Lobatto et al. 2011). More particularly, multifunctional nanoparticles combining imaging, specific site-targeting and drug delivery properties constitute promising tools able to report on the early onset of the disease in each individual patient and deliver a suitable therapeutic agent at the pathological site. Here, we report a liposome-based platform providing multifunctional submicronic systems fluorescently labelled, stabilized by poly(ethylene glycol) (PEG) chains and covalently surface-modified by the RXP470 inhibitor (Figure 1).

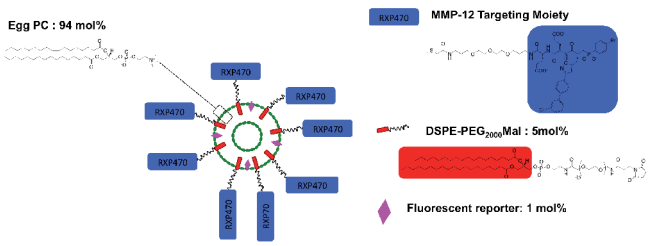

***Figure 1**: Multifunctional nanoparticle combining a MMP-12 selective inhibitor for site-specific targeting and a fluorescent reporter for imaging.*

Fabrication of the liposome and bio conjugation strategy allowing the attachment of the MMP-12 selective ligand, as well as the physical characterization of the final nano system will be discussed. The conserved abilities of the MMP-12 ligand, once present at particle surface, to still interact with its target has been established through in vitro biochemical tests. To further improve these systems, multifunctional particles for in vivo tracking will be developed. Especially, conjugation of fluorescently labeled and magnetic-fluid-loaded liposomes (MFLs) with the MMP-12 selective ligand can be undertaken. Indeed, such liposomes have widely proved to be efficient tools for coupling in vivo imaging and therapy mainly due to their super paramagnetic properties that make them excellent contrast agents for magnetic resonance imaging and magnetic targeting (Martina et al. 2005 and Fortin et al. Radiology 2006). This should allow both MMP-12 in vivo tracking by specific imaging and targeting of atherosclerotic vulnerable plaques.

REFERENCES

• Devel, L., Rogakos, V., David, A., Makaritis, A., Beau, F., Cuniasse, P., Yiotakis, A., and Dive, V. (2006) Development of selective inhibitors and substrate of matrix metalloproteinase-12. The Journal of biological chemistry 281, 11152-11160.

• Fortin-Ripoche JP., Martina MS, Gazeau F, Ménager C, Wilhelm C., Bacri JC., Lesieur S., Clément O.(2006)
Magnetic targeting of magnetoliposomes to solid tumors with MR imaging monitoring in mice: feasibility. Radiology. 239 (2), 415-24.

• Halpert I., Sires U.I., Roby J.D., Potter-Perigo S., Wight T.N., Shapiro S.D., Welgus H.G., Wickline S.A., and Parks W.C., (1996) Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proceedings of the National Academy of Sciences of the United States of America, 93 (18), 9748-53.

• Johnson, J.L., Devel, L., Czarny, B., George, S. J., Jackson, C.L., Rogakos, V., Beau, F., Yiotakis, A., Newby, A.C., and Dive, V. (2011) A selective matrix metalloproteinase-12 inhibitor retards atherosclerotic plaque development in apolipoprotein E-knockout mice. Arterioscler Thromb Vasc Biol 31, 528-535.

• Johnson J.L., George S.J., Newby A.C., and Jackson C.L., (2005) Divergent effects of matrix metalloproteinase-3, metalloproteinase-7, metalloproteinase-9, and metalloproteinase-12 on atherosclerotic plaque stability in mouse brachiocephalic arteries. Proceedings of the National Academy of Sciences of the United States of America, 102 (43), 15575-15580.

• Jormsjo S., Ye S., Moritz J., Walter D.H., Dimmeler S., Zeiher A.M., Henney A., Hamsten A., and Eriksson P., (2000) Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. Circulation Research, 86 (9), 998-1003.

• Lobatto M.E., Fuster V., Fayad Z.A., Mulder W.J. (2011) Perspectives and opportunities for nanomedicine in the management of atherosclerosis. Nat Rev Drug Discov. 21;10 (11):835-52.

• Liang J.Y., Liu E.Q., Yu Y., Kitajima S., Koike T., Jin Y.J., Morimoto M., Hatakeyama K., Asada Y., Watanabe T., Sasaguri Y., Watanabe S., and Fan J.L., (2006) Macrophage metalloelastase accelerates the progression of atherosclerosis in transgenic rabbits. Circulation, 113 (16), 1993-2001.

• Martina M.S., Fortin J.P., Ménager C., Clément O., Barratt G., Gazeau F., Cabuil V., Lesieur S. (2005) Generation of superparamagnetic liposomes revealed as highly efficient MRI contrast agents for in vivo imaging. J. Am. Chem. Soc. 127, 10676-10685.

• Morgan A.R., Rerkasem K., Gallagher P.J., Zhang B., Morris G.E., Calder P.C., Grimble R.F., Eriksson P., McPheat W.L., Shearman C.P., and Ye S., (2004) Differences in matrix metalloproteinase-1 and matrix metalloproteinase-12 transcript levels among carotid atherosclerotic plaques with different histopathological characteristics. Stroke, 35 (6), 1310-5.

NANOSTRUCTURE OF HUMAN TEETH AFFECTED BY ENAMEL CARIES

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INTRODUCTION

Enamel caries is a bacterial infection that progressively destroys the mineral components in tooth enamel [1]. The acidic dissolution of the hard tissue results in complex morphological changes within the affected region. Understanding the caries-induced changes on all length scales down to the atomic level is of uttermost importance for the development of regenerative caries treatments. Enamel micro- and nanostructures have been studied in numerous ways, which include histology, light and electron microscopy, micro- and nano-indentation and X-ray analyses. Based on differences in birefringence, the early carious lesion was divided into four zones [2]. Generally, these techniques grant high-resolution information on restricted areas, rendering data acquisition over large specimens or even volumes time consuming, or vice versa, low resolution over information over macroscopic specimens.

Small-angle X-ray scattering (SAXS) combined with real space scanning [3, 4] allows to extract information about the abundance, orientation and anisotropy of the nanometer-sized components of enamel, thus allowing for the mapping of entire lesions at the level of crystallites and pores in-between. Scanning SAXS has been successfully applied to human hard and soft tissues [5, 6], including dentin and dentinal caries [7], revealing the presence of the unaffected collagen network within moderate lesions.

Experimental data and results

200 µm-thin tooth slices were cut from two teeth presenting representative cases of early/moderate enamel caries, using a diamond band saw (Exakt Apparatebau GmbH, Norderstedt, Germany). One slice was obtained in buccal-lingual direction (specimen H), while the other was cut in transverse direction (specimen V).

Prior to cutting, micro-computed tomography of the teeth was performed on a Skyscan 1174™ tabletop scanner (Bruker CT, Kontich, Belgium) with 50 kV acceleration voltage and 800 µA beam current. 900 equiangular projections with a pixel size of 35.6 µm were acquired over 360°. Tomography data were reconstructed using the software Nrecon (Bruker CT, Kontich, Belgium), based on a modified Feldkamp algorithm.

SAXS data were acquired at the cSAXS beamline (Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland). The tooth slices were scanned through the 18.6 keV photon beam, focussed to 20 µm ´ 8 µm spot size at the specimen position, with raster-scan step sized of 50 µm (specimen V) and 20 µm (specimen H) in horizontal and vertical directions, respectively. Prior to SAXS data acquisition, X-ray transmission of the specimens was recorded at the same raster points. To minimize air scattering, an evacuated flight tube was placed between specimen and detector. The scattering data were evaluated using self-written MATLABÒ (2011a, The MathWorks, Natick, USA) routines (for more details please refer to [3]).

Figure 1, Panel A shows 2D map of the total scattering intensity of specimen H at scattering angles corresponding to the range between 100 and 110 nm. The lesion is clearly visible as bright feature containing alternating bands presenting reduced intensity. In Panel B the full-width-at-half-maximum (FWHM) of the azimuthal intensity distribution of the scattering signal, related to the anisotropy of the specimen nano-components, is mapped. The borders of the lesion, as determined from Panel A, are indicated using the white line. Based on the anisotropy of the scattering signal, the lesion cannot be readily distinguished from unaffected enamel. Panel C shows the main orientation of the scattering signal according to the colour wheel. Again, the caries-affected area cannot be distinguished from unaffected enamel. These results indicate that, despite caries-induced de-mineralization leading to increased porosity, an anisotropic scaffold is still present in the lesion, which can be utilized for hard tissue repair.

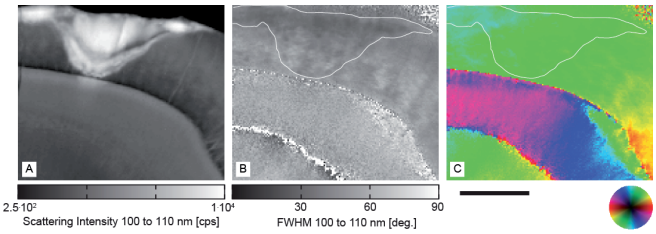

Figure 1. *A: Total scattered intensity in the angular range corresponding to the range 100 to 110 nm. B: Full-width-at-half-maximum (FWHM) of the azimuthal intensity distribution in the range corresponding to the range 100 to 110 nm. C: Orientation of the scattering signal in the angular range corresponding to the range 100 to 110 nm, according to the colour wheel. The length-bar corresponds to 1 mm.*

REFERENCES

[1] Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004;83:Spec No C:C35-C8.
[2] Orams HJ, Phakey PP, Rachinger WA, Zybert JJ. Ultrastructural changes in the translucent and dark zones of early enamel caries. J Oral Pathol 1980;9:54-61.
[3] Bunk O, Bech M, Jensen TH, Feidenhans'l R, Binderup T, Menzel A, et al. Multimodal x-ray scatter imaging. New J Phys 2009;11:123016.
[4] Fratzl P, Jakob HF, Rinnerthaler S, Roschger P, Klaushofer K. Position-resolved small-angle X-ray scattering of complex biological materials. J Appl Crystallogr 1997;30:765-9.
[5] Märten A, Fratzl P, Paris O, Zaslansky P. On the mineral in collagen of human crown dentine. Biomaterials 2010;31:5479-90.
[6] Müller B, Deyhle H, Bradley D, Farquharson M, Schulz G, Müller-Gerbl M, Bunk O. Scanning x-ray scattering: Evaluating the nanostructure of human tissues. Eur J Nanomed 2010;3:30-3.
[7] Deyhle H, Bunk O, Müller B. Nanostructure of healthy and caries-affected human teeth. Nanomed-Nanotechnol 2011;7:694-701.

RODENT CARPA: THE DEVELOPMENT OF A NEW MODEL FOR MEASURING COMPLEMENT ACTIVATION-RELATED PSEUDOALLERGY IN RATS & MICE

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INTRODUCTION

Drug-induced hypersensitivity reactions can limit the use of drugs in the therapy. One type of hypersensitivity reactions is due to complement activation, therefore it is called complement activation-related pseudoallergy (CARPA). CARPA does not involve IgE-mediated acute reactions, or chronic immune changes triggered or mediated by IgG, immune complexes or lymphocytes. CARPA can mainly be observed following treatments with liposomes, nanoparticle-containing drugs, micellar solvents and biologicals that are recognized by the innate immune system as foreign. CARPA is characterized by severe cardiopulmonary changes including arrhythmia, pulmonary edema, hypotension, airway occlusion, respiratory distress and cardiac arrest that in serious cases can cause cardiac (anaphylactic) shock and death. The most sensitive species is the pig that can be used to predict CARPA in man, but its cost and size precludes testing large number of drug candidates1,2. Therefore, the aim of the present study was to develop a model for measuring complement activation-related pseudoallergy in rats and/or mice.

MATERIALS AND METHODS

Mice: The study was performed in male Balb/c mice aged 11-12 weeks. Mice were placed in a restrainer and injected intravenously (i.v.) with one of the following test or control substances. Zymosan (5 mg/kg) suspended in saline served as positive control for complement activation. Ambisome (2.5 mg/kg phospholipid content) dissolved in water was used to test complement activation by liposomal products. Saline was served as negative control. Mice were then euthanized by ether inhalation 5 or 15 min after treatment, and blood was collected from the retro-orbital sinus in tubes containing 10 µl of lepirudin (Refludan, 1 mg/ml) and centrifuged at 3000 rpm for 10 min at 4°C. The plasma was stored at -80°C until analysis.
Rats: The experiments were performed in male Wistar rats weighing 340-420 g. The animals were anesthetized with thiobutabarbital (Inactin, 120 mg/kg i.p.). The left femoral artery and vein, the left carotid artery and the right jugular vein were cannulated with PP50 tubing. The animals were treated with lepirudin (5 mg/kg i.v.). The following test items were administered in different groups of rats. Zymosan (2.5 or 10 mg/kg i.v.) was utilized for direct complement activation. Ambisome (0.25 or 2.5 mg/kg i.v.) served as liposomal complement activator.
Blood sampling: Blood samples of 0.5 ml each were collected from the carotid artery into Eppendorf tubes containing 10 µl lepirudin (Refludan, 1mg/ml) before (time 0), and at different time points (1 - 3 - 5 - 10 - 30 min) after the injection of the test substances. Aliquots of 100 µl blood were drawn into tubes with K2-EDTA for blood picture analysis. Blood was centrifuged at 3000 rpm for 10 min at 4°C, and plasma was stored at -80°C until analysis.
Hemodynamic parameters: The arterial blood pressure of rats was measured by Hemosys data collection and analysis system. Systolic, diastolic and mean arterial blood pressure (MABP) and heart rate were measured for 30 sec before each blood collection.
Complement activation: The total complement activation was determined using the classical C hemolytic (CH50) assay. A fixed volume of optimally sensitized SRBCs was added to serum with appropriate dilution. After incubation, the mixture was centrifuged, and hemolysis was quantified by measuring the absorbance of the hemoglobin released into the supernatant at 540 nm. The amount of complement activity was determined by examining the capacity of test serum to lyse antibody coated SRBCs.
Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

RESULTS

Mice: In mice zymosan increased plasma TXB2 concentrations at 5 min after treatment but these changes were not significant due to huge individual variations. The hemolytic complement levels (CH50) slightly, but consistently increased after treatment with zymosan and Ambisome, which effect was regarded as a sign of plasma extravasation.

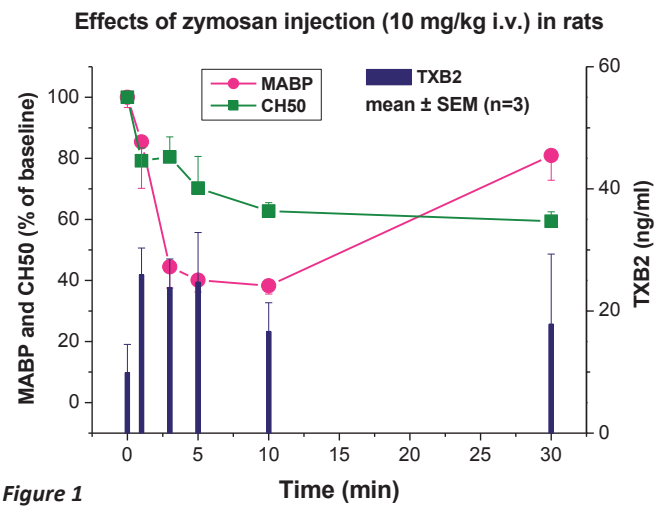

Figure 1

Rats: In rats both zymosan and Ambisome induced characteristic changes. Zymosan at low dose (2.5 mg/kg) decreased MABP by 10 to 15% reaching its minimum at 3 to 5 min after treatment, and recovered by 30 min. Plasma TXB2 levels showed 2 to 3-fold increases

upon zymosan injection. Maximum TXB2 changes were observed at 5 to 10 min after treatment, and TXB2 levels slowly recovered by the end of the study. The CH50 level showed a slight and transient elevation at 3 min that could be attributed to hemoconcentration, but CH50 returned to the control level thereafter. Zymosan at a higher dose (10 mg/kg) induced reversible hypotensive shock (Figure 1). The maximal decreases in MAPB were observed between 3 to 10 min after treatment; systemic blood pressure fell to 40% of control, and then slowly recovered towards the end of the study. Following an initial transient decline, a compensatory increase in heart rate was observed. Parallel to the hemodynamic changes there was a massive, 4-fold increase in plasma TXB2 concentration already 1 min after zymosan injection, lasting for 5 min, and then TXB2 level slowly decreased. Hematocrit also increased. Changes in TXB2 were paralleled with partial complement depletion, reaching 60% of the control by the end of the study.

In the case of Ambisome similar changes to that of zymosan could be seen both at low (0.25 mg/kg) and at high (2.5 mg/kg) doses. The only difference was that the observed changes were generally milder for the hemodynamic parameters, as well as for blood-borne substances. When Ambisome was injected at 2.5 mg/kg (Figure 2), less prolonged hypotonic response was found. MABP dropped to 55 % of control at 3 min, and then started to return towards the control level. Changes in TXB2 were smaller and of a multiphasic type. CH50 revealed transient complement depletion reaching its minimum level (65% of control) at 5 min after Ambisome injection.

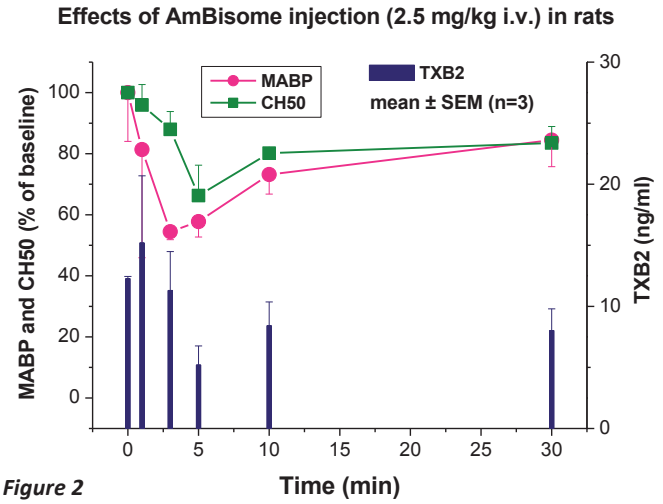

Figure 2

In some cases blood picture was analyzed. Intravenous injection of zymosan at 10 mg/kg i.v. caused a drastic decrease in white blood cell and platelet counts (Table 1). Leucopenia and thrombocytopenia were already observed 1 to 3 min after treatment. White blood cell count rapidly returned to the control level between 10 and 30 min after treatment, while platelet count more gradually increased to the control level between 3 and 30 min after the treatment. Ambisome induced quite similar changes in white blood cell and platelet counts with the difference that Ambisome decreased white blood cell counts less than zymosan.

***Table 1**. Effects of zymosan and Ambisome on white blood cell (WBC) and platelet (PLT) counts in anesthetized rats (n=3/group)*

	Zymosan, 10 mg/kg i.v.		Ambisome, 2.5 mg/kg i.v.	
time, min	WBC	PLT	WBC	PLT
0	9.1±2.3	450.0±51.0	10.7±2.0	476.3±69.8
1	4.7±0.3	252.3±18.7	10.6±2.7	442.5±65.5
3	4.8±0.0	308.0±21.0	9.2±4.3	311.0±33.0
5	4.2±0.3	314.0±21.0	8.8±3.6	303.3±37.3
10	4.8±0.4	408.8±10.2	9.9±1.5	343.8±15.3
30	10.9±2.9	439.7±15.3	11.7±0.7	493.0±67.0

CONCLUSIONS

Zymosan, the activator of the complement cascade, decreased the hemolytic complement level at high dose in rats but not in mice. In rats the consequences of complement activation were hypotension, plasma extravasation, decreases in white blood cell and platelet counts and increases in plasma thromboxane B2 levels. Ambisome, the liposome encapsulated amphotericin B, caused essentially similar effects in rats. Therefore, these results support the use of rats for testing the complement-dependent hypersensitivity reactions of nanodrugs.

Szebeni J. et al., (1994) Complement activation in rats by liposomes and liposome-encapsulated hemoglobin: evidence for anti-lipid antibodies and alternative pathway activation. Biochem Biophys Res Commun, 205:255-63.

Szebeni, J. et al., (2007) Animal models of complement-mediated hypersensitivity reactions to liposomes and other lipid-based nanoparticles. J Liposome Res, 17: 107-117.

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IN-VITRO RELEASE STUDY AND DYNAMIC IN-VIVO IMAGING OF PH- AND MAGNETIC FIELD SENSITIVE HYBRID MICROSPHERES

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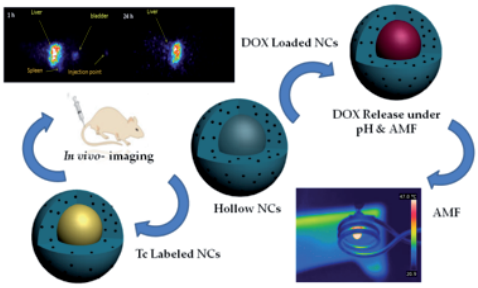
This paper deals with the synthesis, characterization and property evaluation of drug-loaded magnetic microspheres with pH-responsive cross-linked polymer shell. The synthetic procedure consists of 3 steps, of which the first two comprises the synthesis of a Poly Methyl Methacrylate (PMMA) template and the synthesis of a shell, using Acrylic Acid (AA) and Methyl Methacrylate (MMA) as monomers, and Divinyl Benzene (DVB) as cross-linker. The third step of the procedure refers to the formation of magnetic nanoparticles on the microsphere's surface. AA that attaches pH-sensitivity in the microspheres and magnetic nanoparticles in the inner and the outer surface of the microspheres, enhance the efficacy of this intelligent Drug Delivery System (DDS), which constitutes a promising approach towards cancer therapy. A number of experimental techniques were used to characterize the resulting microspheres. In order to investigate the in-vitro controlled release behaviour of the synthesized microspheres, we studied the DOX release percentage under different pH conditions and under external magnetic field. Hyperthermia caused by an Alternating Magnetic Field (AFM) is used in order to study the Doxorubicin (DOX) release behaviour from microspheres with pH functionality. The in vivo fate of these hybrid-microspheres was tracked by labelling them with the γ-emitting radioisotope 99mTc after being intravenously injected in normal mice. According to our results, microcontainers present a pH depending and a magnetic heating, release behaviour. As expected, labelled nanocarriers were mainly found in the mononuclear phagocyte system (MPS). The highlights of the current research are: (i) to illustrate the advantages of controlled release by combining hyperthermia and pH-sensitivity and (ii) to provide non invasive, in vivo information on the spatiotemporal biodistribution of these microcontainers by dynamic γ imaging.



***Scheme 1**. In-vitro and in-vivo study of fabricated MCs*

NOVEL METHOD FOR IN VIVO REAL-TIME IMAGING OF CARBON NANOTUBES LOADED WITH DOXORUBICIN USING THERMOSTABLE LUCIOLA CRUCIATE LUCIFERASE

RAMY EL-SAYED[1,3,2], Mohamed Eita‡, Åsa Barrefelt*, Fei Ye[2], Himanshu Jain[2], Mona Fares[1], Arne Lundin[4], Mikael Crona[4], Khalid Abu-Salah[5], Mamoun Muhammed[2], and Moustapha Hassan[1]
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In this study we present a novel method for in vivo imaging of single-walled carbon nanotubes (CNTs) using green fluorescent thermostable recombinant protein, namely luciferase from Luciola cruciate (LcL). LcL as an imaging moiety overcomes problems associated with other visualization moieties; such as quantum dots, radionucleoutides and fluorophores with drawbacks of toxicity, depth of imaging, and complexity. Here, we have chemically conjugated the LcL to oxidized-CNT (LcL-CNT) using carbodiimide chemistry, keeping the LcL biologically active, thus allowing catalysis of the D-luciferin in the presence of ATP to release significant amount of photons for in vivo imaging. LcL-CNTs were further successfully loaded with doxorubicin (LcL-CNT-DOX) as a chemotherapeutic cargo thus showing the multifunctional competency of the CNTs. Chemiluminescence of LcL allows imaging of CNTs and their cargo in nonsuperficial locations at an organ resolution with no need of an excitation source. In vivo imaging for LcL-CNT as such and LcL-CNT-DOX complexes was done using "IVIS spectrum" after their intravenous (IV) and intraperitoneal (IP) administration to mice. We showed the passage of the LcL-CNT as such and LcL-CNT-DOX complexes to the different organs in mice in a time-dependent manner. The LcL-CNT system is a powerful tool for in vivo imaging and therapeutic molecule delivery and represents an important step for the advancement of the nanomedicine field.

TALKING TO MEMBERS OF VENTURE CAPITAL – HOW TO GET SUCCESS

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Beside the scientific challenges of nanotechnology lead by the up-to-date existing uncertainties in risk and safety issues, a strong cooperation between stakeholders on business and research level is required. To deal with important questions, to bear in mind that an application or a product should have impact to solve social issues, and that via the scientific results – in best case – sozio-economic value can be achieved, will be some key elements for sustainable development of nanotech-sector. It will be important to also enhance the social potential of nanotechnology resulting in benefits for medicine and pharmacy and strengthening of regulators, national authorities and industry. Recent research showed that direct investments seem to be the most welcome way of financing research. Therefore, researchers should be aware of the importance to also attract investors such as venture capitalists, etc. via their scientific work. However, investors need to understand the potential of the scientific results; otherwise they will not identify the ability to gain value out of the investment.
Within this contribution some of the above mentioned key challenges will be addressed: e.g. what can be the right questions, and according to this, the right answers from researchers. Additionally, within this talk the so called tool "Nano-Deals generator", developed in the NANOFORCE project, will be introduced, providing assistance to researchers and investors, to facilitate the communication aiming on project and business development, and to foster the market implementation of nanotechnology application.

DIAMETER AND SURFACE FUNCTIONALIZATION OF SHORT CARBON NANOTUBES INFLUENCE THEIR GENOTOXIC EFFECTS

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INTRODUCTION

Carbon nanotubes (CNTs) are increasingly used in many products. Due to their similarity to asbestos fibres, however, they are suspected to have genotoxic effects. Such effects include induction of mutations of hypoxanthine guanine phosphoribosyltransferase 1, DNA strand breaks 2, micronuclei formation 3, sister chromatid exchanges and chromosome aberrations 4, 5, and increased polyploidy 6. Since high aspect (diameter to length) ratio was identified as negative factor in the biocompatibility of CNTs, short CNTs were developed for medical applications. Potential applications include mainly multimodal cancer therapy and vaccination. In addition to length and diameter also surface functionalization appear to be key parameters for biologic effects of CNTs 7. To assess the hazard of short CNTs, well-characterized short (2 µm) pristine and carboxylated single-walled (SCNTs) and multi-walled (MCNTs) CNTs in different diameters (8 nm, 20-30 nm, 50 nm) were assessed in both human lung adenocarcinoma A549 cells and V79 chinese hamster fibroblasts.

METHODS

V79 cells, as a standard cell line for genotoxicity testing, and A549 cells as representatives for human cells were used. A549 cells are frequently used for the assessment of particle toxicity by respiratory exposure. Prior to genotoxicity testing, cellular uptake of CNTs, cytotoxicity, and the cell's response to oxidative stress (immunoreactivity against hemoxygenase 1 and glutathion levels) were determined. A dose range of 0 – 500 µg/ml was assessed. To identify mutations, a hypoxanthine guanine phosphoribosyltransferase assay in V79 was performed. DNA strand breaks were monitored by a COMET assay and DNA repair by measuring immunoreactivity against activated H2AX protein. The number of micronuclei as well as the number of multinucleated cells was determined.

RESULTS

According to the physicochemical characterization, the diameter of most CNTs were similar to the values indicated by the producer while lengths were much shorter and ranged between 200-450 nm. Plain and carboxylated SCNTs and carboxylated MCNT8 formed bundles with length up to 1 µm. CNTs acted more cytotoxic and, at non-cytotoxic concentrations, more genotoxic in V79 than in A549 cells. In general, CNTs <8nm (IC50 of 120 - 360 µg/ml for V79 cells and 250 – 350 µg/ml in A549 cells) acted more toxic than CNTs >20 nm (in general, IC50 > 500µg/ml in both cell lines). The role of reactive oxygen species was small; increased expression of hemoxygenase 1-immunoreactivity and decrease in glutathione levels were only seen after exposure to carboxylated SCNTs. Only plain CNTs caused mutations in the HPRT assay, usually at 100 µg/ml. Exposure to CNTs only slightly increased immunoreactivity against the DNA damage repair proteins, such as phosphorylated H2AX (Fig. 1).

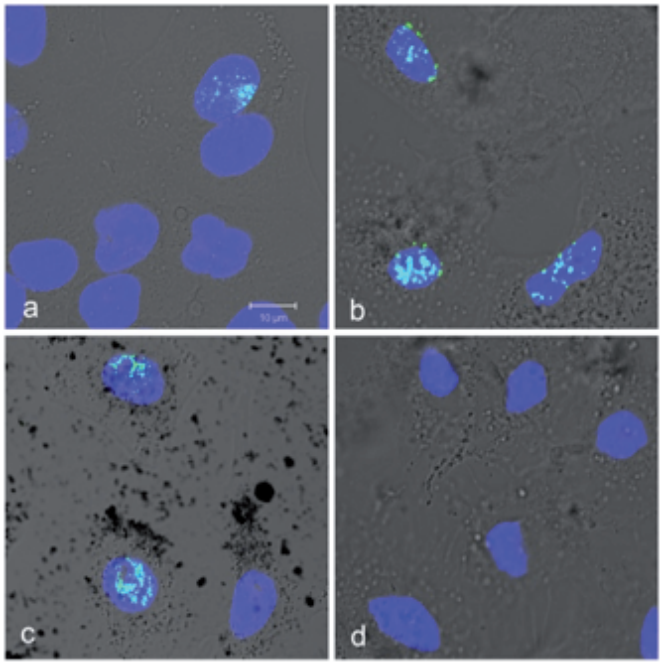

Fig. 1: *Detection of phosphorylated H2AX -immunoreactive foci (green) in nuclei (blue) of A549 cells as indication for activation of DNA repair enzymes. Untreated cells (a), positive control (b), cells treated exposed to carboxylated MCNT50 (c) and antibody negative control (d).*

No increase in the amount of double strand breaks according to tail formation in single cell gel electrophoresis of A549 cells was detected. While all CNTs increased the number of micronuclei in V79 cells in concentrations of 50-200 µg/ml, only 50 µg/ml of plain and carboxylated MCNT50s caused significantly higher rates of micronuclei formation in A549 cells. MCNT50s and bundles of carboxylated SCNTs induced formation of multinucleated cells in both cell types (Fig. 2).

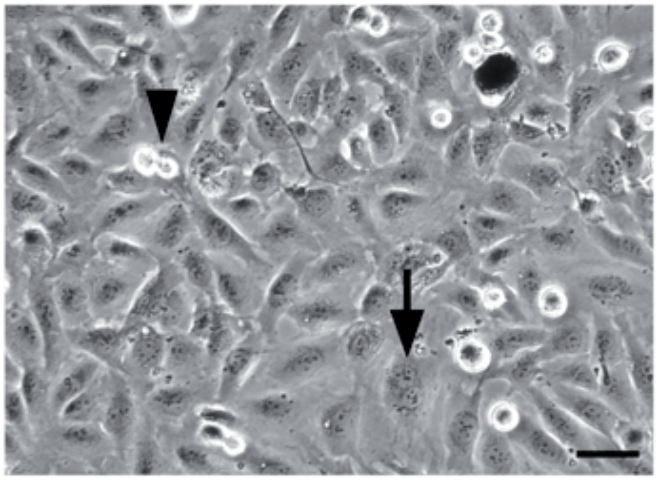

Fig. 2: *Upon exposure to MCNT50 dividing cells (arrowhead) are frequent. In addition, binucleated cells are seen (arrow).*

CONCLUSIONS

While thin (<8 nm) CNTs showed a greater cytotoxic potential, thick (>20 nm) CNTs caused more genotoxic effects. These effects included micronuclei formation and interference with cell division, causing multinucleated cells. Oxidative damage by carboxylated SCNTs in A549 cells did not result in DNA strand breaks, probably because this damage did not exceed the repair capacity of the cells. The greater susceptibility of V79 cells to genetic damage by CNTs could be due to the higher proliferation rate compared to A549 cells.

REFERENCES

1. Manshian, B. B.; Jenkins, G. J.; Williams, P. M.; Wright, C.; Barron, A. R.; Brown, A. P.; Hondow, N.; Dunstan, P. R.; Rickman, R.; Brady, K.; Doak, S. H., Single-walled carbon nanotubes: differential genotoxic potential associated with physico-chemical properties. Nanotoxicology 2013, 7, 144-156.
2. Ursini, C. L.; Cavallo, D.; Fresegna, A. M.; Ciervo, A.; Maiello, R.; Buresti, G.; Casciardi, S.; Tombolini, F.; Bellucci, S.; Iavicoli, S., Comparative cyto-genotoxicity assessment of functionalized and pristine multiwalled carbon nanotubes on human lung epithelial cells. Toxicol. in vitro 2012, 26, 831-840.
3. Patlolla, A. K.; Hussain, S. M.; Schlager, J. J.; Patlolla, S.; Tchounwou, P. B., Comparative study of the clastogenicity of functionalized and nonfunctionalized multiwalled carbon nanotubes in bone marrow cells of Swiss-Webster mice. Environ. Toxicol. 2010, 25, 608-621.
4. Di Giorgio, M. L.; Di Bucchianico, S.; Ragnelli, A. M.; Aimola, P.; Santucci, S.; Poma, A., Effects of single and multi walled carbon nanotubes on macrophages: cyto and genotoxicity and electron microscopy. Mutat. Res. 2011, 722, 20-31.
5. Kato, T.; Totsuka, Y.; Ishino, K.; Matsumoto, Y.; Tada, Y.; Nakae, D.; Goto, S.; Masuda, S.; Ogo, S.; Kawanishi, M.; Yagi, T.; Matsuda, T.; Watanabe, M.; Wakabayashi, K., Genotoxicity of multi-walled carbon nanotubes in both in vitro and in vivo assay systems. Nanotoxicology 2012, Apr 4, [Epub ahead of print].
6. Asakura, M.; Sasaki, T.; Sugiyama, T.; Takaya, M.; Koda, S.; Nagano, K.; Arito, H.; Fukushima, S., Genotoxicity and cytotoxicity of multi-wall carbon nanotubes in cultured Chinese hamster lung cells in comparison with chrysotile A fibers. J. Occup. Health 2010, 52, 155-166.
7. Fröhlich, E.; Meindl, C.; Höfler, A.; Leitinger, G.; Roblegg, E., Combination of small size and carboxyl functionalisation causes cytotoxicity of short carbon nanotubes. Nanotoxicology 2012, Oct 9, [Epub ahead of print]

CAN COMPUTATIONALLY DESIGNED PEPTIDES BE VIABLE ALTERNATIVE TO ANTIBODIES?

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Typical receptors for proteins are antibodies optimized in vivo. However, while monoclonal antibodies are highly successful in therapeutics and diagnostics, they are highly costly. An alternative would be exploiting advances in computing power together with the development of new and powerful algorithms for protein folding, docking, and structure prediction to reproduce the same output, namely an optimized antibody, in silico. While progresses are being made in this direction [1,2], the design of a full antibody from scratch pose a set of challenges due to its structural complexity caused by the large number of monomers forming its primary structure, and it is still out of reach.

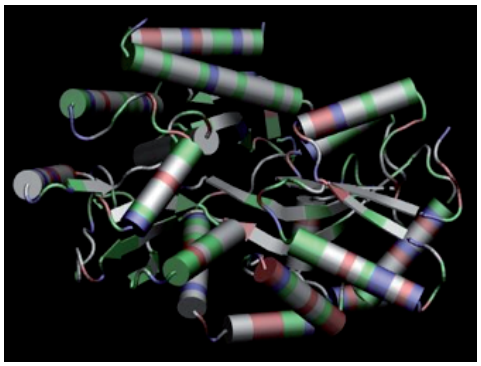

Fig.1 *- The MBP is the prototypical member of the Periplasmic Binding Proteins superfamily. In its crystallographic structure the maltose binding pocket clearly visible in its center. Here colors indicate: non-polar residues (white), basic residues (blue), acidic residues (red) and polar residues (green).*

Here we show that a promising alternative is the in silico design of short peptides, easily synthesizable in vitro by automated synthesis. To this aim (1) we are developing a computational protocol based on replica exchange Monte Carlo [3] for the generation of an optimized set of peptides able to bind with high affinity to a protein; (2) we chose the maltose binding protein (MBP, Fig.1) as a test case and measured the binding affinity of a set of computationally designed peptides to the MBP showing they actually bind to the MBP; (3) we look at the entropic contributions to the peptide-protein complexes to maximize their binding affinity.

1] L. Simonelli, et al., PloS one 2013, 8 (2), e55561;.
[2] Kuroda, D.; et al., Protein Eng. Des. Sel. 2012, 25 (10), 507-521.
[3] R. P. H. Enriquez, et al., J. Chem. Theory Comput. 2012, 8 (3), 1121-1128.

SYNTHESIS AND CHARACTERIZATION OF MESO-TETRAPHENYLCHLORIN TETHERED CHITOSAN BASED NANOCARRIERS FOR PHOTOCHEMICAL INTERNALIZATION

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INTRODUCTION

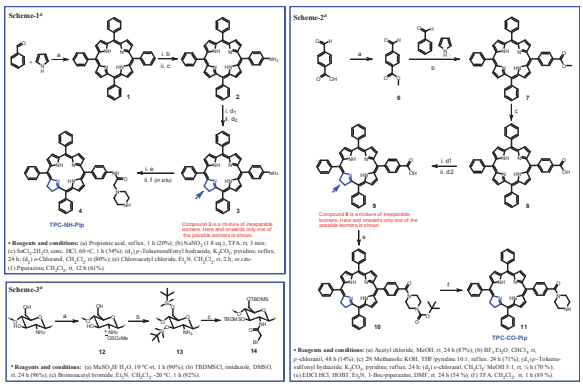
Photochemical Internalization1 (PCI) has been emerged as an advanced novel technology to enhance the site-specific macromolecular therapeutics effect in cancer therapy. PCI found to be very useful to enhance the biological activities of a variety of macromolecules including type-I ribosome-inactivating proteins, immunotoxins, chemotherapeutic agents like bleomycin (Blenoxane®) and doxorubicin. PCI can be highly specific as it applies combination of targeted therapeutics along with the light-activated cytosolic delivery induced by photosensitizer preferentially accumulating in solid tumors which also can contributes to the enhanced antitumor efficacy of the drug. Our, earlier in vitro study based on amphiphilic meso-tetraphenylporphyrin (TPP) conjugated chitosan showed that this type of nanocarriers can be used for PCI.2 However, TPP-conjugates cannot be illuminated with red light, which has deep tissue penetration and this limits their in vivo applicability. In the current study, we synthesized amphiphilic meso-tetraphenylchlorin (TPC) conjugated chitosan nanocarriers. It was expected that they could absorb light in red region and thus allow deep tissue penetration and effective treatment of large lesions in vivo.

RESULTS

We synthesized two different monofunctional (amino and carboxyl) meso-tetraphenylchlorin photosensitizers. The synthesis of mono-aminochlorin TPC(p-NH2)1 3 was achieved in two stages: reduction/ oxidation process with necessary modifications. Firstly, reduction of TPP(p-NH2)1 2 by p-toluenesulfonyl hydrazide in pyridine resulted in a mixture of chlorin and bacteriochlorin. This was confirmed by observing the Q-band peaks at 651 nm (chlorin) and 738 nm (bacteriochlorin) in UV-Vis spectra. This was also confirmed by 1H NMR analysis. Then, the chlorin/ bacteriochlorin mixture was carefully back oxidized by o-chloranil to afford exclusively mono-aminochlorin TPC(p-NH2)1 3, which was confirmed by UV-Vis (rise in peak at 651nm and disappearance of peak at 738 nm), 1H NMR and MS (ESI) analysis. TPC(p-NH2)1 3 was then converted into intermediate TPC-NH-Pip 4 in one pot synthesis (Scheme-1).

Similarly, the second photosensitizer, mono-carboxyl-tetraphenylchlorin TPC(p-CO2H)1 9 was synthesized. Then, the second key nucleophilic intermediate TPC-CO-Pip 11 was prepared by following the synthetic route shown in Scheme-2. Bromoacetyl-chitosan intermediate 14 was synthesized as shown in Scheme-3. The conjugation of photosensitizers TPC and chitosan-polymer could easily be achieved by nucleophilc substitution reaction of either of the intermediate TPC-NH-Pip 4 or TPC-CO-Pip 11 and bromoacetyl-chitosan intermediate 14.

TPC-NH-Pip 4 [0.10 equivalent (per sugar unit)] on treatment with compound 14 afforded compound 15 which upon further treatment with excess NMe3 or 1-methylpiperazine yielded corresponding compounds 16/17 respectively. Similarly, TPC-CO-Pip 11 [0.10 equivalent (per sugar unit)] on treatment with compound 14 afforded compound 20 which after reacting with excess NMe3 or 1-methylpiperazine yielded corresponding compounds 21/22 respectively. Surprisingly, deprotection of the compounds 16, 17, 21 and 22 by TBAF/NMP led to the oxidation of the chlorin-chitosan conjugates to the corresponding undesired porphyrin derivatives. However, finally deprotection of the compounds 16, 17, 21 and 22 by conc. HCl/ MeOH yielded corresponding desired chlorin TPC-chitosan nanocarriers 18, 19, 23 and 24 (Scheme-4).

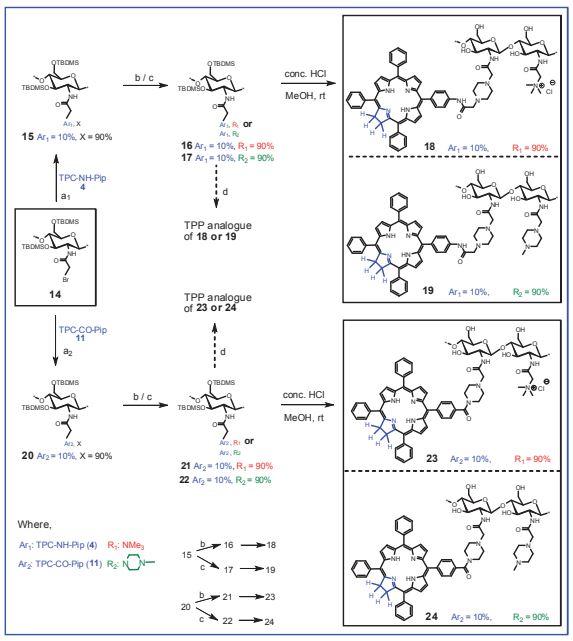


Physicochemical properties of these carriers (Table-1) reveals the formation of stable nanoparticles in the range 140-200 nm. Representative example of 1H NMR analysis of intermediates and final nanocarriers (23, 24) is shown in Figure 1, which confirms the covalent linkage as well as exclusive chlorin derivatives of chitosan.

Biological study. Conjugates 18, 19, 23 and 24 were used as photosensitising agents in PCI to enhance gene delivery. In vitro study of these conjugates indicates substantial enhancement of transfection even at low light doses.

Preliminary in vivo studies of illuminated tumour-bearing mice treated with the conjugates 24 and 19 either alone or together with the cytotoxic anti-cancer agent bleomycin has been done. Untreated animals and animals injected with TPCS2a (the current clinical candidate for PCI) + bleomycin were used as controls. The cancer cells used were permanently transfected to express luciferase so that the extent of the tumours could be monitored by imaging of bioluminescence after injection of luciferin. Chitosan conjugates induced a strong PCI effect and results showing that in the animals treated with compound 24 + bleomycin or 19 + bleomycin; the tumours seem to be completely eradicated.

***Scheme 4a**. Synthetic route for the synthesis of TPC-CS nanocarriers 18, 19 and 23, 24*



a Reagents and conditions: (a1) TPC-NH-Pip (0.1 equivalent), Et3N, CH2Cl2, rt, 2 h (78%); (a2) TPC-CO-Pip (0.1 equivalent), NaHCO3, NMP, 75 oC, 12 h (89 %); (b) NMe3, CH2Cl2, rt, 24 h; (c) 1-Methyl-piperazine, CH2Cl2, rt, 24 h; (d) TBAF, NMP, 55 oC, 12 h.

***Table 1**. Physicochemical Properties of TPC-CS nanocarriers 18, 19 and 23, 24*

CS Derivatives		Relative Φ_F*	Average particle size ** (nm)	Zeta potential** (mV)
$TPC_{NP0.1}$-CS-$TMA_{0.9}$	**18**	0.99	155 ± 96	86 ± 16
$TPC_{NP0.1}$-CS-$MP_{0.9}$	**19**	0.99	194 ± 112	83 ± 5
$TPC_{CP0.1}$-CS-$TMA_{0.9}$	**23**	0.94	148 ± 59	80 ± 4
$TPC_{CP0.1}$-CS-$MP_{0.9}$	**24**	0.94	143 ± 65	83 ± 8

*Fluorescence Quantum Yield (ΦF) measurements were done in DMSO at rt and at excitation wavelength λex.= 420 nm. The observed emission wavelength for all compounds listed in above table is λem. = 653 nm. The calculated ΦF of 4 (TPC-NH-Pip) (0.0135, when anthracene as standard) was used as reference for the relative values. ** DLS and zeta potential measurement of all nanocarriers were done in water.*

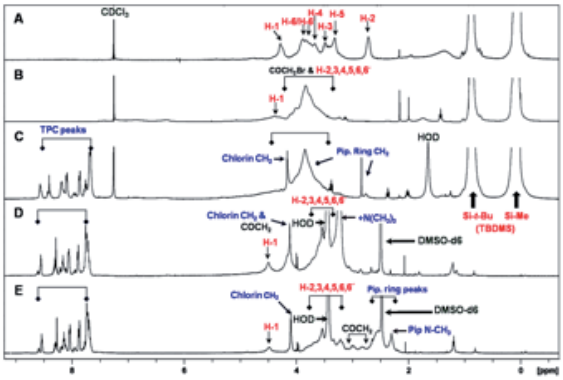


***Figure 1**. 1H NMR spectra overlay of the key intermediates and the final compounds in the synthesis of the chitosan nanocarriers 23 and 24 : (A) 13; (B) 14; (C) 20; (D) 23; (E) 24.*

CONCLUSION

Four distinct amphiphilic meso-tetraphenylchlorin conjugated chitosan carriers were synthesized. The carriers induced PCI transfection of pEGFP-N1 in HCT116/LUC cells showed substantial enhancement of transfection. Preliminary investigation of carriers + bleomycin demonstrated strong PCI effect in nude mice. The nanocarriers also shown substantial tumor selective accumulation due to enhanced and retention (EPR) effect and are therefore promising candidates for PCI in cancer therapy.

Acknowledgement: We would like to thank financial support from Nordforsk Public Private Partnership.

REFERENCES

1. Berg, K.; Selbo, P. K.; Prasmickaite, L.; Tjelle, T. E.; Sandvig, K.; Moan, D.; Gaudernack, G.; Fodstad, O.; Kjolsrud, S.; Anholt, H.; Rodal, G. H.; Rodal, S. K.; Hogset, A., Photochemical internalization: A novel technology for delivery of macromolecules into cytosol. Cancer Res 1999, 59 (6), 1180-1183.

2. Gaware, V. S.; Håkerud, M.; Leósson, K.; Jónsdóttir, S.; Høgset, A.; Berg, K.; Másson, M., Tetraphenylporphyrin Tethered Chitosan Based Carriers for Photochemical Transfection. J Med Chem 2013, 56 (3), 807-819.

PERMEATION ENHANCING EFFECTS OF TMC HOMOPOLYMER AND OTHER N-ALKYL-QUATERNIZED CHITOSAN DERIVATIVES IN BRONCHIAL EPITHELIAL CELLS

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INTRODUCTION

The polysaccharide chitosan has been the subject of numerous studies as a potential permeation enhancer, mainly to increase absorption in the intestinal mucosa. The advantages of the chitosan polymer are its mucoadhesive nature, biodegradability and safety in non-parental dosage formulations. Introducing a permanent positive charge, such as with N,N,N-trimethyl chitosan (TMC), increases the aqueous solubility of chitosan at physiological pH and expands the use of this polymer towards other mucosal membranes.

TMC previously studied had different degrees of trimethylation, often accompanied with O-methylation, thereby reducing the water solubility. In this study, we have used the tert-butyldimethylsilyl protection strategy[1] to synthesize fully trimethylated chitosan without O-methylation in addition to N-alkyl-N,N-dimethyl chitosan derivatives[2] (Figure 1). In addition, we have used recently validated the bronchial epithelial cell line VA10 as a model to study drug permeation.[3]

RESULTS

Di-TBDMS chitosan was methylated by reaction with CH3I in NMP to obtain fully substituted N,N,N-trimethyl chitosan. Furthermore, highly substituted N-alkyl-N,N-dimethyl chitosan derivatives were synthesized and characterized in detail by NMR, confirming the assignments of each peak. Partial N-alkylation (propyl, butyl or hexyl) of di-TBDMS chitosan was found to be likely due to the instability of the imine bond and/or steric hindrance from the protecting groups. Subsequent N,N-dimethylation of the N-alkylated material required dimethyl sulfate [(CH3O)2SO2], a more reactive methylating agent than CH3I. This could be attributed to steric hindrance from the TBDMS group and the existing N-alkyl chain, which can reduce the reactivity of the amino group. Deprotection of the quaternized derivatives required alternative conditions than those previously published,1a with TBAF providing efficient deprotection of the bulky TBDMS protected chitosan derivatives. The resulting N,N,N-trimethyl and N-alkyl-N,N-dimethyl chitosan derivatives were water soluble, with no evidence of O-methylation. This also confirmed that the hydroxyl groups are fully protected in the Di-TBDMS chitosan precursor used in the synthesis. The final products, N,N,N-trimethyl chitosan and N-alkyl-N,N-dimethyl chitosan derivatives, were characterized in detail by FT-IR, 1H and 13C NMR and 1H-1H COSY and 1H-13C HSQC NMR.

In this study, the permeation enhancement was evaluated using the bronchial epithelial cell line VA10 as a model. Transepithelial electrical resistance (TER), a useful indicator of the function of the tight junctions, decreased for all derivatives in a dose-dependent manner. The decrease was different between derivatives, with TER decreasing slowly for TMC and QuatPropyl but a rapid decrease was observed for QuatButyl and QuatHexyl chitosan derivatives.

The decrease in TER values was associated with increased paracellular permeability of FITC-labelled dextran 4 kDa with the highest increase in permeability obtained after treatment with QuatButyl followed closely by QuatHexyl, than QuatPropyl and finally TMC (Figure 2). The highest concentration (1 mg/mL) caused a 55 % decrease in TER for TMC, 73% for QuatPropyl, 91% for QuatButyl and 90% for QuatHexyl (Figure 3). TER measured after 24h recovery showed that QuatButyl was the only derivative that showed full recovery (97% of initial value) followed by 69% recovery of initial value for TMC.

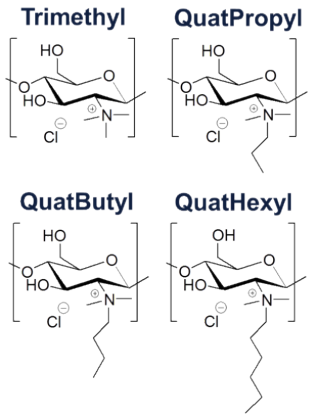

***Figure 1.** Quaternary chitosan derivatives used in the current study.*

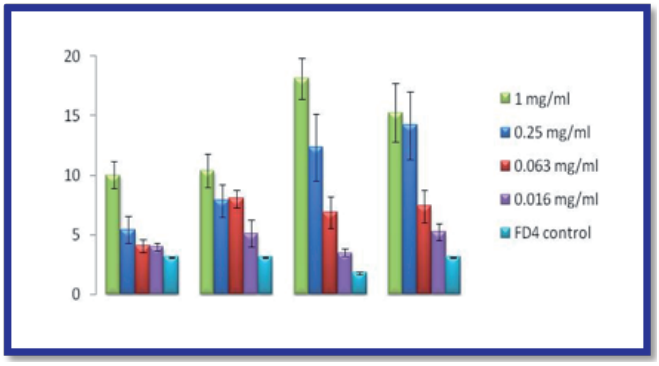

***Figure 2**. Comparison of FITC-dextran 4 kDa Papp values between the quaternary chitosan derivatives.*

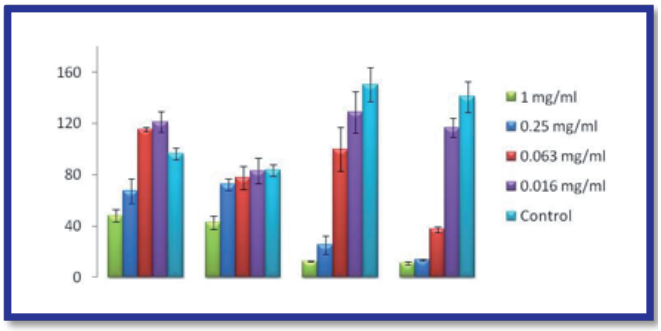

***Figure 3**. Effect of quaternized chitosan derivatives on transepithelial electrical resistance (TER) after 2h treatment in the VA10 lung epithelium (n = 3-5).*

CONCLUSION

In the present study, we demonstrate that Di-TBDMS chitosan polymers can be chemically modified under homogenous conditions in common organic solvents at moderate temperatures under different reaction conditions. Di-TBDMS chitosan thus represents an ideal precursor for further N-selective modifications. All quaternary chitosan derivatives increased the paracellular epithelial permeability Longer N-alkyl chain in N,N-dimethyl chitosan results in a more prominent increase in the paracellular epithelial permeability compared to shorter alkyl chains. These chitosan derivatives could be an important contribution towards the development of drug delivery systems for macromolecular drugs. This study will soon be published.[4]

Acknowledgements: We would like to thank financial support from the Icelandic Technical Development Fund and Nordforsk Public Private Partnership.

REFERENCES

1. (a) Runarsson, O. V.; Malainer, C.; Holappa, J.; Sigurdsson, S. T.; Masson, M., tert-Butyldimethylsilyl O-protected chitosan and chitooligosaccharides: useful precursors for N-modifications in common organic solvents. Carbohyd Res 2008, 343 (15), 2576-2582; (b) Song, W. L.; Gaware, V. S.; Runarsson, O. V.; Masson, M.; Mano, J. F., Functionalized superhydrophobic biomimetic chitosan-based films. Carbohydrate Polymers 2010, 81 (1), 140-144.
2. Benediktsdottir, B. E.; Gaware, V. S.; Runarsson, O. V.; Jonsdottir, S.; Jensen, K. J.; Masson, M., Synthesis of N,N,N-trimethyl chitosan homopolymer and highly substituted N-alkyl-N,N-dimethyl chitosan derivatives with the aid of di-tert-butyldimethylsilyl chitosan. Carbohydrate Polymers 2011, 86 (4), 1451-1460.
3. Benediktsdottir, B. E.; Arason, A. J.; Halldorsson, S.; Gudjonsson, T.; Masson, M.; Baldursson, O., Drug delivery characteristics of the progenitor bronchial epithelial cell line VA10. Pharm Res 2013, 30 (3), 781-91.
4. Benediktsdottir, B. E.; Gudjonsson, T.; Baldursson, O.; Masson, M., N-alkylation of highly quaternized chitosan derivatives affects the paracellular permeation enhancement in bronchial epithelia in vitro. Eur J Pharm Biopharm 2013.

EX VIVO INHIBITION OF TORC1 IMPROVES THE THERAPEUTIC ACTIVITY OF SENESCENT HUMAN CARDIAC STEM CELLS IN A MOUSE MODEL OF MYOCARDIAL INFARCTION

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Cardiac Stem Cells (CSC) expanded in vitro from explanted failing hearts (E-) are characterized (with respect to those obtained from healthy donors, D-) by: reduced proliferation and migratory capabilities, shorter telomeres, and a larger fraction of cells showing both telomere-associated damage foci and senescence markers.
Aim of the study was to verify if the senescence of CSC impairs their in vivo reparative potential. Additionally, we investigated the pathways involved in E-CSC senescence to identify drugs able to reduce this process in vitro and to restore CSC reparative ability in vivo.
Methods and results: the ability of E-CSC to repair a myocardial infarction in SCID/beige immunodeficient mice was outperformed by D-CSC (Figure 1). E-CSC showed a trend towards a higher activity of TORC1 complex, reduced cAMP response element–binding protein (CREB) activation, an arrested autophagic degradation and an altered secretome. Inhibition of TORC1 and enhancement of CREB signaling with a combination of 10nM rapamycin + 0.5µM resveratrol significantly reduced the fraction of senescent E-CSC in vitro and restored both their protective secretome in vitro and reparative ability in vivo. This latter was associated with a reduction in myocyte apoptosis and senescence and with a significant increase in the tissue density of c-Kit+ CSC.
Conclusion: in vitro pretreatment of E-CSC with rapamycin and resveratrol enhances their reparative ability in vivo suggesting the use of such approach to enhance CSC therapy.

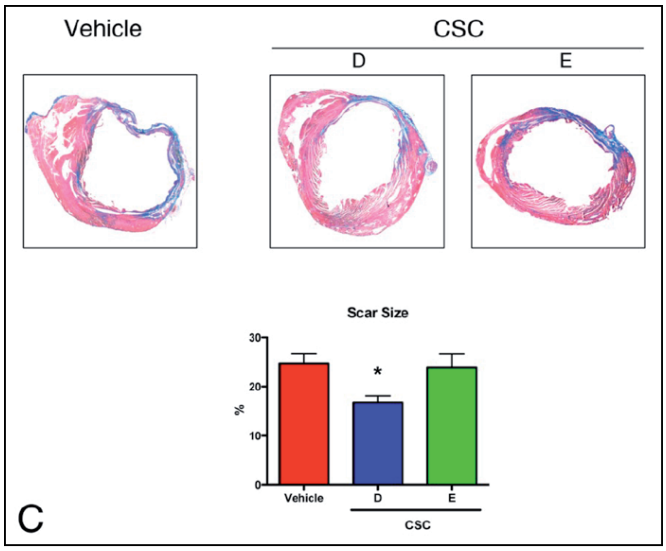

***Figure 1.** Azan Mallory staining of transverse sections of infarcted mouse hearts injected with Vehicle, donor-derived cardiac stem cells (D-CSC) or patient-derived cardiac stem cells (E-CSC). Bar graphs show the volume fraction of myocardium occupied by the scar (blue). *, p<0.05 versus Vehicle. Cesselli D et al. submitted.*

ENHANCED ABSORPTION OF THE RADIOTRACER, (99MTC-MIBI) THROUGH THE ENTRAPMENT IN THE PHEROID® SYSTEM

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BACKGROUND

99mTechnetium-mibi is used for myocardial imaging. 99mTc-mibi Injection is a myocardial perfusion agent that is indicated for detecting coronary artery disease by localizing myocardial ischemia (reversible defects) and infarction (non-reversible defects), in evaluating myocardial function and developing information for use in patient management decisions (Sun et al., 2003). 99mTc-mibi evaluation of myocardial ischemia can be accomplished with rest and cardiovascular stress techniques. It is usually not possible to determine the age of a myocardial infarction or to differentiate a recent myocardial infarction from ischemia.

PURPOSE

Previous studies showed that formulation of active pharmaceutical ingredients in the Pheroid® delivery system enhances absorption of the active pharmaceutical ingredient (API), and may contribute to an increase in the quantity of the API present at the site of interest (Grobler, 2009). Increased drug levels at the active site should increase the effectiveness of a drug with a narrow therapeutic index (TI). The API used in this study is the radiotracer, 99mTechnetium-methoxyisobutylisonitrile (99mTc-mibi). Besides its use in detecting coronary arty disease, the radioactive tracer molecule can also be used to pinpoint the exact location of delivery of the tracer and can thus confirm delivery at the site of action. The radiotracer will accumulate in different locations to that of Pheroid® vesicles, if it is not entrapped inside a vesicle.

METHODS

In this study the intravenous administration of the radiotracer were investigated. The test formulation is defined for the purpose of this study as the radiotracer 99mTc-mibi entrapped in the Pheroid® system and the reference formulation as non-entrapped radiotracer. The comparative study design included twenty-four Sprague Dawley Rats. As shown in the figure 1 below, twelve animals each received the test and reference formulations respectively. These two groups of 12 rats were then divided into three groups of four rats each. Each of these groups were sacrificed at different time intervals (30 minutes, 1 and 2 hours) after administration. All organs were harvested and radioactivity of 14 organs were determined for each animal using a Capintec Beta Detector CRC®-Ultra Dose Calibrator and a TriathlerTM Nal Well Counter.

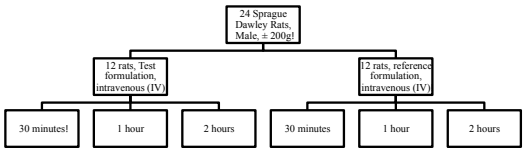

***Figure1**: Schematic representation of the study design*

RESULTS

The graphs below illustrate the percentage of the injected dose per gram of organ after IV administration of the test and reference formulation respectively.

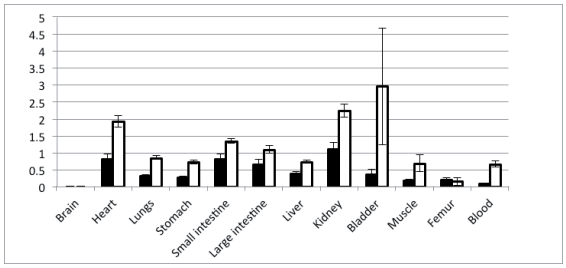

***Figure 2**: Percentage of injected dose per gram of organ. Data depicted as mean ± SE of reference (black) and test (clear) formulations. Rats were sacrificed 30 minutes after administration.*
The total body accumulation of the test formulation is more than double of that of the reference formulation. This means that the Pheroid® system enhanced absorption of the radiotracer and this will lead to better imaging of the desired organs.

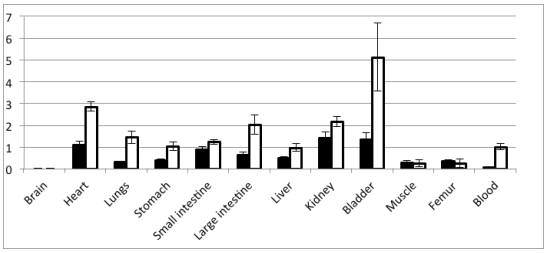

***Figure 3**: Percentage of injected dose per gram of organ. Data depicted as mean ± SE of reference (black) and test (clear) formulations. Rats were sacrificed 1 hour after administration.*
Accumulation of the drug levels in the heart are more than double when the radiotracer is entrapped inside the Pheroid® delivery system. This may enable us to decrease the dosage of the given radiotracer and still get the desired results. This result was even more pronounced 2 hours after administration as shown in figure 4 below.

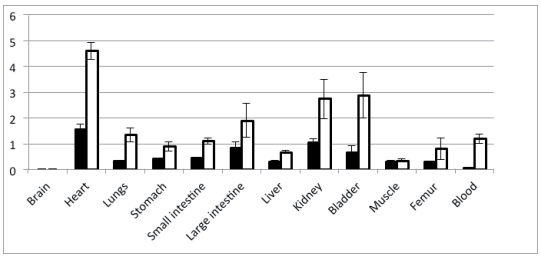

***Figure 4**: Percentage of injected dose per gram of organ. Data depicted as mean ± SE of reference (black) and test (clear) formulations. Rats were sacrifised 2 hours after administration.*
Conclusion: Entrapment of the radiotracer in the Pheroid® delivery system results in a statistically significant increase in the accumulation of the radiotracer in the target organ; in this case the heart. This will lead to better imaging of the organs. The clinical potential is exciting since diagnostic imaging plays a major role in enhanced diagnosis and clinical outcomes.

POLYETHYLENIMINE FUNCTIONALIZED MAGNETIC NANOPARTICLES FOR NUCLEIC ACID TRANSFECTION OF PRIMARY FIBROBLASTS

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Nucleic acid delivery to somatic cells such as fibroblasts are of interest for diverse biomedical applications. Somatic cell nuclear transfer (SCNT) using porcine fetal fibroblasts as donor cells has played a central role in the cloning of animals to produce recombinant therapeutic proteins or to breed animals for the purpose of xenotransplatation. Fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSs) by delivery of pDNA coding for the defined pluripotency factors, and the resulting iPS cells can then be differentiated into variety of specific cell types. The aim of this study was to explore the potential of the plasmid DNA delivery to the primary fetal pig and primary mouse fibroblasts using magnetic delivery vectors and gradient magnetic fields, an approach known as magnetofection.

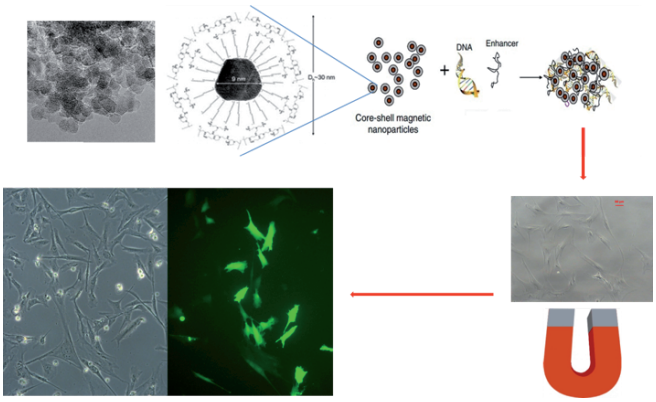

Figure 1. *Schematics of the procedures used to transfect the primary Mouse Embryo Fibroblast and Porcine Fetal Fibroblast cells by magnetofection.*

PEI-Mag2 iron oxide nanoparticles with a magnetic core of 9 nm and a coating from fluorinated surfactant Zonyl FSA and 25 kD branched polyethylene imine and different liposomal transfection reagents were used to formulate magnetic pDNA lipoplexes. The complexes were characterized in regards to their size, magnetic moment and electrokinetic potential. Lipofection of the pig and mouse fibroblasts resulted in similar reporter gene expression patterns. Magnetofection of the pig and mouse fibroblasts with optimal magnetic lipoplexes resulted in improvement of the tranfection efficiency in terms of the luciferase reporter gene expression and in the percent of the transfected cells. Respiration activity of the pig and mouse fibroblasts measured after 48h incubation with optimal magnetic transfcetion complexes was not inhibited up to applied plasmid dose of 8 pg per cell.

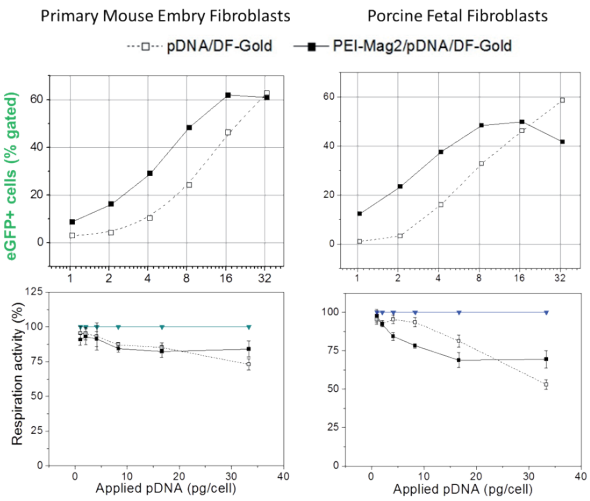

Figure 2. *Magnetofection vs. Lipofection in adherent (A) Primary Mouse Embryo Fibroblasts (PMEF) and (B) Porcine Fetal Fibroblasts (PFF).*

NANOSTRUCTURE AND LOCAL RIGIDITY OF RED BLOOD CELLS (RBC) UNDER INFLUENCE OF MEMBRANE MODIFICATORS AND IONIZING RADIATION

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V.A. Negovsky Research Institute of General Reanimatology, Russian Academy of Medical Sciences, Moscow

INTRODUCTION

The purpose of the present work is to study the alterations of the average local rigidity of RBC membranes under the action of membrane modificators.

One of the most important problems of microrheology is the studying of deformability of RBC. This problem may be decided using atomic force spectroscopy. This method allows to measure the average local rigidity of RBC membranes. The assessment of this parameter is important for microrheology, because the measurement of RBC membranes rigidity allows to determine the deformability of RBC. Deformability index is associated with the rigidity of the membrane by the logarithmic dependence. The greater rigidity, the lower the index of deformability.

The analysis of the average local rigidity of RBC membranes by the action of membrane modificators in vitro is carried out in the present work. Agents having both chemical and physical nature were selected as modificators. Zinc ions induces clusterization of band 3 proteins, hemoglobin; solution of NaNO2 causes lipids peroxidation, verapamil is a calcium channel blocker. Ionizing radiation causes the development of oxidative processes in the blood, namely, peroxidation of membrane lipids and hemoglobin oxidation. Furthermore, the study of perfluorocarbon corrective action on the RBC membranes after influence of various modificators and ionizing radiation was carried out.

MATERIALS AND METHODS

Blood sampling was produced from six healthy donors during preventive examination into microvette (Sarstedt AG and Co., Germany), containing EDTA. In accordance with ethics commission of the Scientific Research Institute of General Reanimatology RAMS all donors signed the informed consent form.

Suitable agents (solutions of ZnSO4 and NaNO2, verapamil) were added to blood in vitro in therapeutic concentrations, and substantially more than therapeutic. Monolayers of RBC were prepared on slides using the device V-Sampler (Austria). All measurements were performed in air. Cell images were obtained using an atomic force microscope (AFM) NTEGRA Prima (NT-MDT, Russia). Rigidity of the membranes was evaluated by atomic force spectroscopy method by power curves, which were registered in seven areas of the cell. Measurements were made on the 4 cells of 1 sample. The value of the coefficient of rigidity for different areas of a cell and different cells has dispersion. What is why a statistical distribution of the local rigidity was built and the average local rigidity of RBC membranes was estimated for each exposure.

The effect of ionizing radiation on the blood was studied. For this purpose ultraviolet radiation of germicidal lamp was used, λ = 200 nm.

RESULTS

Images of RBC under the action of each noted modificators were obtained. Specific changes for each type of exposure were observed. The rigidity of RBC changed differently for different modificators compared with the control.

AFM images of nanostructure of RBC membranes after action of Zn2+ ions are presented on Fig.1.

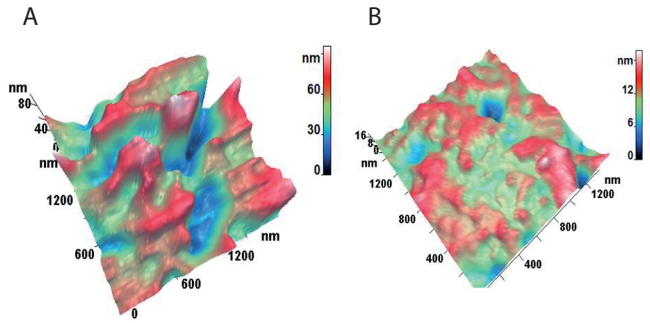

Fig.1. *AFM images of nanostructure of RBC membranes after action of Zn2+. A. Damaged area; B. Undamaged area.*

Along with the change of the membrane nanostructure average local rigidity of the membrane changed. Statistical data for the local rigidity for the control cells, the action of zinc, and subsequent corrective action of perfluorocarbon are shown in Table 1.

Table 1.
Average local rigidity of membrane after influence of zinc ions (M ±m)

	Control	Zinc	Zinc+Perfluorocarbon (60% measurements)	Zinc+Perfluorocarbon (40% measurements)
K (отн.ед.)	4,3±2,6	8,7±2,2*	4,7±2	8,4±1,1*

* -p<0,05 compared with control.

NaNO2 modificator in a concentration of 25 µl/ ml of blood changed nanostructure of membrane and cell shape (Fig. 2B) compared with control (Fig. 2A). Perfluorocarbon corrected alterations and return the membrane topology and shape of the cells to the original (Fig. 2C).

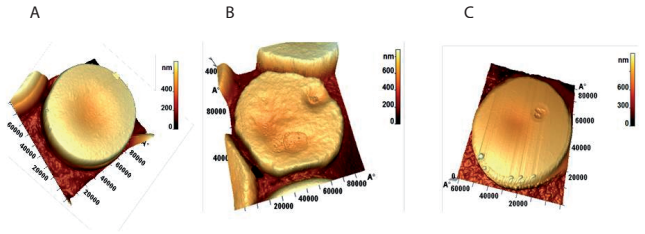

Fig.2. *A.Control cell. B. The cell after influence of NaNO2. C. After influence of NaNO2 and the subsequent addition of perfluorocarbon.*

After the influence of NaNO2 the rigidity increased compared with the control value in 1.95 times ($p < 0.05$) in 95% of cases (Fig. 3). Subsequent influence of perfluorocarbon returned the rigidity of the membrane to the original values in 90% of cases. The rigidity of membrane of remaining 10% of areas on the cells remained high ($p < 0.05$) (Fig. 3).

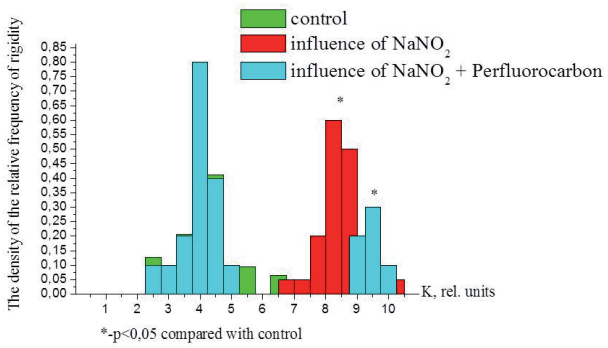

Fig. 3*. Histograms of membrane rigidity in the control, after influence of NaNO2 and after influence of NaNO2 and the subsequent addition of perfluorocarbon.*

Under the influence of verapamil there was a change of cell shape and nanostructures (Fig. 4). The topology of membrane changed on the membrane of cells in certain local areas, the dips formed (Fig. 4). Image of nanostructures of such local distructions presented in 4B and the characteristic size of such dips d = 300-700 nm, h = 150-200 nm. But the rigidity after influence of verapamil almost was not changed.

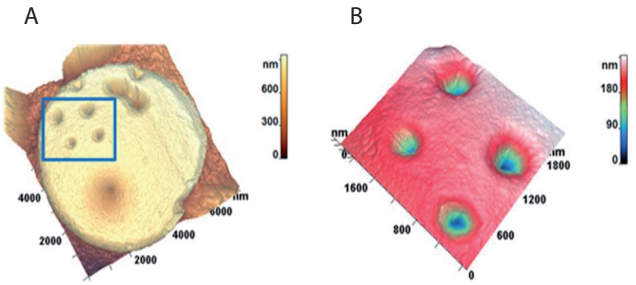

Fig. 4. *The result of influence of verapamil: A. Red blood cell with deep cavities. B. The surface of the area of red blood cell cavities, isolated with blue square in Fig. 4A.*

Under the action of ionizing radiation on the suspension of RBC local alteration of nanostructure of membrane was happened, which was accompanied by a release of K+ ions and hemoglobin molecules from the cell.

CONCLUSIONS

Studying of nanosurface of membranes and measurement of the local rigidity using AFM is a promising method to study the mechanisms of influence of various physical and chemical factors on the RBC membranes and to produce the correct tactics of treatment in critical states.

REFERENCES

1. Elena K. Kozlova, Alexander M. Chernysh, Victor V. Moroz, Artem N. Kuzovlev. Analysis of nanostructure of red blood cells membranes by space Fourier transform of AFM images. Micron, 44 ,2013,218–227.
2. A. M. Chernysh, E. K. Kozlova, V. V. Moroz, V. A. Sergunova, O. Ye. Gudkova, and M. S. Fedorova. Reversible Zinc-Induced Injuries to Erythrocyte.Membrane Nanostructure. Bulletin of Experimental Biology and Medicine, Vol. 154, No. 1, November, 2012.
3. Elena K. Kozlova, Alexander M. Chernysh, Victor V. Moroz, Victoria Sergunova, Olga Gudkova, Maya Fedorova, Artem N. Kuzovlev. Opposite effects of electroporation of red blood cell membranes under the influence of zinc ions. Acta of Bioengineering and Biomechanics, Vol. 14, No. 1, 2012.

TARGETING OF MESANGIAL CELLS WITH LIGAND-CONJUGATED QUANTUM DOTS

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Diabetic nephropathy (DN) is the most common cause for end-stage renal disease in the United States requiring dialysis [1]. Ultimately, between 20 % and 40 % of all patients with Diabetes mellitus will develop nephropathy. Mesangial cells represent one third of all cells in the renal glomerulus and constitute the central stalk. They have a tremendous influence in the pathogenesis of diabetic nephropathy as they regulate the turnover of the mesangial matrix [2]. Mesangial cell hypertrophy and the expansion of the mesangial matrix due to elevated blood glucose levels are two of the earliest features of DN leading to glomerulosclerosis [3]. Lately, mesangial cells emerged as an interesting target for nanoparticular drug delivery as they can be passively targeted with colloids smaller than 100 nm. This is possible due to the size of the endothelial fenestrations the mesangium is located behind [4]. Here we want to show a ligand-mediated targeting of rat mesangial cells with highly fluorescent quantum dots (Qdots) coupled to angiotensin II, the physiological receptor agonist of the angiotensin II receptor type 1 (AT1). Commercially available amine-terminated Qdots were covalently labeled with an N-terminal thiolated angiotensin II derivative using the heterobifunctional linker sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC). The sulfhydryl moiety was introduced at the N-terminus of angiotensin II with the help of 2-iminothiolane. To show that mesangial cells express a functional Gq-coupled AT1 receptor, intracellular calcium measurements were conducted using the ratiometric calcium indicator Fura-2AM. Angiotensin II as well as the agonist-coupled Qdots displayed a strong intracellular calcium signal. In flow cytometry experiments the ligand-modified Qdots exhibited a high binding to the mesangial cells that was displaceable with the AT1 receptor antagonist EXP3174, the active metabolite of losartan. The binding of these ligand-coupled nanoparticles to receptor-negative cell lines was significantly lower.

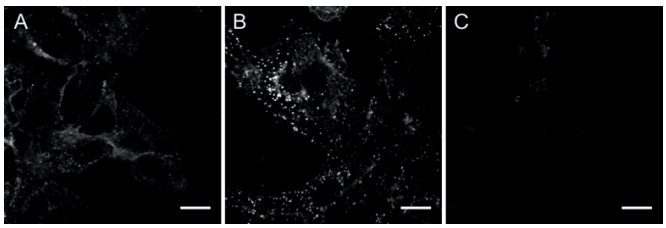

Figure 1*: Confocal microscopic pictures of mesangial cells incubated with non-modified Qdots (A) or Angiotensin II-modified Qdots (B). The spot-like fluorescence of ligand-coupled Qdots diminishes in the presence of EXP3174 (C). Scale bar: 20 µm.*

Confocal laser scanning microscopy showed the distinct uptake of the modified Qdots into vesicle-like structures throughout the cytosol (Figure 1B), which is a prerequisite for nanoparticle delivery to cells. The intracellular accumulation of the Qdots could also be blocked by coincubation with EXP37174 (Figure 1C), underlining the receptor-mediated nature of the nanoparticle endocytosis. Non-modified Qdots showed only minor unspecific binding to the cell membrane (Figure 1A).

In conclusion we could show that ligand-modified Qdots can be used to target mesangial cells in vitro. The nanoparticles specifically bind the AT1 receptor and are taken up by the cells whereas off-target cells do not interact with the Qdots.

[1] A. J. Collins et al., Am. J. Kidney Dis. 2013, 1 Suppl 1, A7, e1-476
[2] D. Schlöndorff et al., J. Am. Soc. Nephrol., 2009, 6, 1179–1187
[3] H. E. Abboud, Exp. Cell Res., 2012, 9, 979–985
[4] C. H. Choi et al., Proc. Natl. Acad. Sci. U.S.A., 2011, 16, 6656–6661

MODELING AND UNDERSTANDING THE INTERPLAY OF CROWN DE- AND REMINERALIZATION TO REPAIR TEETH USING NANOMEDICINE APPROACHES

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In teeth, early carious lesions can be reversed by ion delivery to the demineralized tissue [1, 2]. This process is limited by the severe reduction of the guiding enamel matrix and the ionic diffusion that mainly mineralizes the surface and prevents further mineralization inside the lesion. Delivery systems based on nanoparticles are promised to overcome these limitations because of their reaction potential resulting from the large surface-to-bulk ratio. The mineral nanoparticles can enter the lesion, approach the desired site and release gradually the containing ions for the mineralization of enamel and dentin [3]. Recent studies have shown that even after severe demineralization due to caries the overall nanostructural framework of the crown tissue remains intact [4, 5]. As a result, the remineralization of moderate carious lesions can become feasible.

Since mineral grains grow from controlled nucleation and dissociate from controlled dissolution processes [6, 7], the simulation of these phenomena will provide a deep insight into the interplay of de- and remineralization taking place in the human oral cavity every day. The role of individual parameters and quantities on the atomic or molecular level such as time-dependent concentrations of calcium ions, anisotropic crystal scaffolds and crystallite sizes can be studied in detail.

In order to repair the crowns, the simulations will be extended incorporating two remineralization processes. The release of ions from intentionally delivered nanoparticles increase the concentration of calcium. The growth of the calcium phosphate crystallites mediated by nucleation and aggregation of available calcium ions decreases their concentration. The solution of ions and the growth of crystallites are rather complex biomineralization processes but can generally be described by dissolution theory [8], mean-field nucleation theory, scaling theory, and rate equation analysis [9]. Based on these first principles, mathematical models were derived to cover the entire process chain ranging from the delivery via nucleation to mineralization. The underlying kinetics were partly understood and published [10]. Quantities such as the size of the critical nucleus and the activation barriers for monomer migration and binding have to be derived from the spare literature data and later be deduced from experiments.

The rate-equation approach involves a set of deterministic coupled reaction-diffusion equations describing the time dependence of the average island density while conserving mass. Figure 1 illustrates one of the simplest situations, namely the two-dimensional growth of one-atom-high islands on a square lattice assuming that the dimer is the smallest stable island. The individual parts are explained using key words and schematic drawings. The time-dependent monomer density is increased by the incoming monomers and decreased by migration-limited nucleation and aggregation processes. The island density is increasing by the island formation and decreasing by coalescence of islands. Further processes including dissociation phenomena can be easily included and the extension towards the third dimension is available [11].

The in silico experiments will not only provide a detailed understanding of the fundamental processes in vitro and in vivo but also promote the optimization of the nanoparticle efficacy. The input arguments of the optimization will include the size of the nanoparticle core and its surface or coating including composition, which determines the kinetics of the dominating physico-chemical processes involved. Both the amount of materials and their kinetics affect the final outcome of the mineralization profile. The challenge consists of maximizing the homogeneity of the profile while minimizing the transport loss of the delivery. The results will not only be restricted to teeth, but also applied to bone and later to soft tissue nanocomponents.

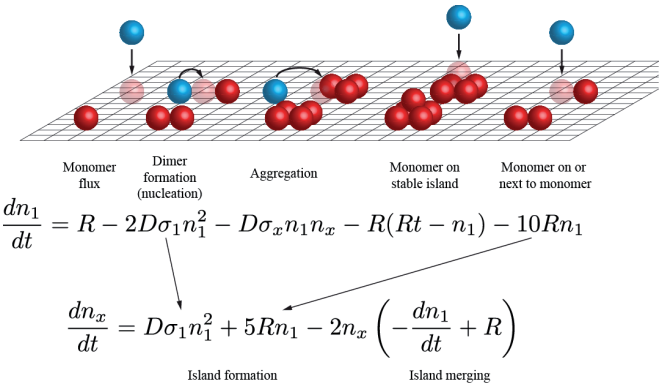

Figure 1. *Rate equation analysis of two-dimensional island formation on square lattices with the dimer being the smallest stable island, where n1 represents the monomer density, nx the density of stable islands, D the migration coefficient, R the deposition rate, t the time, s1 and sx the capture number of monomers and stable islands, respectively (for details see [9])*

REFERENCES

[1] Deyhle H, Hieber SE, Müller B. Nanodentistry. In: Bhushan B, Winbigler HD, editors. Encyclopedia of Nanotechnology Berlin Heidelberg: Springer Verlag; 2012. p. 1514-8.
[2] Hieber SE, Müller B. Nanodentistry. In: Logothetidis S, editor. Nanomedicine and Nanobiotechnology. Berlin Heidelberg Springer-Verlag; 2012. p. 95-106.
[3] Vollenweider M, Brunner TJ, Knecht S, Grass RN, Zehnder M, Imfeld T, et al. Remineralization of human dentin using ultrafine bioactive glass particles. Acta Biomaterialia. 2007;3:936-43.
[4] Imbeni V, Kruzic JJ, Marshall GW, Marshall SJ, Ritchie RO. The dentin-enamel junction and the fracture of human teeth. Nat Mater. 2005;4:229-32.
[5] Gaiser S, Deyhle H, Bunk O, White SN, Muller B. Understanding Nano-Anatomy of Healthy and Carious Human Teeth: a Prerequisite for Nanodentistry. Biointerphases. 2012;7.
[6] Turnbull D, Fisher JC. Rate of nucleation in condensed systems. Journal of Chemical Physics. 1949;17:71-3.
[7] Wang LJ, Nancollas GH. Calcium Orthophosphates: Crystallization and Dissolution. Chemical Reviews. 2008;108:4628-69.
[8] Dokoumetzidis A, Macheras P. A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System. International Journal of Pharmaceutics. 2006;321:1-11.
[9] Müller B. Natural formation of nanostructures: From fundamentals in metal heteroepitaxy to applications in optics and biomaterials science. Surface Review and Letters. 2001;08:169-228.
[10] Brunner TJ, Grass RN, Bohner M, Stark WJ. Effect of particle size, crystal phase and crystallinity on the reactivity of tricalcium phosphate cements for bone reconstruction. Journal of Materials Chemistry. 2007;17:4072-8.
[11] Venables JA, Spiller GDT, Hanbucken M. Nucleation and growth of thin films. Reports on Progress in Physics. 1984;47:399-459.

MULTIMODAL MAGNETIC NANOPARTICLES FOR THE TREATMENT OF BREAST AND PANCREATIC CANCER (MULTIFUN-PROJECT)

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Currently, therapy of cancer is mainly based on different modalities, which are distinctly selected depending on the cancer stage and tumor entity to be treated. Individually, the modalities of surgery, radio-, chemo-, hormone, immune therapy and hyperthermia are not highly specific, leading to side effects as well as long and painful patient recovery. Experience from the clinical and preclin cal studies has shown that a dedicated combination of these modalities could markedly increase the therapeutic outcome and patient compliance. In this context, the project MULTIFUN evaluates the synergy of therapeutic modalities with regard to breast and pancreatic cancer cells at early stages of tumour development. The multimodal therapeutic strategy is based on particularly designed multifunctional magnetic nanoparticles to selectively apply magnetic heating and targeted delivery of anti-cancer agents to the tumor region. This large scale European project with partners in Spain, Ireland, Great Britain, France, Belgium, Germany, and Czech Republic started in June 2011. Since then, we have being evaluating dedicated nanoparticle formulations in terms of their heating capabilities, morphologic and magnetic features, in vitro biocompatibility in isolated cells, pharmacokinetic properties and their therapeutic efficacy in vivo.

Synthesis and functionalization of nanoparticles. MULTIFUN has developed novel methods of synthesis for the production of biocompatible magnetic nanoparticles (MNP) with excellent magnetic properties for MRI detection and magnetic heating. The optimized MNPs are multifunctionalized with distinct molecules in order to selectively target and to deliver anti-cancer agents inside the tumor cells. Different immobilization strategies have been developed to functionalize MNPs with small molecules, peptides, antibodies and siRNAs.

Biological effects on breast and pancreatic cells. To assess the nanotoxicity and biocompatibility of multifunctional nanoparticle (MNP) formulations used in the study an in vitro high content screening approach was adopted. Four breast cancer cell models (MCF-7, BT-474, MDA-MB-231, SK-BR-3) and the MCF-10A normal-like breast cell model were monitored for cell count reduction, changes in lysosomal intensity and pH and an increase in cell permeability following 24 h exposure to a range of MNP concentrations, as previously reported [Prina-Mello et al., IEEE Trans Magn 2013]. Cytotoxicity was observed in a concentration dependent manner with some MNPs at the highest dose of 0.2 mg/mL elucidating a cytotoxic response. Below this concentration cytotoxic effects became less pronounced and similarities in measured parameters between cell models became evident.

Outcome of screening in terms of cytotoxicity, ultramacroscopy and mechanism of internalization were then used as a basis for the Pharmacodynamic work.

Pharmacodynamics. Pharmacodynamic studies were based on the pharmacokinetic (PK) properties of the MNP in rat and pig models and their biodistribution in mouse and pig modes was assessed. All measurements were carried out by the particle electron paramagnetic resonance (pEPR) technique and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique. The PK study of MNPs was described with a twocompartmental model and PK parameters, such as half-life or clearance were calculated. To assess the biodistribution of the MNPs, in vivo MR imaging (at 7 T and 3 T) was used. Post scanning, animals were humanely sacrificed under anesthesia and organs harvested. The quantity of MNPs in the harvested organs was then evaluated by pEPR and ICP-MS for comparative analysis. Results from MRI, pERP and ICP-MS show time-dependent organ-specific accumulation of the MNPs and subsequent clearance, which allow fort the hyperthermia treatment in vivo.

Effects in the in vivo situation in tumor bearing mice. To evaluate the therapeutic effect oft he MNP and the tolerance of the treatment in vivo human breast or pancreaticcells were implanted subcutaneously in the mice. The heating effects oft he NMP were monitored through measurements oft he body weight oft the animals and the tumor volume over 28 days after MNP application and/or exposure to an allernating magnetic field (magnetic heating). Using nanoparticle dosages of 0.3 mg/100 mm3 tumor tissue, local temperature increases above 42°C were achieved. The magnetic heating treatment was highly tolerable, since body weights and hemograms of treated animals were not different from non-treated controls. In most oft he cases a reduction o tumor volumes was observed. The data show the highly localized therapeutic efficacy of the heating modality of magnetic nanoparticles.

CONCLUSION

MULTIFUN project is aimed to deliver theranostic nanoparticle treatment to two types of cancer (breast and pancreatic). The approach taken by the consortium is based on the use of purposely designed multifunctional superparamagnetic iron oxide nanoparticles for contrast imaging and hyperthermia treatment.

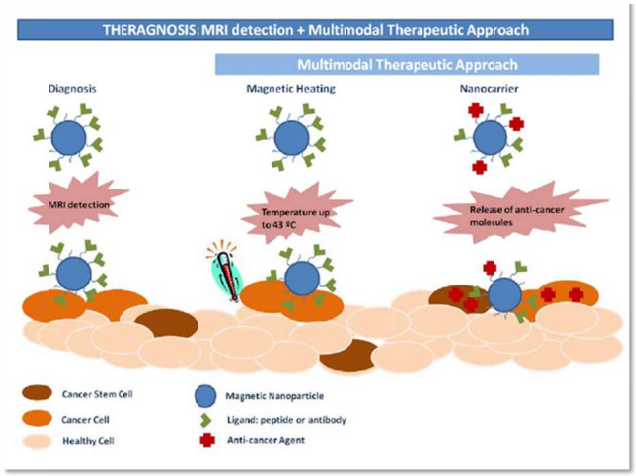

***Figure 1**. Schematic presentation of magnetic nanoparticle related functionalities pursued in the MULTIFUN project to treat breast and pancreatic cancers in a multifuncional approach.*

NANOEMULSIONS WITH ANTIRETROVIRAL DRUGS TO ASSESS THE EFFICACY AGAINST LIVE LABORATORY STRAIN OF HIV-1 VIRUS IIIB

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[1]Department of Molecular and Clinical Pharmacology, University of Liverpool. [2]Department of Chemistry, University of Liverpool.

AIM

Oil in water nanoemulsions have been developed and successfully loaded with antiretroviral drugs Lopinavir (LPV) and Efavirenz (EFV). The purpose of this study was two-fold, firstly to determine the safety of this new formulation in terms of immunogenicity, and secondly to assess the efficacy against a live laboratory strain of HIV-1 virus IIIB.

IMMUNOLOGICAL SAFETY ASSESSMENT

Immunological assessment was performed using three separate assay procedures. For all experiments, peripheral blood mononuclear cells were used, extracted from whole blood using a Ficoll centrifugation gradient method.

Initially, the expression of cell surface activation markers (CD25, CD44, CD69 and CD95) were determined using both CD4+ and CD8+ cells separated from the PBMC's by magnetic separation using the MACS system (Miltenyi Biotech). The data showed that there was no increase in the expression of the activation markers as a result of exposure to the nanoemulsion formulation, indicative of a non-immunogenic compound, at least in vitro. Indeed, the nanoemulsion was comparable to a standard aqueous solution in terms of its impact upon expression of cell surface markers (Figure 1).

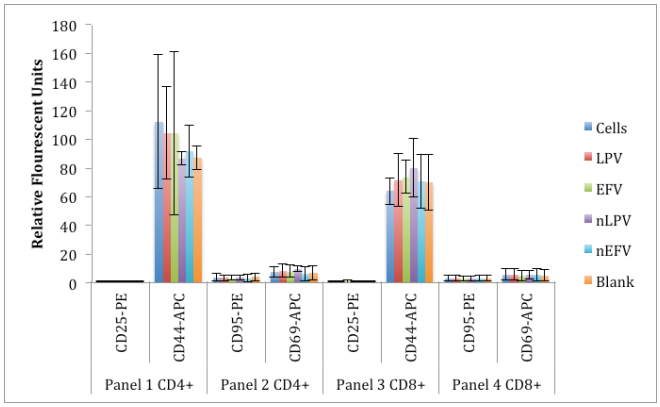

Figure 1. *Expression levels of cell surface activation markers after 24 hour exposure to aqueous or nanoemulsion (n) formulations of LPV and EFV.*

The extracellular media of the expression assay was isolated and analysed for the secretion levels of cytokines IL-2, IL-10 and IFNγ using a Bioplex 200 system and assay kit (Bio-Rad). Data for cells that were pre-stimulated with a T-cell activation kit (Miltenyi Biotech) showed that the levels of cytokines secreted by those cells exposed to nanoemulsions similar to those observed after exposure to aqueous solutions of the same drugs. In the case of IFNγ, the LPV nanoemulsion was more comparable to the control with respect to its secretion profile, than was the aqueous solution of LPV (Figure 2). The same experiment was conducted on un-stimulated cells, with the results showing undetectable levels of cytokines across all conditions (data not shown).

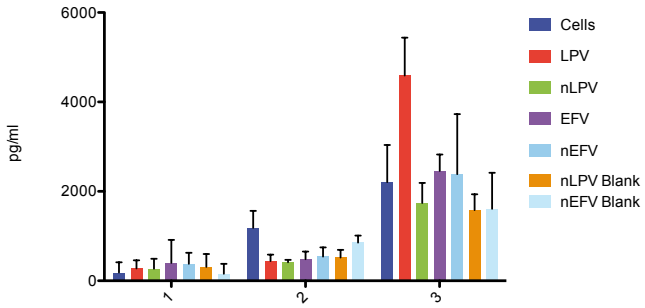

Figure 2*. Secretion levels of cytokines IL-2 (1), IL-10 (2) and IFNg (3) after 24 hour incubation with aqueous or nanoemulsion ARV formulations.*

The effect of the nanoemulsion on the cellular proliferation of PBMCs was also investigated, using a 3H-Thymidine incorporation assay. Briefly, PBMCs were grown for 72 hours in the presence of aqueous solution or nanoemulsion equivalent, with 3H-Thymidine added during the last 16 hours of incubation. Radiometric analysis was used to determine any effect of these incubations on proliferation. The experiment was conducted with and without the addition of Phytohaemagglutinin (PHA), used to stimulate the cells to proliferate. The data showed that across all formulations and conditions, no differences were observed between treated and untreated cells (Figure 3).

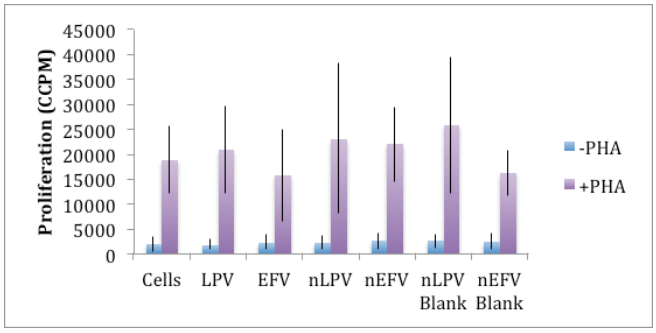

Figure 3*. Proliferation of PBMC's grown in the presence of aqueous or nanoemulsion formulations of ARVs.*

ANTIVIRAL ACTIVITY

The antiviral activity of the nanoemulsion formulations was assessed against the laboratory-adapted strain HIV-1 IIIB, using an MTT assay to determine the viability of a human lymphocyte CD4+ cell line (MT4), after exposure to the virus (with and without aqueous or nanoemulsions). Cells were incubated for a period of 5 days. The data showed that the antiviral activity for the nanoemulsion formulations was similar to that of aqueous solutions, with IC50 values of 0.46 µM and 0.18 µM for LPV and EFV, respectively. The dose response curve for nanoemulsion EFV was identical to that of aqueous EFV, whereas nanoemulsion LPV had a greater viral kill at lower concentrations, as compared with aqueous solution (Figure 4.).

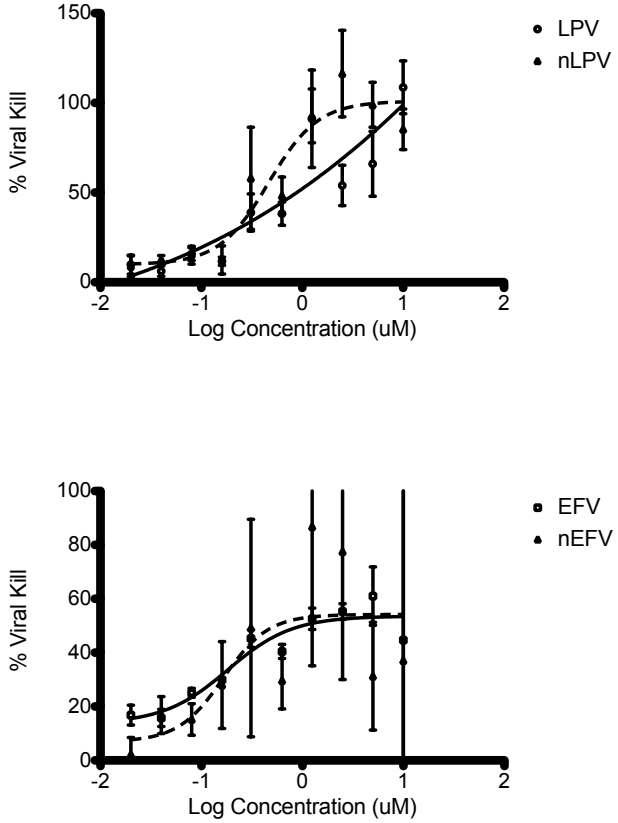

Figure 4*. Viral kill curves of HIV-1 IIIB based upon viability of MT4 cells incubated with either Aqueous or nanoemulsion formulations of LPV (top) and EFV (bottom).*

In summary, we have successfully developed a nanoemulsions system for the poorly water-soluble drugs LPV and EFV. These nanoemulsions had no overt immunological effects in human PMBCs, and the antiviral activity of the drug was not affected by encapsulation within the emulsion. Drug-loaded nanoemulsions may have utility in HIV therapy and further work is now warranted.

DEVELOPMENT OF NOVEL NANOTECHNOLOGY BASED DIAGNOSTIC SYSTEMS FOR RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS NANODIARA

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Inflammatory joint disorders like Rheumatoid Arthritis (RA) together cause the destruction of articular cartilages in about 2-3 % of the population. These disorders are accompanied by significant pain, morbidity and mortality, leading to a reduction in the capacity to work. Osteoarthritis (OA), a degenerative arthritis, is the leading cause of disability among the elderly population affecting over 50% of those over 60 years of age. Effective disease modifying drugs called biologics are now available to treat RA. But in OA drugs only reduce pain but not slow the disease. This often finally results in joint replacement in OA, or treatment may cause side effects in RA.

Existing methods often do not permit early diagnosis of arthritis nor its progression and early responses to therapies if available.
Based on such clinical unmet needs the recent research in safe nanoparticles and in biomarkers of RA and OA disease onset and activity offer the new invaluable technology that will address these as yet unmet needs. The main objective of the project is therefore to develop nanotechnology-based novel diagnostic tools for easy and early detection of arthritic disease and its activity using modified superparamagnetic iron oxide nanoparticles (SPION) for ex-vivo application of sensitive biomarker micro-immunoassays for use with body fluids and in-vivo detection using functionalized nanoparticles targeted to detect by MRI molecular events in joints involved in arthritis onset and activity long before structural damage occurs that is presently detected by available imaging technology.
Superparamagnetic nanoparticles whose surfaces can be derivatised and coated with special targeting molecules used in the NanoDiaRA project exhibit their special magnetic properties only in the nano-range between 5 and about 20 nm. To become highly specific and capable of being targeted to specific cells, and even more specifically to specific cell surfaces or cell compartments or to specific biomolecules present in biofluids, it is necessary to functionalize the surface of the particle such that it is able to "recognize" and bind to specific joint tissues and components thereof. This "targeted tailoring" of nanoparticles will be made possible using specific coatings and functionalization steps and thereby attach targeted molecules already developed or under development in the NanoDiaRA project. For this to happen expert collaborations must be formed for pre-clinical and clinical applications by involving the multidisciplinary expertise of researchers at universities and research institutes.
The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 228929.

DELIVERY OF ALBENDAZOLE NANOPARTICLES TO THE OVARIAN CANCER SYNTHESIS TO IN-VIVO STUDY

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Albendazole (ABZ), an anti-anthelmintic drug, inhibits microtubule farmation leading to disrupted mitosis and cell death and exhibit potential antitumor activity with experimental evidence. Recent studies from our group have shown that ABZ is a potent inhibitor of cell proliferation of a wide variety of cell lines, such as hepatocellular, colorectal, pancreatic and ovarian cancer cells[1,2]. It has also been observed that ABZ inhibits peritoneal growth of human colorectal cancer cells (HT-29) by inhibiting VEGF (vascular endothelial growth factor) production and abolishes tumor angiogenesis and ascites formation in OVCAR-3 xenograft tumor model.[3] Collectively, these findings are indicative of significant clinical potential for using ABZ in the systemic treatment of cancer.
However, the low solubility of ABZ remains a challenge limiting its use as systemic anticancer agents. Among the potential drug carrier systems, albumin is an attractive carrier that has been shown to be biodegradable, nontoxic, non-immunogenic and its solubility in water allowing ease of delivery by injection. A high concentration of drugs can be loaded due to different drug binding sites present into the albumin. Moreover, albumin allows the adsorption of various drugs molecules resulting in the formation of nano-size albumin aggregates (nab-technology).4 More importantly, albumin nanoparticles can easily accumulate in tumor tissue due to the enhanced permeability and retention (EPR) effect[5, 6] In addition, studies have also suggested that the accumulation of albumin based nanoparticles within the tumor tissue is also caused by transcytosis. The binding of albumin to 60KDa glycoprotein (gp-60) receptor results in the binding of gp60 with caveolin-1 and the consequent formation of transcytotic vesicles.7 Therefore, the nab-technology platform is now hailed as promising drug delivery pathway in oncology.
The ABZ loaded albumin nanoparticles were prepared by a pH sensitive desolvation process. The particle size was measured by dynamic light scattering (DLS) and confirmed by transmission electron microscope (TEM). The loading and encapsulation efficiency of the drug and release kinetics were measured by high performance liquid chromatography (HPLC). The cytotoxicity of the drug was measured using the well-established SRB assay to determine the IC50 of the drug. The cellular internalization was observed by confocal laser scanning microscopy and quantitatively measured by flow cytometry. In-vivo efficacy of the drug was observed by establishing the OVCAR-3 intraperitoneal xenograft model.
Our prepared nanoparticles were designed and optimized to achieve a particle size in the range of 150-300 nm with drug encapsulation efficiency 70%- 90%. Cell viability was only 20% in SKOV-3 cells and 80% in HOSE(Human ovarian surface epithelial cells) after 72 h treatment using an ABZ concentration (0.4 µM), which represents a significant improvement to the drug alone. Cellular uptake studies were performed in SKOV-3 and OVCAR-3 cell lines using fluorescent labeled albumin. It wasobserved that mostly drug carriers (100µg/ml) were internalized within 1 h of treatment with the uptake reaching saturation within 2 h of treatment. Drug release studies also revealed that pHsensitive release behavior was observed in a controlled release fashion.After 72 h 20% of the drugs were released in PBS at pH7 and 70% were released in PH4. Efficacy of nab-ABZ was performed in OVCAR-3 bearing intraperitoneal xenograft tumor model volume and ascites fluids. We analyzed the findings and found that the particle size could be the key factor affecting inhibition of tumor growth. Further plan was ahead to see the in-vivo fate of ABZ NPs comparing different particle size in subcutaneous xenograft tumor model.
In conclusion, these results have great significance in developing and optimizing effective intracellular delivery of ABZ to the tumor tissue while minimizing damage to healthy cells. Therefore, ABZ albumin nanoparticles may have great translational potential for the treatment of ovarian cancer patients.

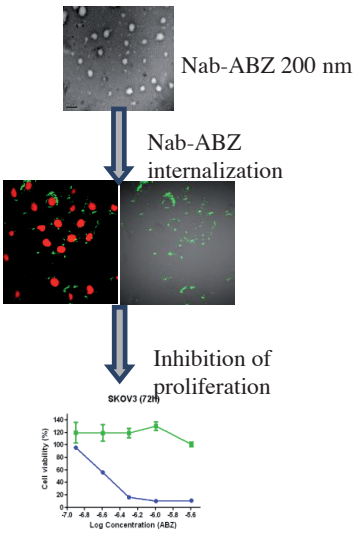

***Figure1**: Sequence of events, nab-ABZ was synthesized and internalized into the SKOV-3 cells followed by inhibition of proliferation and reduced cell viability.*

REFERENCES

1. Pourgholami, M.H., Akhter, J., Woon,L.,Almajd, R., Bowery,P., Morris, D.L. In vitro and in vivo suppression of growth of hepatocellular carcinoma cells by albendazole. Cancer Letters 165, 43-49 (2001).
2. Pourgholami, M.H., Akhter, J., Wang, L., Lu, Y. & Morris, D.L. Antitumor activity of albendazole against the human colorectal cancer cell line HT-29: in vitro and in a xenograft model of peritoneal carcinomatosis. Cancer Chemotherapy and Pharmacology 55, 425-432 (2005).

3. Pourgholami, M.H., Yan Cai, Z., Lu, Y., Wang, L. & Morris, D.L. Albendazole: a potent inhibitor of vascular endothelial growth factor and malignant ascites formation in OVCAR-3 tumor-bearing nude mice. Clinical Cancer Research 12, 1928-1935 (2006).
4. Elsadek, B. & Kratz, F. Impact of albumin on drug delivery - New applications on the horizon. Journal of Control Release 157(1):4-28 (2012)
5. Maeda, H., Wu, J., Sawa, T., Matsumura, Y. & Hori, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. Journal of controlled release : official journal of the Controlled Release Society65, 271-284 (2000).
6. Maeda, H. Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting. Proceedings of the Japan Academy.Series B, Physical and biological sciences88, 53-71 (2012).
7. Desai, N. et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. Clinical cancer research : an official journal of the American Association for Cancer Research12, 1317-1324 (2006).

PREPARATION OF POROUS NANOPARTICLES BY SALT-LEACHING AND THEIR USE AS CONTROLLED RELEASE VEHICLES

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Biodegradable micro/nanoparticles have emerged as promising drug delivery vehicles. Porous and non-porous PLGA nanoparticles are widely used as drug carriers in targeted drug delivery applications. We report salt-leaching as a novel approach to synthesize porous PLGA nanoparticles and their deployment as controlled release systems. Bovine serum albumin (BSA) was used as model drug and loaded into porous and non-porous nanoparticles. Water-in-oil-water (w/o/w) double emulsion method was used to prepare these nanoparticles (porous and non-porous). Double emulsion method prevented BSA from getting degraded in an organic solvent like chloroform. Porous nanoparticles were synthesized using sodium bicarbonate as the extractable porogen. The physical characterization of porous and non-porous nanoparticles was done using laser scattering, zeta potential analyzer and scanning electron microscopy (SEM). SEM micrographs of synthesized nanoparticles showed spherical morphology for porous and non-porous nanoparticles with average diameters of 525.2 nm and 321.8 nm respectively. In vitro drug release study for both types of nanoparticles was carried out for 30 days. Porous nanoparticles showed better and sustained release behavior over the same length of time. The drug loading efficiencies for porous and non-porous PLGA nanoparticles were determined to be 65.5% and 77.59% respectively. Similarly, the cumulative release for porous and non-porous PLGA nanoparticles was also measured to be 87.41% and 59.91%, respectively. The preparation of porous nanoparticles using this rapid and inexpensive technique opens the door to investigate many other water-soluble salts for their use as extractable porogens. Enhanced drug release and high stability of porous PLGA nanoparticles with potential for various alternative porogens may facilitate controlled release of encapsulated drugs.

INTRODUCTION

Poly(lactic-co-glycolic acid) (PLGA) micro/nanoparticles have evolved as effective drug carriers due to their large surface area, low-price, high-stability, and biodegradability [1, 2]. Various approaches that include single and double emulsion methods to synthesize these polymeric micro/nanoparticles have been reported before. PLGA-based particles with porous structures on their surface offer better drug stability and optimized therapeutic efficiency by evading the accelerated polymer degradation [3, 4]. Porous nanoparticles have significant impact in controlled drug release systems but require efficient synthesis techniques.

MATERIALS AND METHODS

Water-in-oil-in-water (w/o/w) double emulsion method was used to prepare porous and non-porous PLGA nanoparticles where freeze-drying was used for the phase separation. Sodium bicarbonate was used as the extractable porogen to introduce pores on the surface of nanoparticles. The water-soluble salt was extracted through the salt-leaching process. SEM micrographs and dynamic light scattering (DLS) readings were recorded to measure the particle size, polydispersity and zeta potential of the synthesized nanoparticles. Drug loading efficiency was calculated using the indirect method that used the amount of unloaded drug in the supernatant to reflect the amount of loaded drug. Finally, drug release activities for both porous and non-porous nanoparticles were observed. The particles were placed in separate dialysis bags which were submerged in PBS solution. After regular intervals, the samples were collected from the surrounding PBS to determine the drug released at that particular time point. Pierce BCA protein assay was employed for quantitative study of the drug released.

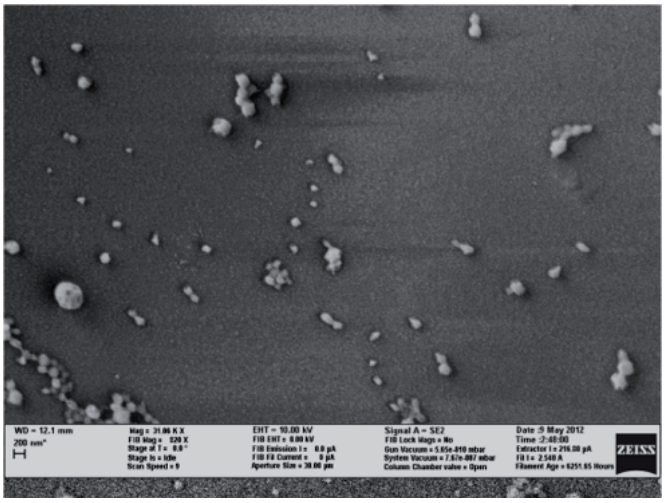

Figure1. *SEM micrograph shows that porous nanoparticles synthesized by salt-leaching process have spherical morphology with an average diameter of 525.2 nm. The inset shows transmission electron micrograph of non-porous nanoparticles.*

RESULTS AND DISCUSSION

Sodium bicarbonate incorporated by the PLGA nanoparticles was leached out at later stage and left porous features on the surface of the nanoparticles. Both types of nanoparticles (porous and non-porous) exhibited spherical shape as shown in Figure 1. The average diameter for porous and non-porous nanoparticles was recorded to be 525.2 nm and 321.8 nm respectively. Porous particles showed larger size possibly due to the incorporated salts. Polydispersity and zeta potential values confirmed that both the particles were highly stable in colloidal suspension and displayed very uniform size distribution.

The drug loading efficiency for BSA loaded porous and non-porous nanoparticles was calculated to be 65.50% and 77.59% respectively. Porous nanoparticles lost a part of loaded drug during the salt-leaching process, resulting in poorer loading efficiency. The drug release behavior of porous and non-porous nanoparticles showed that porous particles gave much better release profile over the same time. Porous features on the particles' surfaces aided easy release of drug in conjunction with an increased surface area [5, 6].

The cumulative drug release for porous and non-porous PLGA nanoparticles over a period of 30 days was calculated to be 87.41% and 59.91% respectively. The release behavior could be optimized by varying the porosity which was directly controlled by the porogen concentration used. Such a controlled and sustained release is highly desired for controlled drug delivery systems.
Conclusion: We present a simple and straightforward approach to synthesize porous nanoparticles. Sodium bicarbonate was used as porogen which was extracted through the salt-leaching process and rooted the formation of porous structures on the nanoparticles surface. The drug release study revealed that porous nanoparticles offered much more controlled and sustained release behavior, owing to their large surface area and ease of drug release through the leaky structures on the particle surfaces. This study shows possibility of new investigations of using several other cheap and easily accessible water-soluble salts as extractable porogens to prepare porous nanoparticles for controlled drug delivery systems.

REFERENCES

[1] S. Gelperina, O. Maksimenko, A. Khalansky, L. Vanchugova, E. Shipulo, K. Abbasova, R. Berdiev, S. Wohlfart, N. Chepurnova, and J. Kreuter, "Drug delivery to the brain using surfactant-coated poly (lactide-co-glycolide) nanoparticles: influence of the formulation parameters," European Journal of Pharmaceutics and Biopharmaceutics, vol. 74, pp. 157-163, 2009.
[2] B. Magenheim and S. Benita, "Nanoparticle characterization: a comprehensive physicochemical approach," STP Pharma Sciences, vol. 1, pp. 221-241, 1991.
[3] L. Fan and S. K. Singh, Controlled release: A quantitative treatment: Springer-Verlag Berlin, 1989.
[4] D. Klose, F. Siepmann, K. Elkharraz, S. Krenzlin, and J. Siepmann, "How porosity and size affect the drug release mechanisms from PLGA-based microparticles," International Journal of Pharmaceutics, vol. 314, pp. 198-206, 2006.
[5] M. S. Arayne and N. Sultana, "Porous nanoparticles in drug delivery systems," Pakistan Journal of Pharmaceutical Sciences, vol. 19, p. 158, 2006.
[6] J. F. Chen, H. M. Ding, J. X. Wang, and L. Shao, "Preparation and characterization of porous hollow silica nanoparticles for drug delivery application," Biomaterials, vol. 25, pp. 723-727, 2004

RGD-FUNTIONALIZED CHITOSAN NANOPARTICLES FOR DIRECTED TISSUE ENGINEERING

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INTRODUCTION

In complex wounds such as ulcers, chronic wounds or larger burns, spontaneous skin barrier restoration that protects from infection and maintains tissue hydration is impaired. To promote cell migration during the healing process, we propose to expose the cells at the wound edges to polymeric nanoparticles functionalized with adhesion peptides. For this purpose, a chitosan derivative functionalized with Gly-Arg-Gly-Asp-Ser (GRGDS) peptides was synthesized.

EXPERIMENTAL METHODS

Polyelectrolytic nanocomplexes (PEC) were prepared using complex coacervation between a GRGDS-grafted chitosan and chondroitin sulfate (CS). GRGDS-functionalized chitosan derivate was synthetized in 3 steps: chitosan trimethylation, carboxymethylation and finally GRGDS grafting by carbodiimide chemistry1. Nanocomplexes size and properties were investigated by photon correlation spectroscopy (PCS). Polymers and nanocomplexes were tested in vitro for biocompatibility by XTT proliferation tests and for their ability to induce adhesion on human dermal fibroblasts (HDF)2 and immortalized keratinocytes HaCaT.

RESULTS AND DISCUSSION

Chitosan was functionalized with 69 nmol/mg GRGDS. The PECs were shown to be in the size range of 170 nm in water, with 10-fold stable swelling in DMEM. The RGD-functionalized chitosan derivate was shown to slightly decrease the cell viability in its soluble form, but no toxicity was observed when complexed with CS even 1 mg/mL concentration. A spread phenotype with a 3-fold cell area increase was observed on GRGDS-nanocomplexes when compared to control inactive SDGRG peptide (Fig. 1). Cell number was not significantly different for both conditions. Using keratinocytes, biocompatibility was confirmed but no clear increase of cell adhesion to the polymer or phenotype change could be shown.

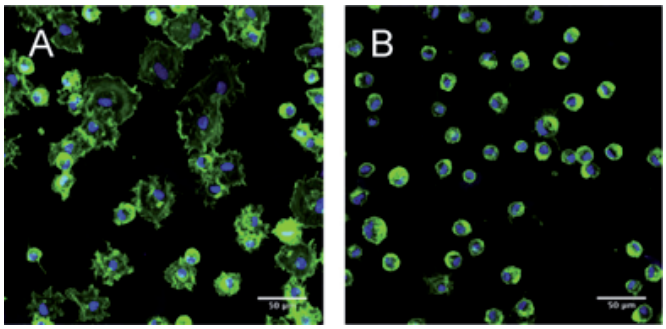

***Fig. 1**: Confocal images of HDFs on coatings of 0.1 mg/ml (A) GRGDS- and (B) SDGRG-CM-TMC nanoparticles. Actin-fibers are shown in green and nuclei in blue, scale bar 50 mm.*

CONCLUSION

Nanocomplexes functionalized with RGD-peptides were developed to be used to direct cell behavior. They were shown to be stable, biocompatible and to support fibroblast adhesion and spreading. However, lack of keratinocyte spreading indicates either a too low GRGDS concentration or that GRGDS is not an appropriate ligand for the integrins expressed by keratinocytes. Further studies directed towards higher adhesion peptide concentration or combination with other adhesion peptides are needed before evaluating in vivo nanocomplexes activity.

REFERENCES

Hansson, A., et al., Carbohydr Polym (2012), 90: 1494-500.
Hansson, A., et al, Int J Pharm (2012) 439:73-80.

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SIMPLE COATING TECHNIQUE TO OBTAIN GOHAP™ LAYER ON POLYMER SCAFFOLDS FOR BIO-APPLICATIONS

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The idea of bone regeneration scaffolds is to facilitate bone regrowth when the gap in it is too large for its natural healing. This can frequently happen after severe bone fractures, removal of bone tumours, craniomaxillofacial surgery, etc. The presently tested scaffolds do not meet clinician's criteria. The bone regrowth process is too slow, the voids filling is incomplete, inflammatory processes may take place, and mechanical strength of the scaffold is low. In present work an ultrasonic technique was used to coat polymer scaffold with hydroxyapatite nanopowder. As a result it is expected to obtain a nano-coating which will enhance bone regrowth.
The hydroxyapatite (GoHAP) nanopowder is produced in our laboratory by means of a microwave solvothermal synthesis process MSS [1]. The solubility test (according to ISO 10993-6:2007) of

above nanopowder showed higher solubility compared to commercial hydroxyapatite nanopowders [2]. The ultrasonic coating technique is a simple, relatively cheap process.

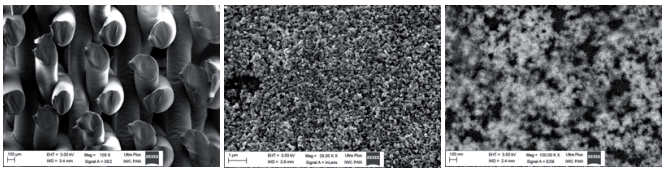


Fig. 1: *Images of hydroxyapatite coating on scaffold surface.*

The coatings were investigated by Scanning Electron Microscopy (SEM) (Fig. 1.), Atomic Force Microscopy (AFM), and Energy Dispersive Spectroscopy (EDS) in order to test the coating texture, composition and strength of ceramic-to-polymer joint. Furthermore, the wetting angle for water was measured.
Wetting angle values for pure polycaprolactone surface and coated with hydroxyapatite nanoparticles.

Wetting angle:
Pure polycaprolactone: 71,8
Polycaprolactone coated with hydroxyapatite nanoparticles: 33,7

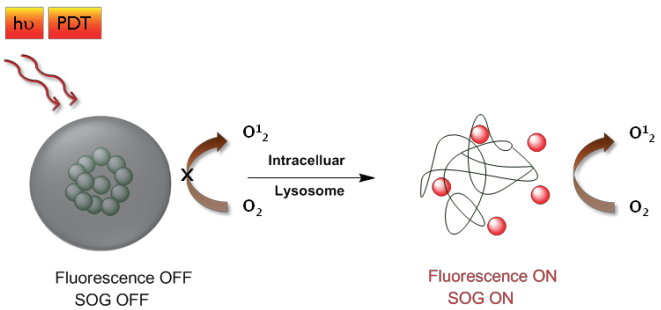
REFERENCES

1 Patent application P-369906, The method of nanoplates and method of nanopowder with nanoplates obtaining from synthetic hydroxyapatite. 2 Smoleń, D. et all, J Nanomaterials (2012), 2012, doi:10.1155/2012/841971 ACKNOWLEDGEMENTS: This work was supported by the infrastructure project CePT and SONOSCA project (NCBiR/ERA-NET-MATERA/04/2011), as well as BIO-IMPLANT project (POIG.01.01.02-00-022/09).

PHOTOSENSITIZER-CONJUGATED POLYMERIC NANOPARTICLES FOR ACTIVATABLE FLUORESCENCE IMAGING AND PHOTODYNAMIC THERAPY OF CANCERS

HYUNJIN KIM, Yongdoo Choi*

In this study, we developed activatable photodynamic therapy (PDT) agent by conjugating second generation photosensitizer chlorin e6 (Ce6) with hyaluronic acid (HA) through GSH-cleavable disulfide linker. The GSH-cleavable Ce6-HA conjugate forms nanoparticles with 254±51.5 nm diameters. Fluorescence and singlet oxygen generation of Ce6 were quenched after forming nanoparticles. We expected that this smart PDT agent can be accumulated into tumor tissues by EPR effect, and then its fluorescence and phototoxicity will be activated after entering cancer cells in the tumor tissues. When DTT at different concentration was added to Ce6-HA nanoparticle solution, fluorescence and singlet oxygen generation were increased depending on the concentration of DTT added. In particular, singlet oxygen generation was increased about 5 times after treating the Ce6-HA nanoparticles with 5 mM DTT while no increase in fluorescence and singlet oxygen generation were observed in the 5 mM DTT-treated sample. In vitro cell studies showed potential utility of this activatable PDT agent as a biocompatible optical theranostics for near-infrared fluorescence imaging and subsequent PDT of cancers.



FOLIC ACID FUNCTIONALIZED QUATRO-NANOCONTAINERS AS TARGETED AGENT: IN VITRO AND IN VIVO STUDY

GEORGE KORDAS[a] *, Eleni K. Efthimiadou[a]*, Christos Tapeinos[a], Leto Tziveleka[a], Eirini Fragogeorgi[b,c] and George Loudos[b]
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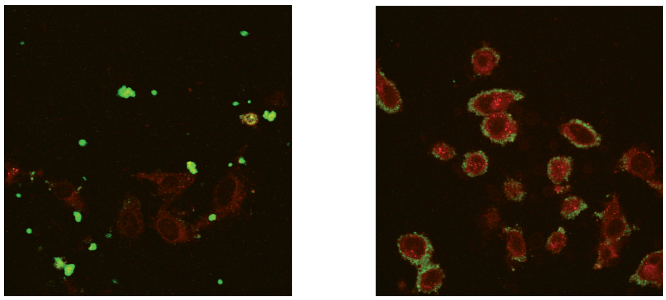
Nanocontainers (NCs) can serve as highly versatile platforms for the controlled functionalization and delivery in the last decade as DDS. The responsiveness of the functionalized NCs could be attained by choosing monomers with specific sensitivities, such as, sensitivity in temperature (e.g. NIPAAm, HPMA, DMAEMA), pH (e.g. AA, MAA) and reductive-oxidizing (redox) environments, which are desired for selective release in tumor area. Nanospheres present many advantages such as stability, presence of many active groups that can be easily functionalized with specific molecules for targeted therapy and coated with polymers improving their circulation time in bloodstream and inducing therefore, stealthy properties. For the targeted therapy through the NCs, the small molecule of folic acid has been employed. Because folate receptors are known to be overexpressed and actively internalized through folate receptor mediated endocytosis in various types of cancer cells, folic acid has been considered to be a suitable ligand for improving the cellular uptake of drugs and macromolecules into cells. The NCs were modified by carbodiimide chemistry with FITC and/or Folic Acid. Ovarian cells, HeLa, which overexpress the folic acid receptor, were incubated for 72h with the empty and drug loaded NCs aiming at studying the targeted internalization mechanism with and without folic acid.



Without Folic Acid With Folic Acid

Figure 1. *Cellular trafficking of FITC-labeled and FA- FITC-labeled NCs in HeLa cells. The cells were incubated for 1h with the FITC-labeled NCs in the presence of LysoTracker Red (10 min) at 37 oC, followed by live cell imaging. In all experiments cells were treated with either 3 μM folic acid functionalized NCs.*

DESIGN OF ELECTROSPUN NANOFIBERS FOR DRUG DELIVERY AND REGENERATIVE MEDICINE

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PURPOSE

Today's interest in nanomedicine keeps growing because the application of nanotechnological tools into development of structures at the molecular level enables the improvement of interactions between material surfaces and biological entities (1). The cells in vivo are in close contact with extracellular matrix (ECM) which provides biological and physical support for cell growth and exerts topographical and structural features at nanometre scale (2, 3). The interactions between the cells and the ECM or biomaterials (na-

noparticles, nanofibers, nanodevices) influence cell growth, guide cell adhesion and mobility (2). Compounding this, processing biocompatible polymers into nanofibers and networks represents the initial step toward mimicking some aspects of native ECM structure from at least the mechanical (tensile strength and elasticity) and biological perspectives (4). One of the direct implications for characterizing the fiber is the closest estimation of what cells actually experience in their surroundings.

The objective of this study was to prepare and evaluate the morphology, mechanical properties, thermal and swelling behavior of single polyvinyl alcohol (PVA), or composite PVA/hyaluronic acid (HA) nanofibers with respect to their variations in the composition, thickness and alignment on cell growth in order to discover the crucial properties for clinical use. HA is an appropriate candidate for a tissue-engineering scaffold matrix because it is a native component of ECM and a non-immunogenic polymer which plays a vital role in regeneration and healing processes (5).

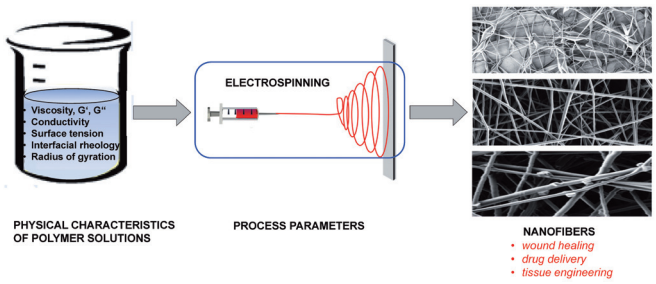

***Fig. 1**: Scheme of electrospinning process and SEM images of PVA nanofibers*

MATERIAL AND METHODS

Investigated nanofibers made of PVA (Mowiol® 20–98, Mw = 125 000 g/mol) and HA sodium salt from Streptococcus equi were prepared by electrospinning. It is a process for preparing nanofibers from a polymer solution by the application of a high voltage that induces the formation of a thin liquid jet at adequate experimental conditions (Fig.1). Morphological and mechanical properties and swelling behavior of produced nanofibers were investigated combining atomic force microscopy (AFM), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and attenuated total reflectance spectroscopy (ATR). Cells (keratinocytes or fibroblasts) were seeded on tested support (randomly and aligned nanofibers or glass coverslip as a control). The speed of cell adhesion was determined by counting of unattached cells, while cell migration by agarose drop assay. Proliferation was tested by MTS assay and effect of nanofibrillar support on cell morphology with confocal fluorescent microscope (6, 7). Additionally, the effects of prepared nanofibers on cell gene expression after 3 and 6 days of incubation were determined by PCR.

RESULTS

A standard electrospinning system is simple in terms of equipment, however, the physics governing the process is extremely complex, which requires a lot of tuning parameters (6). AFM evaluation revealed the formation of solid nanofibers with non-porous surfaces, a random orientation and diameter of 279.7 nm (PVA) and 159.3 nm (PVA/HA).

From the experimental point of view, during electrospinning due to the enlarged surface-to-volume ratio of the polymeric solution and produced nanofibers, surface effects become more significant and impact parameter in a size-dependant manner. Moreover, we suggest that the internal structure has a dominant role in polymer nanofiber reinforcement, which is evident from the AFM measurements (3). More precisely, fibers tend to be stiffer only in the direction in which their molecules are oriented. In contrast, fibers are much more elastic if they are bent at right angles to their molecular orientation. In the range from 200 to 300 nm is not observed reasonable a size-dependant surface effect for the PVA nanofibers, while less than 160 nm, a much more prominent increase in stiffness has been achieved. The more elastic nature of PVA nanofibers in comparison to PVA/HA can be related to a higher degree of structural order because oriented molecules much more easily slip under applied force (Fig.2). Molecular interactions between the PVA and HA, confirmed with the DSC and ATR measurements, comprised a more compact structure of PVA/HA nanofibers, which resulted in higher bending E.

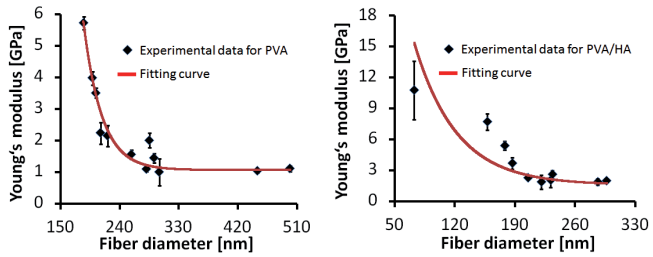

***Fig. 2** - Young's moduli as a function of nanofiber diameters*

In the study influence of nanofibers on cell behaviour we proved lower adhesion rate of keratinocytes on well characterized PVA nanofibers in comparison with a glass substrate, but the strength of the adhesion was higher. The effect of nanofibrillar aligment on metabolic activity and cell morphology was clearly shown. Randomly oriented nanofibers limit cell mobility comparing to aligned. The cells, which grow along aligned nanofibres morphologically, differ from those which are seeded on glass (control) (Fig. 3). Nanofibrillar support with nanosized interfibrillar pores enables efficient cell proliferation and could accelerate wound healing, but does not support 3D tissue regeneration. Randomly oriented nanofibers increase proliferation rate of keratinocytes, while decrease proliferation of fibroblasts. Oriented nanofibers even more increase proliferation of keratinocytes, while the proliferation of fibroblasts is comparable to the proliferation of fibroblast grown on cover glass. Additionally, nanofibers significantly affect expression of particular genes in keratinocytes and fibroblasts, at both controlled time points.

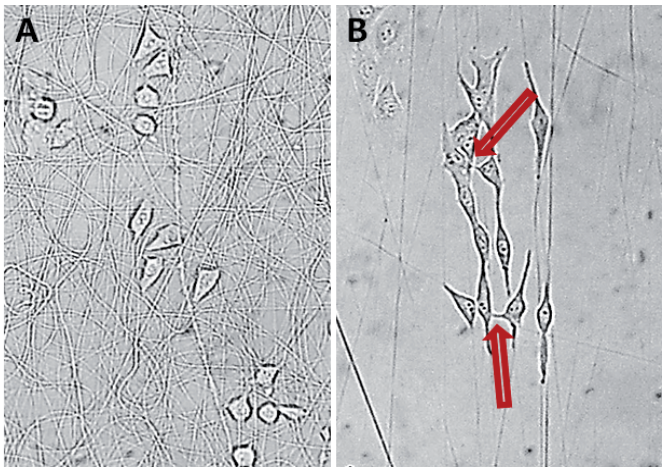

***Fig. 3**-Morpholohy of keratinocytes grown on A - randomly and B - aligned nanofibers.*

CONCLUSIONS

Our research work represents a significant step towards production and use of nanofibrillar scaffolds in clinical practice after resolving some of critical effects of nanofibers on cell growth. These results especially highlight the finding that the mechanical properties of polymeric nanofibers can be tuned by changing the fiber size as well as the composition in order to match the mechanical properties of native tissues. Consequently, it should be emphasized that experimental results show that: (a) the morphology of the investigated cells and distribution are a consequence of topographical and mechanical properties of nanofibers; (b) cellular behavior is very different due to their self-organization at the interphase with nanofibers using flagella and pili and the production of extracellular material. Nanofibers based on PVA and PVA/HA are more appropriate for scaffolds engineering various tissues such as nerves, wounds, skin, and so on.

LITERATURE REFERENCE

1. Bhardway N., Kundu S.C. Biotechnol. Adv. 28, 2010, 325-347
2. Pelipenko J. et al, Eur. J. Pharm. Biopharm. DOI: 10.1016/j.ejpb.2012.09.009
3. Janković B. et al. Int. J. Pharm. In Press
4. Rim, N.G., et al., Biomed. Material 8, 2013, 1-14.
5. Du, F., et al., J. Nanosci. Nanotechnol. 11, 2011, 6718–6725.
6. Rošic R. et al., Eur. Polymer J. 48, 2012, 1374 -1384 and 49, 2013, 290-298.
7. Anselme, K., et al., Acta Biomaterialia, 6, 2010, 3824-3846.

RATIONAL DESIGN AND DEVELOPMENT OF LIPOSOMES FOR DRUG DELIVERY

ANNE KÜMMEL, Sabine Schaedelin, Matthias Machacek

Drugs delivered with liposomal formulations can have a more favorable therapeutic index than naked formulations. However, the design and selection of an appropriate liposomal formulation is greatly complicated by the interplay of liposomal pharmacokinetics (PK), drug release and naked drug PK. Here we propose a quantitative pharmacology approach that includes integration of experimental data and mechanistic knowledge to assist the rational design and development of liposomal formulations.

The proposed approach is centered on the desired PK properties in human. Typically, desired PK properties would include maximal peak plasma concentration, a certain half-life, and tissue distribution. These requirements will be driven by the PKPD relationship and the inadequate therapeutic index of the naked drug. A mathematical model used to predict the tissue distribution for the (free) drug given as a liposomal formulation. This model is used to assess different liposomal formulations and different release rates in order support the selection of most promising liposomal formulations to meet the targeted drug PK properties.

The mathematical model integrates several components into a physiology-based pharmacokinetic approach. Part of the model is a database containing tissue distribution values of a large number of different liposomal formulations thus providing the information on how liposomal PK depends on physico-chemical properties. Drug release rates are informed through in-vitro experiments and naked drug PK parameters from animal PK experiments.

To date, the tissue distribution database and the PBPK models that describe data from release experiments or tissue distribution studies have been established. As a next step, their predictive power for in vitro/in vivo or between species translation need to be assessed. This will not only evaluate the utility of the modeling but also reflect the knowledge and data gaps that need to be addressed for the rational design of liposomal formulations.

"SOLVOTHERMAL SYNTHESIS AND CHARACTERIZATION OF NANOMATERIALS IN THE LABORATORY OF NANOSTRUCTURES FOR PHOTONICS AND NANOMEDICINE, CENTER OF BIO-NANOMATERIALS, CEPT "

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[1] Expertise of Laboratory of Nanostructures for Photonic and Nanomedicine IWC PAN lies in synthesis and comprehensive research on nanomaterials. Synthesis of nanomaterials requires the use of state of the art, advanced reactors. For more than 10 years the Laboratory of Nanostructures for Photonic and Nanomedicine IWC PAN has been developing solvothermal technologies of nanoparticles production. The process is based on the MSS Microwave Solvothermal Synthesis. The precursors of the reaction (solutions, suspensions) are enclosed in a pressure vessel and as a result of heating with the microwave energy, the temperature increases above the boiling point. The MSS process allows to prevent contamination of synthesis, by sealing the reaction vessel, which is made of chemically inert material, so an ultra-pure product is obtained. The mixing effect occurs in a microwave reactor, so that the obtained product is homogeneous. MSS technology innovation is the possibility to control the size of crystallite nanoparticles in a narrow distribution of size. Thanks to this technology, we are able to obtain nanoparticles in the range from 9 to 100 nm in ultra-short synthesis time. We can obtain power density in liquid reaching up to 10 W/ml. We specialize in the production of: HAp, ZnO and ZrO2 nanopowders.

We have constructed innovative reactors MSS-1 and MSS-2 (Fig. 1) and our reactors were awarded a gold medal at:
- MSS-2 International Fair in Poznan, Innovation Technology Machines Poland, 2011
- MSS-1 International Exhibition in St. Petersburg in 2009.

MMS-2 technical parameters:
- Maximum working pressure to 10 MPa
- Temperature up to 260°C
- Capacity 470 cm3
- Microwave Heating at 2450 Mhz
- Microwave power up to 3 kW
- Chamber material PTFE and ceramic Al2O3

***Fig. 1**. Microwave Solvothermal reactor MSS-2*

Research directions of the Laboratory of Nanostructures for Photonic and Nanomedicine IWC PAN are focused on the characteristics of nanomaterials and the possibility of their application in medicine, optics, optoelectronics pharmacy and cosmetics.

The correct characterization of nanometric-materials is necessary in order to determine the properties of materials. Research in these areas requires advanced, sophisticated measuring equipment. With co-financing from the European project CePT (Centre for Preclinical Research and Technology) laboratory has purchased devices such as:
- particle size distribution and zeta potential analyzer with an automatic titrator MPT-2 model Zetasizer Nano-ZS, Malvern
- particle size distribution analyzer model NS500, NanoSight
- ultra high resolution scanning electron microscope, model ULTRA plus, Zeiss with X-ray spectroscopy analyzer model Quantax 400, Bruker
- thermal stability analyzer TGA/DSC model STA 449 E1 Jupiter, Netzsch coupled with mass spectrometry analyzer model Gas Analitycal System QMS 403C, Netzsch and FTIR spectroscope analyzer System TENSOR27, Bruker
- viscometer analyser model DV-II+Pro, BROOKFIELD
- bubble pressure analyser model BPA-1P, Sinterface
- particle dispersion analyzer model Turbiscan Lab, Formulaction
- OptiMax™ synthesis workstation for chemical and process development, Mettler-Toledo
- multi-parameter Instrument model ProLab 2000, SI Analytics, pH and conductivity measurement

In addition, the laboratory has such a measuring devices as:
- x-ray powder diffractometer model X'Pert PRO, Panalytical, and model D8, Bruker
- gas displacement density analyzer AccuPyc II 1340 Pycnometer, Micromeritics and AccuPyc 1330 Pycnometer, Micromeritics
- surface area analyzer, model Gemini 2360, Micromeritics

Laboratory of Nanostructures for Photonic and Nanomedicine IWC PAN deals with the characteristics of nanomaterials obtained by our team, as well as provides research services such as analysis of density, surface area, morphology, phase composition, thermogravity of nanomaterials, surface tension, viscosity, stability and the size of nanoparticles in colloidal solutions and suspensions. We cordially invite you to collaborate with us in the area of nanoparticles synthesis and functionalization, their characterization, and applications in nanomedicine.

COMPLEMENT AND LIPOSOMES LOADED WITH LIPOPHILIC PRODRUGS OF METHOTREXATE AND MELPHALAN

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The advantages offered by incorporation of lipophilic derivatives of antitumor agents into lipid bilayer of liposomes include efficient loading and retention of the prodrugs in the nano-carrier and a simplified liposome preparation procedure. Previously, we demonstrated that 1,2-dioleoylglyceride esters of the widely used antitumor agents methotrexate (MTX-DOG) and melphalan (Mlph-DOG) incorporate virtually completely (10 mol. %) into the lipid bilayer of 100-nm liposomes built from natural phospholipids [1] (Fig. 1). Early proof-of-concept in vitro [1] and in vivo [2] experiments proved the approach promising. In a panel of in vitro tests the liposomes were found to be well tolerated by blood cells yet MTX-DOG, and not Mlph-DOG liposomes, induced complement (C) activation [3]. Indeed, modification of the liposome surface disturbs the liposome–plasma protein interface, which may result in both biocompatible and bioadverse effects. Large amount of data has been generated on hypersensitivity reactions that are induced by commercial liposomal drugs (e.g. Doxil®) and proceed, at least in part, through activation of C [4]. Here, we summarize the data on interactions between liposomes carrying lipophilic prodrugs and complement obtained thus far.

Liposomes (L) were prepared of egg phosphatidylcholine (PC) – yeast phosphatidylinositol (PI) – MTX-DOG/Mlph-DOG mixture, 8 : 1 : 1 by mol, by standard extrusion technique. The liposomes were incubated with human blood or plasma at 37°C for 15 min. The rate of C activation in the presence of liposomes was assessed by C3a (anaphylatoxin released upon C activation) and SC5b-9 (final product of activation) ELISA determination. Besides, residual C activity of plasma samples towards sensitized heterologous red blood cells was determined after blood incubation with liposomes. To learn the molecular details of the liposome–protein interactions, complexes formed upon the liposome short-term (15 min) incubation with human plasma at 37°C were isolated via gel permeation chromatography on a Sepharose CL-4B column. Fractions containing liposome-bound proteins were identified using UV spectrometry (MTX-DOG: λmax 302 nm, ε 25000; Mlph-DOG: λmax 258 nm, ε 19700) to detect the prodrugs and a modified Lowry assay to quantify protein. These fractions were pooled, delipidated, and analyzed by SDS-PAGE coupled to immunoblotting.

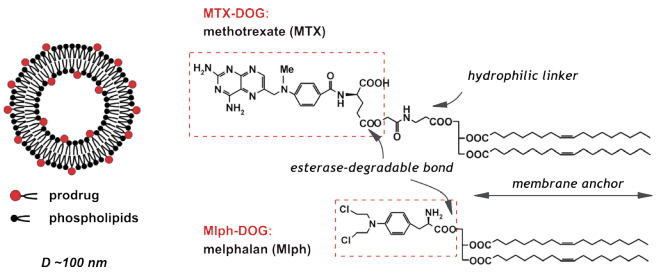

Fig. 1. Liposome schematic representation on the left and 1,2-dioleoylglyceride prodrugs of methotrexate and melphalan, on the right.

In contrast to Mlph-DOG containing liposomes, MTX-DOG-L induced elevated C3a levels in the in virto tests in a concentration dependent manner (Fig. 2). The reactivity of liposome surface was not influenced by the presence or absence of PI, an anti-opsonizing component of the formulation, or by introduction of a targeting tetrasaccharide ligand [3]. Decrease in the liposome loading with MTX-DOG from 10 to 2.5% resulted in considerably reduced impact of the prodrug on C activation by liposomes.

The results were supported by the residual C activity measurements. Surprisingly, none of the liposomal samples induced elevated SC5b-9 plasma levels according to the ELISA test [3]. There have been no reports of this kind of assay discrepancy. We assume that the terminal complex formed upon C activation by MTX-DOG-L prefers to incorporate into the liposome bilayer present in access, rather than to bind S-protein into the SC5b-9 complex; then, (S) C5b-9 is still recognized by the assay antibodies in complex with rigid membranes of the formulations reported previously, but not with the fluid-phase bilayers of the liposomes under study.

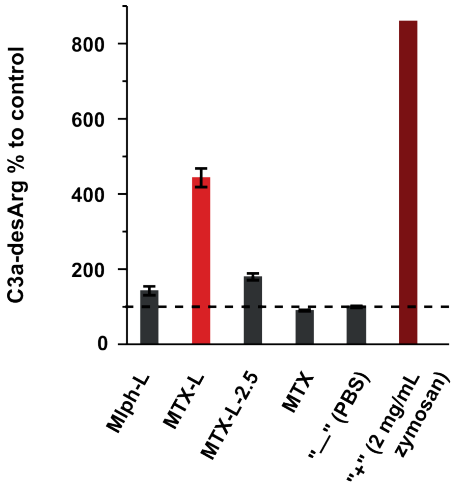

Fig. 2. C3a-desArg (stable metabolite of C3a) generation in blood upon incubation with liposomes according to ELISA data. Bars are mean ± SE.

According to GPC data, both Mlph-DOG-L and MTX-DOG-L bound considerable amount of protein (~80 g protein/mol lipids). However, repertoires of bound proteins were different (Fig. 3). While C4b-binding protein of C system was detected among proteins associated with both liposome species, only MTX-DOG-L bound component C3 and complement regulatory factor H. Decrease in MTX-DOG content in the bilayer (10 to 2.5 mol %, sample MTX-L-2.5) resulted in the absence of C3 or factor H from the liposome surface. Thus, the data are in agreement with the increased complement consumption by MTX-DOG-L and C3 and factor H should be involved in C activation. Decreased protein binding and C activation by MTX-DOG liposomes with less prodrug was probably due to lower surface charge density and/or a different pattern of functional group distribution over the liposomes surface. Indeed, functional groups that MTX moiety may expose on liposome surface include aromatic amino functions and the free α-carboxyl group. Meanwhile, amino groups arranged in a certain manner may attack the internal thioester bond of the C3 factor accelerating its hydrolysis and C activation.

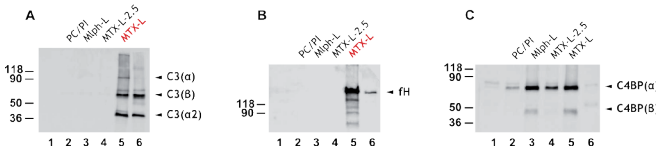

*Fig. 3. Identification of liposome-associated plasma protein by immunoblotting using antibodies against C proteins (**A**) C3, (**B**) factor H, and (**C**) C4b-binding protein. Lane 1 corresponds to PBS and 6, to whole plasma diluted ×500.*

Finally, encouraging results were obtained in preliminary experiments employing the sensitive porcine model used to investigate hypersensitivity reactions [5]. At a therapeutically relevant dose (0.1 mg total lipids/kg), bolus injections of MTX-DOG-L induced no changes in such cardiovascular parameters as systemic and pulmonary arterial pressure and heart rate and demonstrated tachyphylaxis. The dose of 1.0 mg/kg broke through the tachyphylactic effect and evoked an unusual (severe and retarded) immunotoxic cardiovascular reaction. However, the effect yet needs further confirmation and exploration.

REFERENCES

• Kuznetsova, N. et al. Liposomes loaded with lipophilic prodrugs of methotrexate and melphalan as convenient drug delivery vehicles. J. Drug Del. Sci. Tech. 19, 51–59 (2009).

• Vodovozova, E. et al. Antitumour activity of cytotoxic liposomes equipped with selectin ligand SiaLeX, in a mouse mammary adenocarcinoma model. Eur. J. Cancer 36, 942–949 (2000).

• Kuznetsova, N. R. et al. Hemocompatibility of liposomes loaded with lipophilic prodrugs of methotrexate and melphalan in the lipid bilayer. J. Control. Release 160, 394–400 (2012).

• Szebeni, J., et al. Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: Prediction and prevention. Adv. Drug Deliv. Rev. 63, 1020–1030 (2011).

• Szebeni, J. Hemocompatibility testing for nanomedicines and biologicals: predictive assays for complement mediated infusion reactions. Eur. J. Nanomedicine 4, 33–53 (2012).

INFLUENCE OF THE PEG COATING ON THE DELIVERY PERFORMANCE OF STEALTH LNCS LOADED WITH A FERROCENYL DRUG: APPLICATION THROUGH ECTOPIC GLIOMA TUMOR TREATMENT

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ABSTRACT SUMMARY

The anticancer activity of a novel ferrocenyl drug (c-FcdiOH) was assessed in an intradermic gliosarcoma model through one-step stealth Lipid NanoCapsules (OS LNCs) recently designed, in comparison to conventional PEG post-inserted-LNCs (PI LNCs). These two stealth nanocarriers were obtained via two different PEGylation techniques. Interestingly, significant tumor growth inhibition was observed with OS c-FcdiOH-LNCs whereas PI c-FcdiOH-LNCs only showed a slight trend for growth reduction suggesting enhanced tumor delivery and potent anticancer activity with OS c-FcdiOH-LNCs.

INTRODUCTION

Malignant brain tumor remains one of the main medical challenges for scientists as no life-saving treatment has been found to date despite aggressive therapy modalities. As the cancer ability to evade apoptotic cell death mechanism is one of the principal causes of treatment failure, new effective strategies are needed. Since the discovery of the outstanding performance of the cisplatin as anticancer agent, metal-based drugs are considered as new hopeful therapeutics [1], expected to provide alternative cell death approaches. In this context, a new ferrocenyl derivative [2] was synthesized and preliminary in vitro studies showed potent activity on various cancer cell lines from the National Institutes of Health. Considering the hydrophobic characteristic of this drug, the use of a delivery system was required for further preclinical evaluations. Nowadays, nanocarriers have appeared as a compelling drug delivering system offering great benefits such as increased bioavailability, protection against in vivo degradation and enhancement of pharmacokinetic properties. Additionally, the discovery of the enhanced permeability and retention (EPR) effect [3] has represented a milestone in nanomedicine development. Consequently, many researches have been devoted to the formulation of EPR effect-based nanocarriers to achieve a great and specific drug accumulation into the cancer site. A commonly used technique to obtain stealth nanocarriers, essential to promote the EPR effect, consists in coating nanoparticles with a polyethylene glycol (PEG) based molecule. As a result, the hairy layer formed by the polymer constitutes a steric hindrance to complement protein interaction preventing monocyte uptake [4]. Our laboratory have developed and patented a drug delivery system based on a temperature phase inversion method called lipid nanocapsules [5]. Currently, standard PEGylation of LNCs is achieved via the post-insertion technique of DSPE-mPEG 2000 (1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy-(polyethyleneglycol)-2000]) involving a prior LNC formulation step and a subsequent incubation step [6]. In order to reduce the formulation time and to limit the DSPE-PEG content for toxicity concerns, one step-stealth LNCs were recently designed and showed high loading capacity, great physical stability and extended blood circulation time. As a result, OS LNCs were suitable candidates as passive tumor targeting delivery system. Although the biological mechanism of action of this innovative ferrocenyl complexe is still not fully elucidated, an in vivo experiment was performed on a 9L gliosarcoma model through OS c-FcdiOH-LNCs compared to PI c-FcdiOH-LNCs. Consequently, the aim of this study was to assess the c-FcdiOH anticancer activity within an in vivo environment allowing ultimately the comparison of the drug delivery performance by the two PEGylated LNCs.

EXPERIMENTAL METHODS

The LNCs were prepared according to the phase inversion method as previously described [5]. Briefly, the preparation process consisted in mixing all the excipients (Solutol® HS15 (17.2 % w/w), Lipoid® (1.5 % w/w), Labrafac® (20.8 % w/w), NaCl (0.5 % w/w) and water (60 % w/w)) under magnetic stirring. Three cycles of progressive heating and cooling between 90 and 60 °C were then carried out. At the last cooling step, the LNC suspension was gently stirred until reaching room temperature and finally diluted with water. For the c-FcdiOH encapsulation, the drug was added as powder with all the components and then temperature cycles were performed. The OS LNCs were obtained by adding DSPE-mPEG into the initial mixture of LNCs while the post-inserted-LNCs were achieved via an additional incubation step between LNCs and an aqueous DSPE-mPEG solution at 60°C during 2hrs. The average hydrodynamic diameter and the zeta potential of nanocapsules were determined at 25°C, in triplicate, using a Malvern Zetasizer® (Nano Serie DTS 1060, Malvern Instruments S.A., Worcestershire, UK).

In vitro antiproliferative assay was performed in order to compare the activity of the free drug or encapsulated into LNCs. 9L cell survival percentage was estimated by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) survival assay after 72h exposure to increasing c-FcdiOH concentrations ranging from 0.01µM to 10µM.

The antitumor activity of c-FcdiOH was assessed in vivo on an intradermic gliosarcoma model into female Fisher rats through OS LNCs compared to PI LNCs and control groups including saline and unloaded LNCs (both types PI and OS). Intravenous injections (20mg/kg) were dosed 5 days per week, over 2 weeks. Tumor growth evolution was monitored via physical tumor volume measurement.

RESULTS AND DISCUSSION

Regarding the physicochemical properties of the various LNCs, the encapsulation of the drug markedly decreased the LNC hydrodynamic diameter compared to that of unloaded LNCs from approximately 50 nm to 40 nm, likely due to the drug arrangement at the LNC interface. The coating with DSPE-mPEG 2000 by the post-insertion method slightly increased the particle size whereas no size alteration was observed through the one-step process. With both PEGylation techniques, the DSPE-mPEG presence into the shell was evidenced by a greater negative surface charge detected via the zeta measurement. This is typical of PEG coating onto nanoparticle surfaces as previously described for other nanocarriers such as polymeric micelles [7], and liposomes.

As a PEG coating might hinder nanoparticle uptake by the tumor cells, antiproliferative assay on 9L gliosarcoma cells was conducted to compare the efficiency of the free drug in DMSO to that of the drug entrapped into PEGylated LNCs. As expected, c-FcdiOH showed a low IC50 value reaching around 0.1 µM which proved its potent therapeutic activity. Additionally, this cell survival assay revealed similar IC50 for both c-FcdiOH-LNCs and free c-FcdiOH. Accordingly, both PEG coatings did not alter the drug activity.

Unlike the in vitro assay, the in vivo study showed different responses to treatment according to the LNC type. As observed in Fig 1, tumor volume increased dramatically for the control groups including saline and the unloaded LNC groups. Interestingly, OS c-FcdiOH-LNC treatment substantially slowed down the tumor growth from D7 compared to the two control groups whereas PI

c-FcdiOH-LNCs merely showed a slight tendency for tumor growth reduction. It has been shown that PEG barrier could limit the cargo diffusion through the extracellular matrix and reduce cell interactions due to steric repulsions and slight negative charged nanocarrier surfaces. This consideration is in accordance with the results since the OS LNCs contained, by far, less PEG amount and showed successful outcomes.

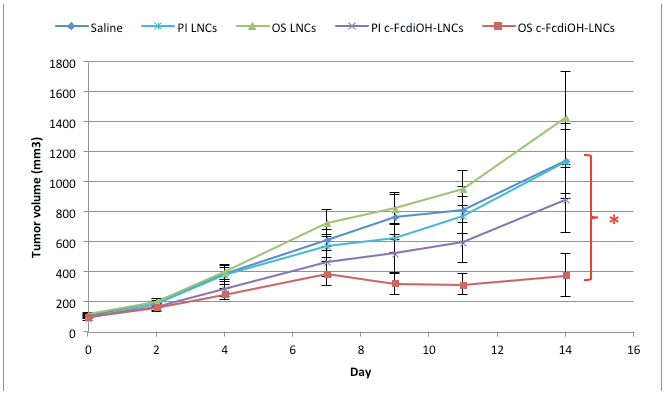

***Fig. 1**: Comparison of the tumor growth evolutions according to different treatments including saline, unloaded LNCs (PI and OS) and c-FcdiOH-LNCs (PI and OS). Mann and Whitney Test :*p<0.05*

CONCLUSION

The study has evidenced the in vivo antitumor activity of c-FcdiOH on an ectopic glioma model and the enhanced drug delivery performance of OS LNCs over PI LNCs. With the growing interest in EPR-based nanocarriers, this result highlights the importance of carefully devise the PEG coating in order to achieve optimal delivery performance.

REFERENCES

1. Lainé A-L, et al. Curr. Opin. Pharmacol. 2012, 12:420–426.
2. Nguyen A, et al. Chimia. 2007, 61:716–724.
3. Maeda H, et al. J. Control. Release. 2000, 65:271–284.
4. Vonarbourg A, et al. J. Biomed. Mater. Res. A 2006, 78:620–628.
5. Huynh NT, et al. Int. J. Pharm. 2009, 379:201–209.
6. Perrier T, et al. Int. J. Pharm. 2010, 396:204–209.
7. Wang J, et al. J. Drug Target. 2005, 13:73–80.

PENTABLOCK COPOLYMER-BASED NANOPARTICLES FOR EFFICIENT RECEPTOR MEDIATED SIRNA DELIVERY

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INTRODUCTION

In the field of cancer research, the future direction lies within siRNA therapeutics, considering a huge potential for personalized cancer treatment. Specific small interfering RNA (siRNA) can interfere with a specific gene and therefore suppress cancer growth. Due to their polyanionic characteristics, siRNA molecules are not able to spontaneously cross the cell membrane and thus require a delivery platform, which allows cellular uptake as well as cytosolic release for efficient gene knockdown. Despite the numerous platforms, which have been developed a major drawback is their cationic nature. Positive charged nanoparticle often show unspecific binding, increased cytotoxicity and binding to serum proteins, which inhibits cellular uptake of the particles.

The aim of this research project was the design of a polymer based siRNA delivery system with a low overall surface charge for highly suitable actively targeted gene knockdown.

METHODS

Characterization of the size and morphology of the polymer complexes has been done by dynamic light scattering (DLS) and transmission electron microscopy (TEM), respectively. Zeta potential measurements were conducted to analyze the surface charge of the particles. Determination of the siRNA loading capacity on the polymer nanoparticles has been investigated by gel electrophoresis studies to quantify unbound siRNA by variation of the N/P ratio. For knockdown analysis, non-targeted and targeted polymer siRNA complexes as well as free siRNA were added to GFP expressing HeLa cells in vitro. The knockdown efficiency was evaluated using fluorescence microscopy.

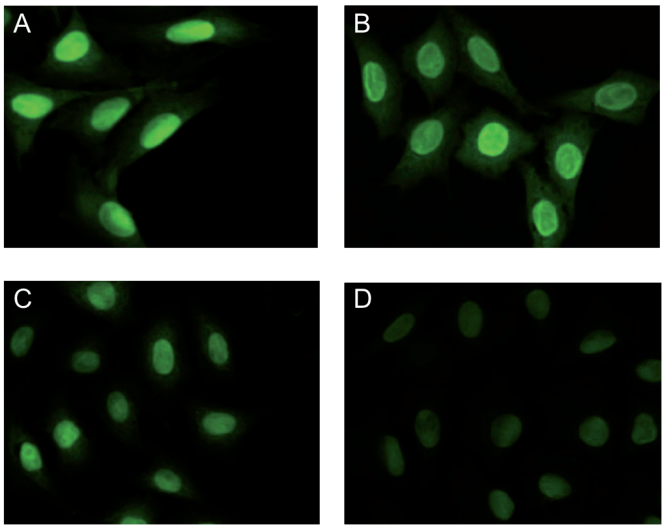


***Figure 1**: Evaluation of GFP knockdown in GFP expressing HeLa cells by fluorescence microscopy. A) Non-treated GFP expressing HeLa cells. B) HeLa cells treated with free siRNA. C) HeLa cell treated with non-targeted polymer-siRNA complexes. D) HeLa cells treated with targeted polymer-siRNA complexes.*

RESULTS

Our results showed stable encapsulation properties of the polymer-siRNA complexes (gel electrophoresis studies) as well as a monodisperse size distribution behavior as seen by DLS and TEM measurements. The low positive zeta potential values comprises high suitability for further in vivo studies due to the overcoming of charge related unwanted binding properties. Actively targeted delivery of the siRNA in GFP expressing HeLa cells could be achieved by the functionalization of the polymer with a receptor specific ligand. The polymer-siRNA complexes revealed a clear receptor mediated increase in GFP knockdown compared to non-targeted complexes. The almost neutral surface charge and the clear receptor mediated gene knockdown turns our polymer based delivery system into a highly suitable application for further in vivo studies.

ACKNOWLEDGEMENTS

This work is financially supported by the Swiss National Science Foundation research program NRP 62 “Smart Materials”.

ABC-EFFLUX TRANSPORTERS GET MORE TOLERANT TOWARDS PH VARIATION AT HIGH CATALYTIC ACTIVITY

XIAOCHUN LI-BLATTER, Päivi Äänismaa, Andreas Beck, and Anna Seelig

ATP-binding cassette (ABC) exporters found in prokaryotes and eukaryotes translocate or flop solutes (allocrites) from the cytoplasmic to the periplasmic leaflet of lipid bilayers. This mechanism serves different purposes; if allocrites are lipids as e.g. in the case of the exporter MsbA from Gram-negative bacteria, it helps to preserve the lipid asymmetry of membranes, if allocrites are toxins or drugs as e.g. in the case of the human P-glycoprotein (Pgp, ABCB1, MDR1), it helps to protect the cytoplasm from intruding allocrites which can lead to multidrug resistance.

The minimal functional ABC exporter unit is formed by two cytoplasmic nucleotide-binding domains (NBDs) that bind and hydrolyse ATP to drive the transport cycle, and two transmembrane domains (TMDs), each comprising six a-helices that are responsible

for allocrite recognition and translocation. Whereas the TMDs are more diverse and generally determine allocrite specificity the NBDs are highly conserved among ABC transporters. A highly conserved glutamine of the Walker B motif and a histidine of the switch motif constitute a catalytic dyad for ATP hydrolysis in which the glutamate acts as a general base to activate a water molecule attacking the gamma-phosphate of ATP.

We measured the pH dependence of the basal and allocrite-stimulated ATPase activity of the bacterial ABC exporter Sav1866 from Staphylococcus aureus and the human P-glycoprotein in lipid vesicles in the range of pH 5.0 – pH9.0. The ATPase activity of Sav1866 in lipid vesicles showed a maximum at about pH 6.8 and decreased to negligibly low values below pH 5.0 and above pH 8.0. Data were fitted to a Gaussian peak distribution yielding an optimum for ATP hydrolysis at pH 6.7 and half-maximum activity, V50%, at pH 5.9 and pH 7.6. In the presence of the allocrite C12EO8 the pH sensitivity decreased especially towards high pH.

The pH dependence of Pgp in plasma membrane vesicles in the absence of allocrites was measured in an analogous manner. It was broader than that of Sav1866 in lipid vesicles, especially towards high pH. In mixed micelles (with C12EO8 at concentrations above the CMC) the pH dependence of Pgp remained similar in width, and somewhat lower in activity over the whole pH range. Because C12EO8 induced maximum ATPase activity at low concentrations we also measured the pH dependence of Pgp under these conditions. Under these conditions the activity vs. pH curve broadens further, again inducing higher activity towards high pH values.

Our results suggest that stimulation of ATP hydrolysis may lead to higher proton production and a concomitant decrease in the local pH value which may, in turn, allow for protonation of the catalytically relevant histidine in the NBD even at high bulk pH values. They further suggest that the robustness of ABC exporters towards bulk pH variation (e.g. in the intestine) increases with increasing catalytic activity in the presence of allocrite (drugs or toxins).

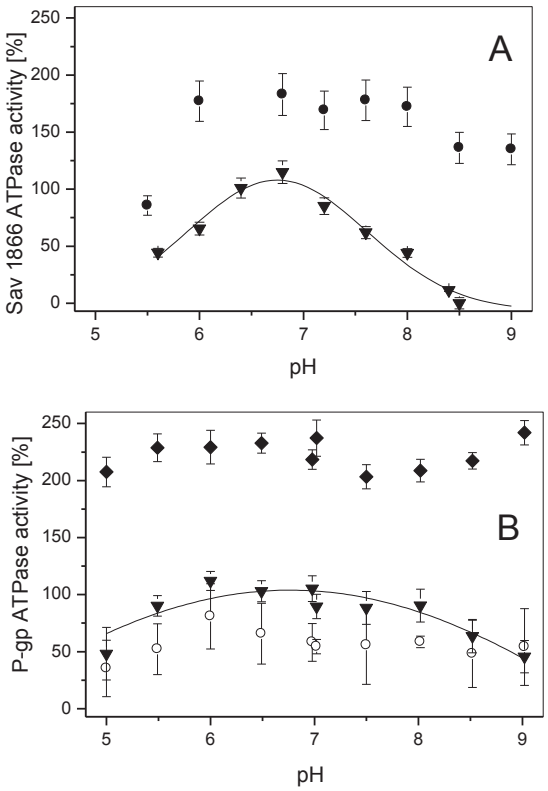


Fig1. *A, B. The Basal Sav1866 and P-gp-ATPase Activity Plotted as a Function of pH. A: ATPase activity of Sav1866 in lipid vesicles (triangles) and mixed lipid/C12E8 micelles (solid circles) measured in the range of pH5.6 – pH9. B: ATPase activity of P-gp in plasma membrane vesicles (triangles), plasma membrane vesicles with C12E8 (C = 1.6 mM) (lozenges) and mixed lipid/C12E8 micelles (C = C12E8 163.2 mM) with (open circles) was measured in the range of pH5.0 – pH9.0.*

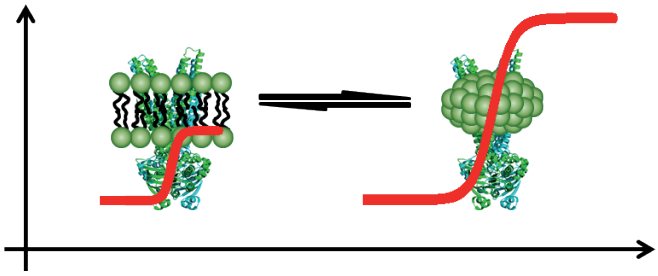


Fig2. *The illustrate plot of Sav1866 ATPase activity. It gives stronger ATPase activity in mixed lipid/C12E8 micelles than in lipid vesicles.*

Reference: our manuscript (biochemistry in press).

THE BIOZENTRUM BIOPHYSICS FACILITY

XIAOCHUN LI-BLATTER and Tim Sharpe

The Biozentrum Biophysics Facility supports researchers in the use of sophisticated instrumentation to measure the interactions, reactions, stability and size of biological molecules with accuracy and precision.

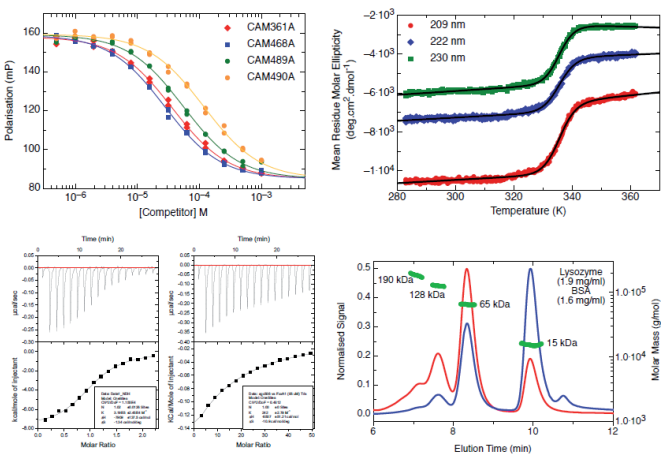
A growing range of techniques has been developed to characterise the physical properties of biological macromolecules such as proteins and nucleic acids. These biophysical techniques enable us to investigate how those molecules gain and retain structure, perform reactions, and interact when they assemble into larger structures, in order to play their essential roles in living cells.

We support researchers who wish to use these techniques, from the first stages of experimental design to the final stages of data interpretation and presentation. Depending on individual requirements, we can perform experiments for users or train them to operate instruments and to collect high-quality data. Although the facility is based in the Biozentrum, we also have users from other departments in the University of Basel, other universities in Switzerland and from industry.

We will identify the best combination of methods to answer each question. The facility has instruments for micro-calorimetry (DSC, ITC), circular dichroism and fluorescence spectroscopy, surface plasmon resonance (Biacore), micro-scale thermophoresis, differential scanning fluorimetry (Thermofluor), analytical ultracentrifugation and light-scattering measurements (DLS, MALLS). We are responsible for maintaining and improving these instruments to meet the future needs of users.

EXAMPLES OF TYPICAL APPLICATIONS ARE

- Measuring the energies of macromolecular interactions
- Determining the size and shape of complexes
- Assessing the impact of mutations on protein structure and stability
- Measuring the size and size-distribution of nanoparticles
- Screening small molecules libraries to identify chemical probes that bind to a particular macromolecular target



INTERACTION OF GOLD NANOPARTICLES WITH HUMAN LYMPHOCYTES: IMPACT OF THE INTRODUCTION OF A "MIX MATRIX" CAP

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Metal nanoparticles have many properties that make them attractive for a number of biomedical applications. They have shown great promise in many developments such as highly sensitive diagnostic tests, imaging and radiotherapy enhancement, modulated drug delivery and photothermal cancer therapy. Gold nanoparticles (AuNP) are amongst the most chemically stable nanoparticles available. However, it should be noted that from a biological perspective stability refers to their solubility or dispersal in a physiological environment and the lack of aspecific interactions with targets other than those required for their desired function. No simple conclusions have emerged from the available studies on nanoparticle toxicity due to the variability of parameters such as the physical and chemical properties of the particle, cell type, dosing parameters, and the biochemical assays used. Bulk gold is well known to be "safe" and chemically inert, and gold-based compounds have been used in the clinic as anti-inflammatory agents to treat rheumatoid arthritis. Furthermore, radioactive gold microparticles have been effectively used in local radioisotope cancer therapy. In most of the imaging and therapeutic applications that are being investigated, the gold particles are ~5 nm or larger. At sizes larger than 5 nm, the general assumption is that gold is chemically inert like the bulk.

An important disadvantage of note is that AuNP interact with serum proteins within the body, which adsorb onto the surface, thereby producing a 'corona' of proteins that can affect their structure and biological function. By impacting the ability of AuNP to interact with and enter cells, this may modify in vivo behaviour. Prevention or reduction of protein corona formation may be one way of modulating the interaction of AuNP with the immune system. A mixed matrix (also referred to as 'capping') is one potential approach that can be applied to stabilize and protect AuNP from aspecific interactions and ligand exchange. The mixed matrix utilised in this study comprises a self-assembled ligand monolayer and can be formed by the mixture of HS-PEG and alcohol terminated peptides. AuNP that possess this mixed matrix layer are resistant to aggregation of electrolytes and proteins. The addition of a mixed matrix to AuNP has been demonstrated previously with the mix matrices imparting a unique stability in physical, chemical and biological terms to AuNP.

The aim of the present study was to examine the impact of AuNP on the function and phenotype of primary lymphocytes and to assess if the presence of a mixed matrix alters these interactions. T cells are particularly important to the orchestration and resolution of an immune response, particularly CD4+ T helper cells. Several studies have addressed the influence of nanoparticles on Th1 and Th2 responses. Large (>1 µm) industrialized particles induced the Th1 response, whereas smaller ones (<500 nm) were associated with Th2. However, there are also contradictory reports in the literature that show induction of Th1 responses with smaller (<500nm) nanoparticles. This serves to highlight the importance of structure activity relationships in immune interaction studies, particularly with respect to any surface modifications that these nanoparticles may undergo.

We have used flow cytometry to assess the expression of markers of T cell activation (CD25 and CD44) in CD4+ T cells from human volunteers ex vivo (using anti-CD2, anti-CD3 and anti-CD28 MACSiBead particles as a positive control for T cell stimulation), Multiplexed bioanalyte measurements of IL-2, IL-10 and IFNγ in cell culture supernatants of peripheral blood mononuclear cells (PBMC; using anti-CD2, anti-CD3 and anti-CD28 MACSiBead particles as a positive control for T cell stimulation) and proliferation of PBMC via incorporation of 3H-thymidine in response to both capped and uncapped AuNP.

Incubation of PBMC with MACSiBead particles resulted in significantly higher expression of CD25 (11-fold difference; P=0.047) and CD44 (1.4-fold difference; P=0.048) in CD4+ T cells, but neither capped nor uncapped AuNP affected expression of these markers (fig. 1).

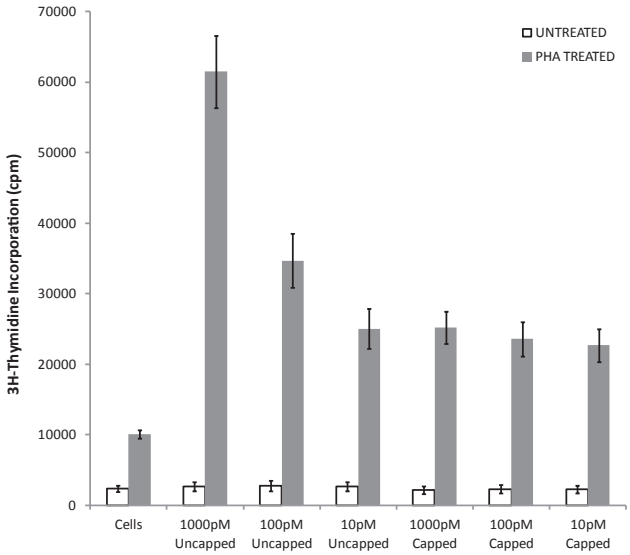

Figure 1. *Impact of AuNP (capped and uncapped) on (a) CD25 and (b) CD44 expression in CD4+ T cells from healthy volunteers. Data shows mean ± SEM.*

Figure 2 shows the secretion of IL-2, IL-10 and IFNγ from PBMC treated with AuNP. There was a 52-fold difference in IL-2 secretion from MACSiBead treated PBMC compared to the unstimulated control. PBMC treated with uncapped nanoparticles (1000pM) also resulted in higher secretion of IL-2 compared to controls (4.4-fold difference) however this was not statistically significant. IL-10 secretion was dramatically affected by uncapped AuNP in an apparent dose dependant manner (1000pM 2.4-fold difference; 100pM 2-fold difference; 10pM 1.5-fold difference). IFNγ secretion was also dramatically affected by 1000pM AuNP (capped and uncapped) however as there was no detectable IFNγ in the untreated control it was not possible to calculate a fold difference.

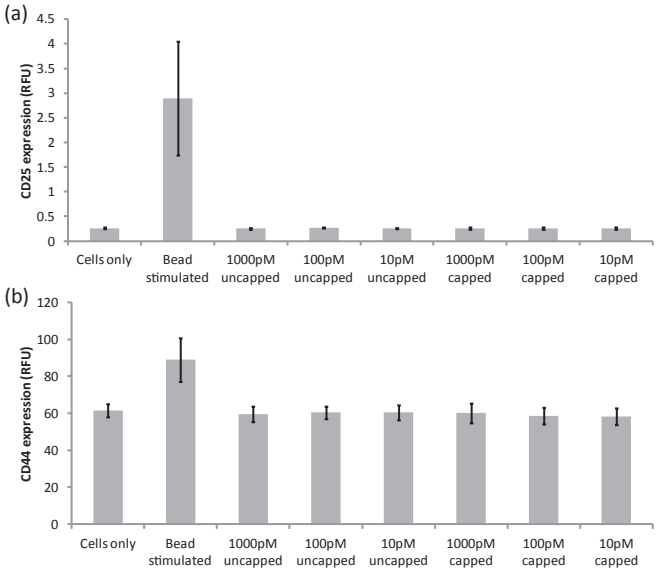

Figure 2. *Impact of AuNP on cytokine secretion from healthy volunteer PBMC.*

Figure 3 illustrates the impact of AuNP on cellular proliferation in cells from healthy volunteers in the presence and absence of phytohaemagglutinin (PHA). There was a greater degree of proliferation in PHA treated cells compared to untreated cells (4.2-fold difference, P<0.0001). Neither uncapped nor capped AuNP had an impact on the cellular proliferation of PBMC in their own right. However, both uncapped and capped AuNP significantly impacted on the proliferation of PBMC treated with PHA resulting in a significant enhancement of proliferation beyond that of PHA alone e.g.,

Uncapped AuNP at 1000pM (6-fold higher proliferation compared to PHA alone; P=0.003) and capped AuNP at 1000pM (2.5-fold higher proliferation compared to PHA alone; P=0.007).

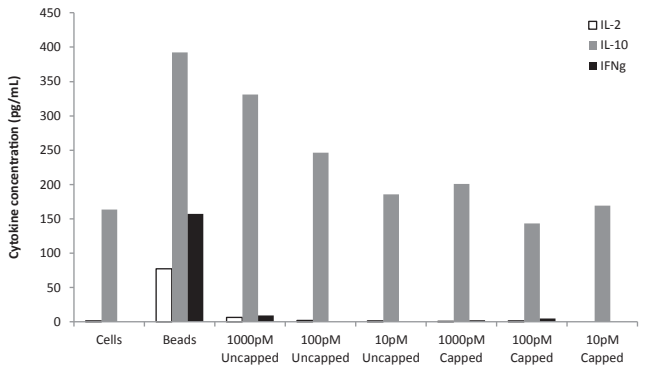

Figure 3. *Impact of AuNP on cellular proliferation in healthy volunteer PBMC in the presence and absence of PHA. Data shown as mean ± SEM.*

The lack of impact on expression of T cell activation markers may suggest that although it is possible for AuNP to activate T cells the lack of a costimulatory signal may result in T cell anergy rather than an upregulation of activation markers. However, this requires further investigation. The stimulation of T cells to release cytokines may be from intracellular stores of cytokines rather than de novo synthesis of new proteins and maybe in line with the lack of change in immunophenotype. There was marked interindividual variability in cytokine secretion from healthy volunteer PBMC, which may explain the lack of statistical significance in this data. Nonetheless, the increase in IL-10 is in line with secretion of Th2 cells and is in accordance with previous reports that smaller nanoparticles may stimulate this type of response. The enhancement of cellular proliferation in PBMC exposed to PHA may initially be explained by the uncapped AuNP taking up PHA onto their surface and thereby making them more immunogenic. However, capped AuNP also enhanced the cellular proliferation of PBMC, albeit to a lesser extent. In summary, we have shown that AuNP impact on the phenotype and function of primary lymphocytes and that this impact is reduced by the addition of a mixed matrix to the AuNP surface. How this mix matrix reduces the interaction of AuNP with immune cells now warrants further investigation. The interaction of capped and uncapped AuNP in other immune cells such as macrophages is now being investigated but the mix matrix approach shows promise for reducing immune interaction.

"METAL OXIDE NANOPARTICLES AND THEIR POTENTIAL EFFECTS ON THE IMMUNE SYSTEM"

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Due to their small size, nanoparticles (NPs) are able to enter cells, interacting with proteins and DNA, so they can modulate the immune system. Metallic nanoparticles (NPs) are widely used in cosmetics, health and food industries, but the interaction of these materials with the components of the immune system and their potential risks for human health are poorly understood.

OBJECTIVE

To assess the potential impact of diverse metallic NPs on immune functions such as cell adhesion and proliferation, chemotaxis, basophil activation and leukocyte oxidative burst after phagocytosis.

MATERIALS AND METHODS

ZnO, CeO2, TiO2 and Al2O3 metal oxide NPs were tested in human peripheral blood lymphocytes (PBLs) and the T cell line Jurkat. After the treatment with the NPs, the expression levels of the integrins LFA-1 and β-1, the chemokine receptor CXCR4 and L-selectin were determined. In addition, Transwell chemotaxis assays towards the chemokine SDF-1α (the ligand for CXCR4), leukocyte oxidative burst, basophil activation tests and T lymphocyte proliferation studies with CFSE in response to PHA (phytohemagglutinin) were also performed. Data were acquired in a flow cytometer.

RESULTS

The expression levels of LFA-1, integrin β-1, L-selectin and CXCR4 were affected in cells incubated with the NPs, except in the case of Al2O3 NPs, which did not induce significant changes in CXCR4 expression levels (Fig. 1 and data not shown). We also observed an increase in the proliferative induced by PHA upon cell treatment with TiO2 and Al2O3 NPs (Fig. 1 and data not shown). In addition, a decrease in the chemotactic response towards the chemokine SDF-1α was found in cells treated with ZnO NPs. Regarding basophil activation (Fig. 2) and leukocyte oxidative burst after phagocytosis (data not shown) no significant changes were observed upon treatment with any of the NPs studied.

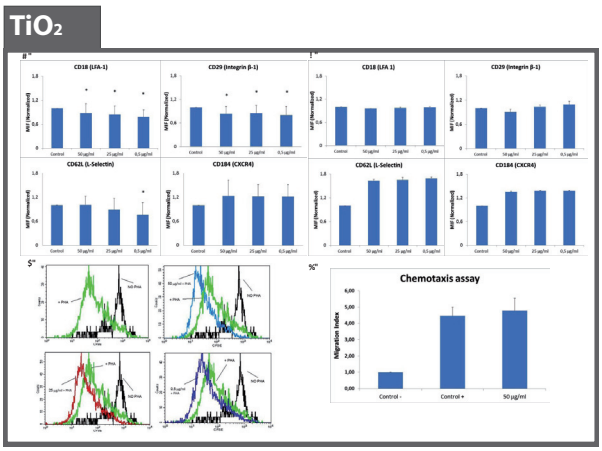

Figure 1. *Titanium oxide NPs (TiO2) induce changes in the expression of adhesion molecules but not in CXCR4 levels in human peripheral blood lymphocytes (PBLs), and also increase the proliferation capacity of cells, but have no effect in cell chemotaxis towards SDF-1α (1A) Changes in the expression levels of LFA-1, β-1 integrin, L-selectin induced by the indicated NP concentrations in PBLs. Data represent the normalized mean intensity of fluorescence ± S. D. obtained by flow cytometry in 20 independent experiments (*p<0.05). Similar results were obtained when experiments were performed in J77 cells (1B). (1C) NPs increase the proliferation capacity of T lymphocytes in response to 20 µg/ml PHA. PBLs were labeled with CFSE and data were obtained by flow cytometry between 2 and 4 days of incubation with PHA. A representative experiment out of 4 performed is shown. (1D) Titanium oxide NPs did not induce significant changes in the migratory response to SDF-1α. Data represent the migration index (mean± S. D.) of 3 independent experiments.*

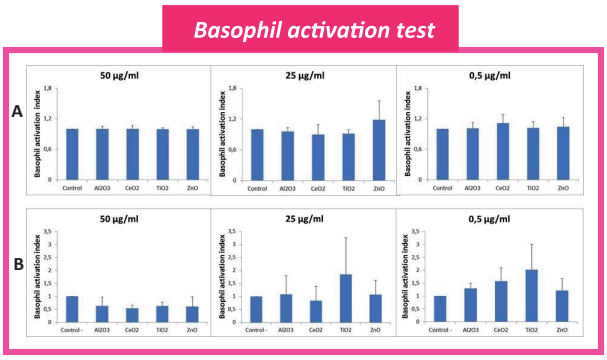

Figure 2*: Metal oxide NPs do not affect the basophil activation state. (2A-B) Heparinized whole blood samples were incubated overnight (2A) or for 40 min. at 37ºC (2B) with different NP concentrations. An*

antibody anti-IgE was added as positive control, and basophil activation level was measured as the percentage of CD63 expression by flow cytometry. Data are represented as basophil activation index (mean± S. D.) of 4 independent experiments.

CONCLUSIONS

Although the presence of metallic NPs did not alter immune functions like leukocyte oxidative burst and basophil activation, these nanomaterials are not inert. They are able to induce changes in the expression levels of integrins, L-selectin and the chemokine receptor CXCR4, all implicated in key immune cell functions such as adhesion, migration and proliferation. These results indicate that NPs could produce important undesired effects in a medical context, such as a modification in the migration and activation capacity of immune cells.

This work has been supported by EU Project HINAMOX NMP4-SL-2009-228825 and ETORTEK 2010 (ETORIE 10-276). We also thanks to the EU [FP7/REGPOT-2012-2013.1] under grant agreement BIOCAPS-316265. Tamara Lozano acknowledges a PhD fellowship (FPU, Spanish Ministry of Education).

SETUP OF IN VIVO BREAST CANCER MODELS FOR NANODRUG TESTING

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Synthetic lethality is a promising concept for future cancer treatment and using siRNAs as the synthetic lethal drug component allows for also covering the space considered as non-druggable by conventional small molecule drugs. Systemic administration of naked siRNA, however, does not result in sufficient cellular uptake without targeting and siRNAs commonly have a short plasma half-life of less than 10 minutes. As a consequence, nanodevices for siRNA targeting and delivery to cancer cells are under extensive investigation, including, for example, chimeric compounds such as siRNA/antibody or siRNA/aptamer conjugates, or carriers such as liposome/chitosan/micelle spheres. As a first step towards testing of the efficacy of siRNA delivery in vivo via different conjugates and complexes, we aimed at developing a standardized breast cancer model system in mice. In this conception, a reporter gene is used for detection of the primary tumor and metastasis and the efficacy of siRNA delivery is measured by reporter gene-targeting siRNAs and in vivo imaging. The use of a uniform siRNA not affecting cellular processes would allow for standardized assessment of siRNA delivery to cancer cells without interferences via differential knockdown efficacies and the readout can directly be performed by quantitative imaging using a Caliper IVIS system. In one line of experiments, we engineered non-metastatic MCF-7 breast cancer cells to express the luminescent reporter firefly luciferase (Luc2) along with a pro-metastatic microRNA. This produced strongly luminescent tumors upon implantation and ex vivo examination of lungs and liver revealed prominent metastatic spread. In a second line, we engineered MCF-7 cells with a near-Infra Red Fluorescent Protein (iRFP) reporter, lacking the pro-metastatic microRNA. The reporter signal followed the increase of the tumor volume and the mice lacked detectable metastasis formation in the liver and the lungs as assessed by ex vivo imaging. In vitro experiments indicated that iRFP-targeting rather than Luc2-targeting siRNAs were effective in silencing the respective reporter without exerting substantial non-specific toxicity to the cells.

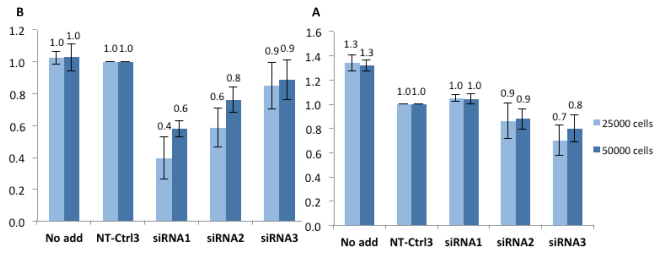

Figure 1. *Effect of iRFP siRNA knockdown on fluorescence relative to cell viability (A) and cell viability alone (B). Non-targeting control #3 was used as relative baseline for quantifications. The experiment was performed three times in 6-plicates. NT-Ctrl3 is Non-Targeting siRNA Control#3 (Dharmacon). The values represent the average values of the three repetitions. The error bars are the standard error of the mean (SEM).*

Considering this in conjunction with the easier handling of the iRFP reporter than the Luc2 reporter during in vivo imaging, the system with the iRFP reporter might represent a more suitable platform for testing siRNA delivery, so that we presently set up a corresponding metastatic mouse model with this system as well.

PEGYLATED CHITOSAN NANOCARRIERS FOR LIGHT ACTIVATED CANCER THERAPY, SYNTHESIS AND CHARACTERIZATION

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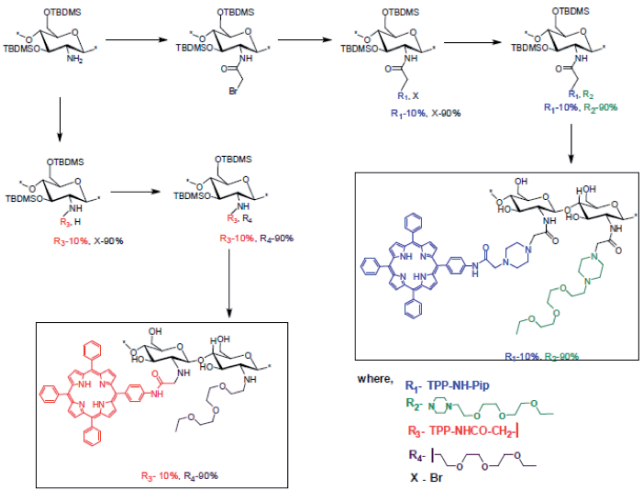
Neutral amphiphilic nanocarriers (drug-polymer conjugates) with suitable properties for photochemical internalization (PCI) were synthesized and characterized. The aim was to synthesize a nanocarrier from chitosan which should be ~10% substituted by tetraphenylporphyrin derivatives (TPP) and 90% substituted by triethylene glycol (TEG) chains. Different approaches to N-modifications were tried, including reductive alkylation, electrophilic and nucleophilic substitution. Specialized TPP and TEG reagents were developed for this purpose. Synthesise scheme over these nanocarriers are shown in the scheme below.



Scheme 1*: Synthetic route from TBDMS-chitosan to chitosan nanocarriers*

Piperazine and tosylate derivatives of PEG and TEG were synthesized and their efficacy in chitosan N-modifications was investigated with 1H NMR by calculating the degree of substitution (DS). Direct N-modifications with electrophilic reaction by TPP-derivative and TEG tosylate directly to chitosans primary amine led to low DS values, only 4 % for TPP and 36 % for TEG tosylate. However the reaction of the nucleophilic piperazine derivatives of TPP and TEG, to the acetylbromochitosan were highly efficient and gave DS of 10

% (TPP) and 87 % (TEG).
Of the two different chitosan nanocarriers synthesized, the one that had less DS for both TPP and TEG had limited aqueous solubility. Partition experiment was done of the chitosan nanocarrier that had higher DS. This experiment revealed that the product is hydrophilic and does not partition into organic phase.

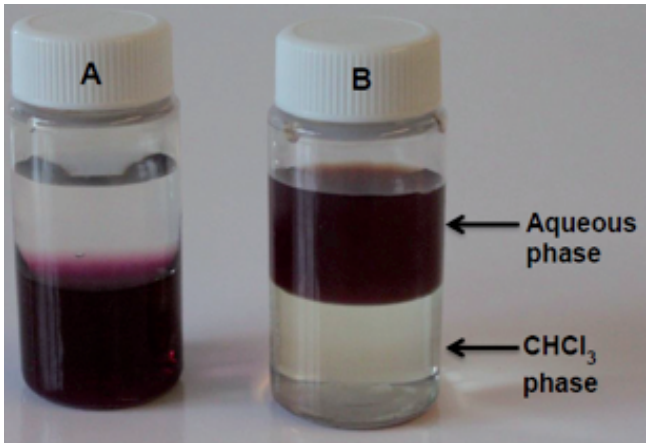


***Figure 1**: Partition experiment of TPP-piperazine and TEGylated TPP-piperazine-chitosan. A) TPP-piperazine is accumulating in the organic phase (CHCl3) B) All the color has gone over to the aqueous phase which indicates that the TEGylated TPP-piperazine-chitosan is no longer lipophilic*

Dynamic light scattering (DLS) study was performed on the TEGylated TPP-piperazine-chitosan to estimate the particle size distribution of the nanoparticles in aqueous solutions and stability.

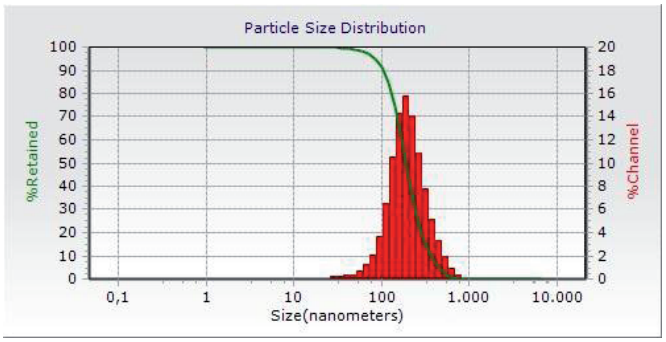


***Figure 2**: Size distribution measurement of TEGylated TPP-piperazine-chitosan nanoparticles by DLS*

From this graph it was found that the mean intensity diameter was 216 nm, and a measurement of Z-potential was 87 mV which indicates that these nanoparticles are stable in aqueous environment. The results of the present study are positive and by most part in accordance to aims that were set in the beginning. Even though a further optimization of methods can be achieved, a novel nanoparticle drug-polymer conjugate has been synthesized with suitable properties for light activated therapy, including PCI where light activated photosensitizers promote the release of endocytosed drug molecules into the cells cytosol.
Acknowledgement: We thank financial support from the Technical Development and the Icelandic Research Centre and from University of Iceland Research Fund.

GASC (GLIOMA-ASSOCIATED STEM CELL)-DERIVED EXOSOMES ENHANCE THE AGGRESSIVENESS OF A GLIOBLASTOMA CELL LINE

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BACKGROUND
Glioblastomas are the most common and aggressive type of malignant gliomas, characterized by a median survival of 15 months. In the last years, several studies highlighted the importance of tumor-supporting cells in cancer progression and the role played by exosomes in tumor-stroma interactions. Recently, we optimized a method to isolate and grow in culture populations of human glioma-associated stem cells. These cells, although devoid of genetic alterations, are characterized by stem cell properties, aberrant growth features and a gene expression profile typical of tumor supporting cells. Moreover, GASC were able to modify the biological properties of the commercially available glioblastoma cell lines A172 and U87.

AIMS
to verify whether GASCs produce exosomes and if they are able to increase the biological aggressiveness of A172.

METHODS AND RESULTS
we isolated exosomes from GASC culture supernatants by ExoQuick-TC precipitation and verified their presence either by FACS (expression of CD9 and CD63) or Atomic Force Microscopy (particles with a diameter ranging from 20 to 110 nm). We compared the capacity of the unfractioned-, exosome-depleted GASC supernatant and GASC-derived exosome to modify growth kinetic, migration ability and anchorage-independent growth of A172 cells. These latter, when grown in the presence of unfractioned GASC supernatant and GASC-derived exosomes, showed a significant decrease in the population doubling time and a significant increase both in migration speed and anchorage-independent growth (Figure 1). These changes were not present when A172 cells were grown in the presence of the exosome-depleted supernatant, supporting the notion that exosomes were responsible for the effects of GASC supernatant on A172 cells.

CONCLUSIONS
Glioma-Associated Stem Cells (GASC) produce exosomes that are able to modify the biological behavior of the glioblastoma cell line A172 increasing its aggressiveness. Studying the molecular cargo of exosomes produced in the glioma microenvironment could be important to understand the tumor biology, opening the way to new therapeutic approaches aimed at interfering with the tumor-stroma interplay.

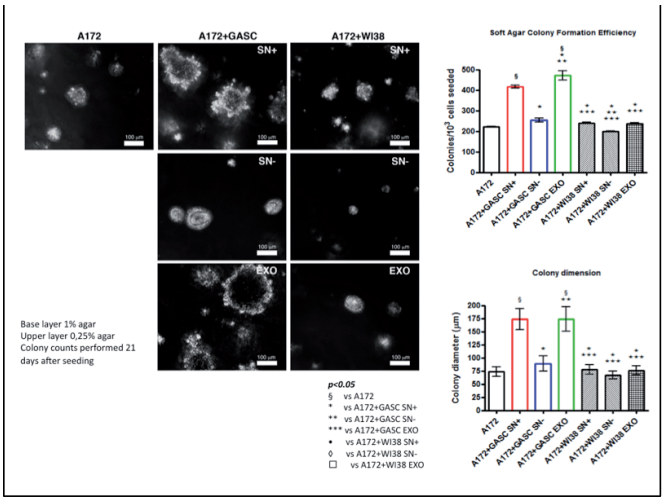

***Figure 1.** Anchorage-independent growth of A172 grown for 21 days in a medium conditioned or not by GASC or Wi38. On the left, representative pictures of A172 grown in the absence of conditioned medium (A172, left panel) or in the presence of either GASC-derived (middle panel) unfractioned supernatant (SN+), exosome-depleted supernatant (SN-) and exosomes (EXO), or Wi38-derived (right panel) unfractioned supernatant (SN+), exosome-depleted supernatant (SN-) and exosomes (EXO). On the right, histograms represent the quantitative analysis of the colony forming efficiency and colony dimension of A172 in the different experimental conditions. Data are presented as mean±standard deviation.*

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SILVER NANOPARTICLES SYNTHESISED USING HERBAL EXTRACT

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INTRODUCTION

Nanocrystalline silver particles have received considerable attention in recent years because of its unique properties and pharmaceutical applications. Physical and chemical methods of synthesis of silver nanoparticles are cumbersome and not eco friendly. Exploration of the plant systems as potential nanofactories has heightened interest in the biological synthesis of nanoparticles. Piper nigrum is a flowering vine belonging to Piperaceae family and various bioactivity studies have established its anti- inflammatory, anti-cancer, antimicrobial properties. The various activities are attributed to the alkaloid Piperine present in Piper nigrum.

OBJECTIVES

Rapid synthesis of silver nano particles using the aqueous extract of unripe fruits of Piper nigrum and to evaluate its anti-inflammatory activity using in-vitro assays.

MATERIALS AND METHODS

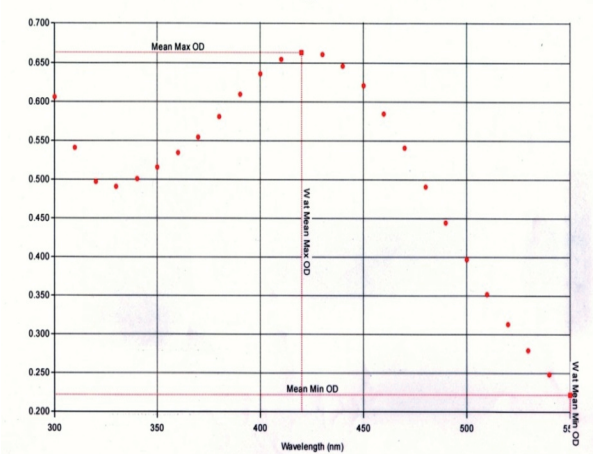
Piper nigrum seed extract was prepared by boiling 10gms of powdered dried fruits with 50 ml of distilled water for 15 mins. The supernatant was then filtered and pH adjusted to 12.

Synthesis of silver nanoparticles

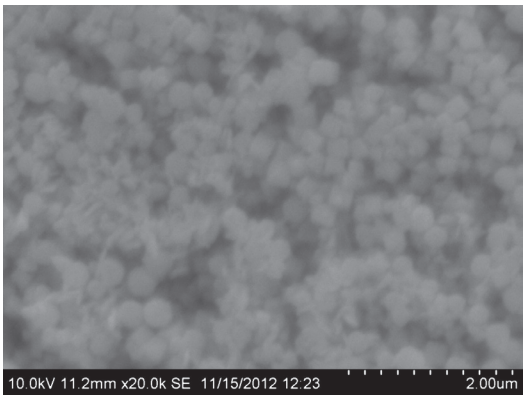
To 100 ml of 1mM AgNO3 solution, 1ml of the extract was added and the mixture was kept in a magnetic stirrer for 2 hrs. The mixture was then centrifuged at 3000 rpm for 10 min and the residue was used for characterization studies.

Characterisation of synthesised silver nanoparticles

UV-Vis Spectroscopic analysis showed an absorbance peak at 420 nm which was specific for Ag nanoparticles(AgNP), and SEM image showed size between 40-80nm.



UV- SPECTROPHOTOMETER- ABSORBANCE PEAK



SCANNING ELECTRON MICROSCOPE PICTURE

EVALUATION OF ANTI- INFLAMMATORY ACTIVITY

MTT assay:

MTT assay was performed as per Mosman et al (1983) in Hep G2 cell line. Pre-confluent Hep G2 cells were seeded in 96-well plates. Cells were treated with different concentrations of synthesised silver nanoparticles (ranging from 10ρg - 1010ρg) after 24 h following plating and incubated at 37°C for one day. At 20 h following exposure, cells were incubated at 37°C with 5 mg/ml MTT for 4 hours. At the end of the experiment, the insoluble formazan product was dissolved in DMSO (200 µL) and kept at least 15 minutes in the dark.

Assay for TNFα & NO

Mouse macrophage cell line was used. Cells were cultured in suitable medium supplemented with 2 mM glutamine, antibiotics (100 U/mL each of penicillin and streptomycin), and 10% heat-inactivated fetal bovine serum and maintained at 37°C in a humidified incubator containing 5% CO2. Cells were seeded onto a 24-well plate (5 × 104 cells/well) for experiments. The cells were pretreated with test drug for 24 hours and subsequently stimulated with 10 µg/mL of LPS

Test drug was dissolved in 0.01% dimethyl sulfoxide (DMSO). For the control group, cells were cultured with 0.01% DMSO alone.

Determination of Nitrite Concentration in Medium

NO was measured as its end product, nitrite, by using Griess reagent The culture supernatant (100 µL) was mixed with 100 µL of Griess reagent for 10 minutes, and the absorbance at 550 nm was measured in a microplate reader. The concentration of nitrite in the samples was determined with reference to a sodium nitrite standard curve. The data represent the mean of eight determinations ± SD.

Levels of TNF-α

The levels of TNF-α and PGE2 in the medium were measured by ELISA. The ELISA assay was performed in duplicate.

RESULTS AND DISCUSSION

The MTT assay showed increasing cytotoxicity of the synthesised silver nanoparticles with increasing concentration. The IC 50 value obtained was 108.6 microgram/ml. The anti-inflammatory assay is currently being done, and results will be presented on the poster.

REFERENCES

• P.H.M. Hoet, I.B. Hohlfeld, O.V. Salata, J. Nanobiotechnol. (2004) 2, 12
• J.Y. Song, H.K. Jang, B.S. Kim, Process Biochem. (2009) 44, 1133
• M.A. Farooqui, P.S. Chauhan, P. Krishnamoorthy, J. Shaik, Digest Journal of Nanomaterials and Biostructures (2010) 5, 43
• Venkat Reddya, Pullela V, Srinivasa B, Phytomedicine, (2004), 11, 697–700.
• El Hamss R, Idaomar M, Alonso-Moraga A, Muñoz SA Food chem. Toxicol. (2003), 41(1): 41-47.
• Pradeep CR and Kuttan G (2004). Int. Immunopharmacol. 4: 1795-1803.
• Reshmi S.K, Sathya E, Suganya Devi P, Journal of Medicinal Plants Research (2010) Vol. 4(15), pp. 1535-1546,
• Vineet K. Shukla, Ravindra P. Singh, Avinash C. Pandey, Journal of alloys and compounds, (2010) ,507 L13–L16
• Tim Mosmann. Journal of immunological Methods. (1983); 65: 55-63.
• Sunila ES, Kuttan G. J Ethnopharmacol 2004; 90: 339-346.

SINGLE-WALLED CARBON NANOTUBES (CNT) AS A FEASIBLE AND POTENTIAL MULTIFUNCTIONAL DRUG CARRIER FOR CONJUGATING INSULIN

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In this work, the carboxylic acid groups on functionalized single-walled carbon nanotube sidewalls were used as a precursor for di-imidation to form amide bonds with the amine groups present on insulin. This was carried out through the reaction of the carboxylic groups with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and N-hydroxysulfosuccinimide (S-NHS) to form an amine-reactive ester group. Results obtained from STEM and TEM imaging showed

the clear conjugation of insulin to the surface of the nanotube sidewalls. Bicinchoninic acid (BCA) assay tests that were conducted on the insulin-conjugated SWCNTs showed that an estimated 0.24 mg of insulin was attached for every 1 mg of carboxylated SWCNT used. The results of this study represent an interesting first step towards the potential development of SWCNT as insulin carriers.

INTRODUCTION

Among the recently explored potential application of SWCNTs is their use as multi-functional drug carriers. The high aspect ratio of carbon nanotubes means that it is not only possible to attach drugs but also other compounds that impart certain desired functionalities. Expanding on this, the use of carbon nanotubes as a multifunctional insulin carrier may be an interesting prospect to explore. As an example, for the oral route, carbon nanotubes that are used as insulin carriers may be functionalized with mucoadhesives, absorption enhancers or enzyme inhibitors to aid the delivery of the attached insulin through the gastrointestinal tract [1]. In this work, the conjugation of insulin onto the sidewalls of functionalized single-walled carbon nanotubes (SWCNTs) through their esterified carboxylic groups is presented. The approach and the mechanism behind the utilization of carboxylic groups on the SWCNT surface for the attachment of insulin groups have been fully elucidated. For the characterization of samples, transmission electron microscopy (TEM) imaging was used to visualize the synthesized SWCNT-insulin conjugates that were purified through a dialysis step. To estimate the amount or concentration of the insulin attached, a bicinchoninic acid (BCA) assay was conducted on the synthesized samples before measuring the absorbance using a UV-Vis spectrophotometer.

MATERIALS AND METHODS

10 mg of human recombinant zinc-insulin (Merck) was dissolved in 2 ml of pH 7.6 phosphate buffer. The resuspended diimide-activated SWCNT mixture was sonicated in an ultrasound bath briefly before the quick addition of the insulin solution. The diimide-activated SWCNT-insulin mixture was left to react for 3 h on an orbital shaker at 180 rpm. Following this, the mixture was centrifuged at 2000 x g for 1 min to remove the unreacted nanotubes. The resulting supernatant was dialyzed using a cellulose membrane tubing (Sigma Aldrich) for 4 days to remove the unbound insulin. For the dialysis process, 0.005 M KH2PO4 buffer was used as dialysate for the first 3 h before changing to ultrapure water. The dialysate was changed three times a day to maintain the diffusion force across the membrane. Dialysis was conducted at around 10-12 °C overnight for the duration of the procedure using refrigerant gel packs to minimize the protein degradation. All BCA assay studies were conducted using a Bicinchoninic acid (BCA) protein assay kit (Thermo Scientific) while the absorbance of the reacted samples was measured using a UV-Vis spectrophotometer (Perkin Elmer Lambda 35). The absorbance-concentration calibration curve was measured using bovine serum albumin (BSA) standards (Thermo Scientific). After adding the BCA containing reagents, the samples were incubated for 30 min before measuring the absorbance at 562 nm. For TEM imaging, samples were dropped on formvar-carbon coated copper grids before staining with 3% phosphotungstic acid for 2 min. The grids were then left to dry sufficiently before subjecting to TEM (LEO 912AB Energy Filter TEM) and STEM (FEI Quanta 650) imaging.

RESULTS AND DISCUSSION

In this investigation, the carboxylic acid groups on the functionalized carbon nanotube sidewalls were used as a precursor for diimidation to form amide bonds with the amine groups present on insulin. This has been achieved through the reaction of the carboxylic groups with EDC and S-NHS to form an amine-reactive ester group. The same technique had been successfully employed to attach proteins such as bovine serum albumin (BSA) as well as ferritin [2, 3]. The EDC/S-NHS activation method was preferred due to its high conversion efficiency and excellent biocompatibility which minimizes any adverse effects on the bioactivity of target molecules [4, 5].

From the primary structure of the human insulin, there are three amino groups located at A1, B1 and B29 that can be used to form amide bonds with the functionalized SWCNT [6]. The EDC reacts with the –COOH groups on the SWCNT defect sites to form an amine-reactive *O*-acylisourea intermediate. The addition of S-NHS helps increase the stability and coupling efficiency by reacting with the *O*-acylisourea groups to form amine-reactive NHS-ester groups which can be stable for hours [4]. The latter can then be used to react with amine groups present on the insulin molecule to form stable amide crosslinks. The result is a stable covalent bond between the functionalized SWCNT and the target insulin molecule.

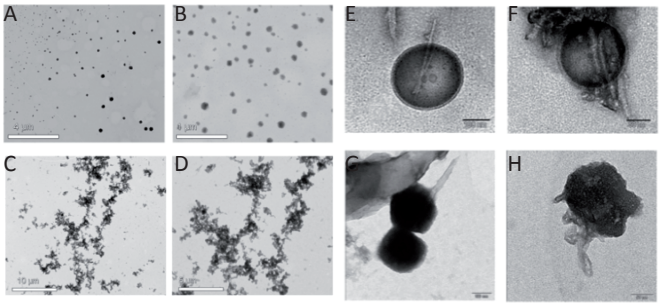

***Figure 1**: (A and B) STEM images of insulin stained with phosphotungstic acid; (C and D) STEM images of insulin-conjugated SWCNTs stained with phosphotungstic acid; (E-H) TEM images of insulin-conjugated SWCNTs stained with phosphotungstic acid.*

STEM images of pure insulin which could be seen in Fig. 1 (A, B) shows that the overall particle size of insulin is in the region of 80-200 nm. The images also show that the insulin proteins are generally spread evenly within the phosphate buffer. A comparison of the STEM images of insulin with that of the insulin-conjugated SWCNT (Fig. 1, C and D) confirms the affinity of the insulin towards the diimide-activated carbon nanotubes. The attachment of insulin onto the functionalized SWCNT sidewalls can clearly be seen from the difference between the insulin which showed affinity to the SWCNT sidewalls in Fig. 1 (C and D) and the relatively dispersed appearance of the pure insulin that can be seen in Fig. 1 (A and B). However, due to the instrument constraints of the relatively low-resolution STEM, it is difficult to resolve images at higher magnification levels, with only the larger aggregates of insulin-conjugated SWCNTs being visible. For a close-up view of the conjugation of insulin onto individual carbon nanotubes, TEM imaging of the samples using the same staining method was conducted. From the TEM images shown in Fig. 1 (E-H), the insulin molecules can clearly be seen to conjugate onto the surface of the individual carbon nanotubes. The images confirm that the insulin molecules are roughly 80-200 nm in size. The image depicts the natural spherical conformation of the insulin molecule in acidic conditions while the dark colour is a result of the phosophotungstic acid stainer enveloping the insulin molecule.

To estimate the concentration of protein conjugated onto the SWCNT surface, the purified SWCNT-insulin samples were analyzed using a BCA protein quantitation assay. From the analyzed results, it can be observed that for every 1 mg of diimide-activated SWCNT, approximately 0.24 mg of insulin was conjugated. It should be noted that as the unbound insulin purification might not be complete, the actual amount of conjugated insulin should be slightly lower than the one reported for samples after 4 days of dialysis. If necessary, there are a couple of possible methods that can be used to adjust the conjugation efficiency and the amount of insulin that could be attached to the SWCNT sidewalls. For example, the number of carboxylic group on the functionalized SWCNTs can be controlled during the carboxylation process. In addition, the ionic strength of the buffer used during the SWCNT-insulin preparation may also have an effect on the final amount of attached insulin [5]. The adjustment of the ionic strength can also be done by varying the pH of the buffer used for the amidation process.

CONCLUSIONS

Insulin was successfully conjugated on the surface of functionalized carbon nanotubes through the formation of amide bonds with the esterified carboxylic groups. From the BCA assay studies, it was found that approximately 0.24 mg of insulin was attached for every 1 mg of carboxylated SWCNT used. The results of this study represent an interesting first step towards the potential use of SWCNT as insulin carriers.

ACKNOWLEDGEMENTS

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REFERENCES

• G.P. Carino, E. Mathiowitz, Advanced Drug Delivery Reviews, 35 (1999) 249-257.
• W. Huang, S. Taylor, K. Fu, Y. Lin, D. Zhang, T.W. Hanks, A.M. Rao, Y.-P. Sun, Nano Letters, 2 (2002) 311-314.
•K. Jiang, L.S. Schadler, R.W. Siegel, X. Zhang, H. Zhang, M. Terrones, Journal of Materials Chemistry, 14 (2004) 37-39.
• C. Wang, Q. Yan, H.B. Liu, X.H. Zhou, S.J. Xiao, Langmuir, (2011).
• H. Jans, K. Jans, T. Stakenborg, B. Van de Broek, L. Lagae, G. Maes, G. Borghs, Nanotechnology, 21 (2010) 345102.
• T. Blundell, G. Dodson, D. Hodgkin, D. Mercola, Advances in Protein Chemistry, 26 (1972) 279-402.

MULTIFUNCTIONAL NANOCARRIERS: MNPS LOADED NIOSOMES, PREPARATION AND CHARACTERIZATION

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The use of nanoparticulate pharmaceutical carriers to enhance the in vivo efficiency of many drugs well established itself over the past decade both in pharmaceutical research and clinical setting. Surface modification of pharmaceutical nanocarriers, such as solid lipid particles, polymeric nanoparticles, nanocapsules, liposomes, niosomes, micelles and others is normally used to control and modulate biological properties in a desirable fashion and make them to simultaneously perform various therapeutically or diagnostically important functions. Furthermore, surface modification can enable to obtain responsiveness to local physiological stimuli such as pathology-associated changes in local pH and/or temperature, and ability to serve as imaging/contrast agents for various imaging modalities (gamma-scintigraphy, magnetic resonance imaging, computed tomography, ultrasonography). Nanoparticle formulations have demonstrated clinical relevance by increasing drug efficacy and decreasing toxicity and numerous targeted formulations are under clinical evaluation. There are unique
opportunities to use multifunctional formulations for both diagnostic and targeting purposes. Vesicles formed by surfactants are known as Niosomes or non-ionic surfactant vesicles (NSVs). The self-assembly of non-ionic surfactants into vesicles was firstly reported in the seventies by researchers in the cosmetic field. Niosomes are analogous to liposomes; when compared to phospholipid-based vesicles, surfactant vesicles have several advantages such as greater stability, thus lesser care in handling and storage and lower cost, attractive for industrial applications both in the field of pharmaceutics and cosmetics. Moreover niosomes, like liposomes, are capable of encapsulating both hydrophilic and lipophilic drugs. The superior magnetic properties of iron oxide magnetic nanoparticles (MNPs) along with their non-toxicity, biodegradability and inexpensiveness, have made them a material of choice in many bioapplications, such as contrast probes in magnetic resonance imaging (MRI). In order to confer colloidal suspendability to the particles, additives, typically hydrophilic polymers are added during the particle formation process, in order to passivate the nanocrystal surface and protect against particle aggregation. In this study, the preparation and characterization of MNPs loaded niosomes that offer the opportunity for targeted, non invasive diagnosis by MRI were carried out. Iron oxide MNPs have been encapsulated into surfactant vesicles to develop niosomal contrast agents, i.e., magnetic niosomes (MNs). Iron oxide NPs with a hydrodynamic diameter of 50 nm or less were selected. Such MNPs consist of a magnetic core coated by various materials (hydrophilic coating, e.g., dextran, or lipophilic coating, e.g., lipid or polymaleic acid co-olefin) to improve the solubility and/or stability of the MNPs in colloidal suspension.
This particular feature makes MNs suitable as MRI contrast agents for several biomedical applications and at the same time for active targeting strategies and for hyperthermia based treatments. However, for in vivo applications, the circulation time of MNs in the bloodstream should be long enough to ameliorate target-ligand interaction. This goal can be achieved by steric stabilization of the niosomes (e.g., by adding a poly-ethylene glycol [PEG]-based surfactant to the formulation). Tween 20 is a natural pegylated hydrophilic surfactant (HLB=16.7), it possesses 20 PEG units in its molecule and is able to prevent the rapid recognition and consequent uptake of MNs via the mononuclear phagocyte system. Tween 20 could be an useful surfactant to prepare vesicles, as previously reported, able to entrap hydrophilic MNPs. To entrap lipophilic MNPs, Span 20, a lipophilic surfactant (HLB=8.6) without PEG units in its molecule have been used to prepare MNPs loaded vesicles. The prepared vesicular formulations have been analyzed in terms of chemical and physical characteristics, including particle size, ζ-potential, morphology and stability, surfactant vesicle bilayer polarity and microviscosity, encapsulation efficiency, iron oxide loading capacity and magnetic properties.

Unilamellar vesicles were prepared by means of the 'film' method.
MNPs-loaded vesicles were purified from untrapped substances, for the evaluation of entrapment efficiency (e.e.). Fe e.e. within non-ionic surfactant vesicles was determined using inductively coupled plasma mass spectrometry (ICP-MS) on purified MNs. Measurements were performed by means of a dynamic reaction cell (DRC) ICP-MS (Elan DCR II, Perkin Elmer SCIEX, Norwalk, CT, USA) operating in DRC mode with ammonia as reaction gas (purity of 99.999%) so as to overcome the likely interferences on the Fe determination. Fe e.e. was calculated as the ratio between the mass of the incorporated Fe and that of the Fe used for vesicle preparation.
Dynamic light scattering (DLS) was used to determine mean size and size distribution (polydispersity index, PDI) of non-ionic surfactant vesicles at 25°C using a Malvern Nano ZS90 light scattering apparatus (Malvern Instruments Ltd., Worcestershire, UK) at a scattering angle of 90.0°. The same apparatus was used for the evaluation of ζ-potential of vesicles, which was calculated from the electrophoretic mobility in the Smoluchowsky approximation.
Physical stability studies of MNPs loaded vesicles were carried out to investigate if significant changes in size and ζ-potential of surfactant vesicle dispersion occur during storage. Vesicular formulations were stored at 4◦C and 25 ◦C for a period of 90 days.
Fluorescence experiments on vesicles incorporating pyrene were carried out (Perkin-Elmer LS55 spectrofluorometer with excitation wavelength 319 nm) to evaluate the micropolarity of the hydrocarbon bilayer in vesicles. Since pyrene is solubilized inside the hydrocarbon chain of vesicles, the information obtained from fluorescence of pyrene in our systems refers to the bilayer of the vesicle pigeon-hole.
The sample topography is firstly imaged in AFM standard tapping mode, then the scanning is repeated with the cantilever oscillating far enough from the surface. The phase of the cantilever oscillation is acquired and used as the MFM 'magnetic' signal, thus mapping the magnetic properties of the sample.

The obtained encouraging results confirm that Tween 20 and Span 20 vesicles could be promising carriers for the delivery of hydrophilic and lipophilic MNPs, respectively, thereby prompting various opportunities for the development of suitable theranostic strategies. The analyzed formulations confirm the importance of surfactant physico-chemical characteristics in entrapping the MNPs of different polarity, highlighting the high versatility of niosomal bilayer and structure. These properties make them so appealing among

drug delivery nanocarriers. In the future it could be very interesting to deepen chemical-physical characterization of magnetic niosomes (MNs)_together with their magnetic properties analyses to evaluate their theranostic potentiality, after loading of a model drug.

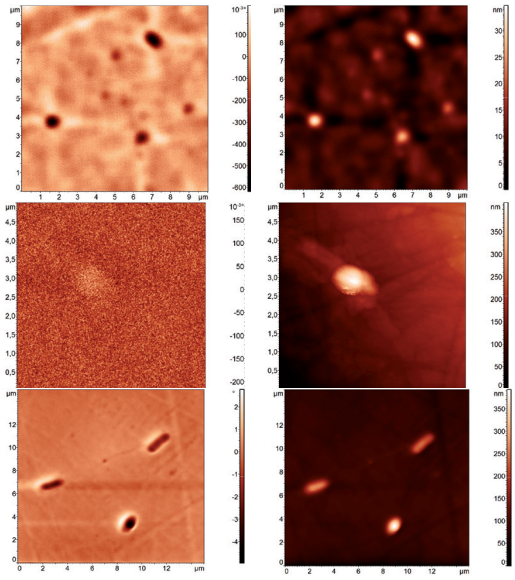

***Fig. 1**: AFM (right column) and MFM (left column) images of from the top: MNPs alone, empty vesicles and MNPs loaded vesicles*

NUMERICAL MODELING OF DRUG LOADED SILICONE MATRIX AND TRANSDERMAL DELIVERY

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Silicones are biocompatible polymers which have been used for different kinds of applications both medical and non-medical. Silicone matrix formulations have many unique characteristics, they are non-biodegradable polymers, suitable for medical use, drug delivery matrices (Snorradottir et al. 2011) and in medicated prosthesis (Gholizadeh et al. 2012). Drug micro- and nanoparticles have been embedded for various types of applications such as birth control implants and transdermal patches. The high stability of the silicone and the biocompatibility offers great advantage as matrix drug formulation and the good reproducibility of drug release from such formulation are advantageous especially when considering modelling drug release from such formulations in nanotechnology. Achieving the goal of an optimal release profile can be challenging when relying solely on traditional experimental work. Accurate modelling complementing experimentation is therefore desirable. Numerical modelling is increasingly becoming an integral part of research and development due to the significant advances in computer simulation technology.
Using two non-linear coupled partial differential equations derived from the Noyes-Whitney and Fick´s second law (Frenning 2003) were solved numerically using MATLAB. The aim was a model which could accurately predict the rate of drug release from silicone elastomers, as well as un-dissolved drug concentration in the material at each point in time. The predictions could be fitted to the experimental data. Drug release from these matrix type systems were conducted with vertical Franz diffusion cells (see fig.1) which offers one dimensional diffusion and is advantageous for definition of release profile from the silicone matrix. Here is presented further modifications of the numerical modelling of multi-layered silicone membranes with the aim of adding the skin into the model. Therefore offering a model with the option of having multi-layered silicone matrix as well as implicating the skin as one barrier/layer in the numerical modelling.

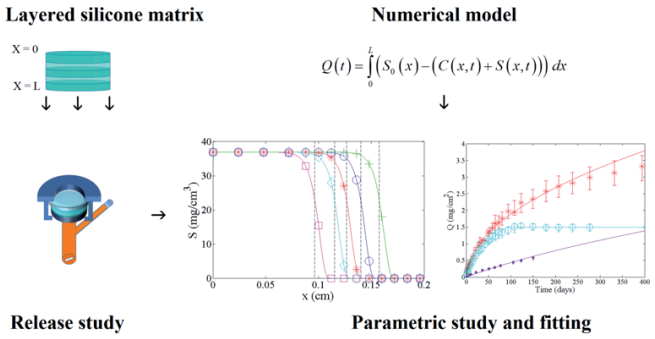

***Fig.1** Schematic overview of the modelling and experimental setup.*

REFERENCES

• Frenning, G., 2003. Theoretical investigation of drug release from planar matrix systems: effects of a finite dissolution rate. J. Control. Release. 92, 331-339.
•Gholizadeh, H., N. A. Osman, M. Kamyab, A. Eshraghi, W. A. Abas, M. N. Azam, 2012. Transtibial prosthetic socket pistoning: static evaluation of Seal-In((R)) X5 and Dermo((R)) Liner using motion analysis system. Clin Biomech (Bristol, Avon). 27, 34-39.
• Snorradottir, B. S., P. I. Gudnason, F. Thorsteinsson, M. Masson, 2011. Experimental design for optimizing drug release from silicone elastomer matrix and investigation of transdermal drug delivery. Eur. J. Pharm. Sci. 42, 559-567.

NANOPARTICLES CONJUGATED IN ORIENTATION–CONTROLLED MANNER WITH HALO-U11 PEPTIDE FOR CANCER CELLS TARGETING

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Multifunctional nanoparticles (MNP), combining unique superparamagnetic properties and fluorescence emission, have been conceived as promising bimodal tracers for noninvasive diagnosis of malignant cells.[1] The selectivity in recognizing specific cancer cells is usually accomplished exploiting the functionalization of MNP with molecules having high affinity for definite membrane receptors.[2] However, the orientation, in which individual homing ligands are immobilized at the interface between the inorganic core and the biological environment, may strongly affect the actual targeting efficiency of the conjugated nanoconstruct.[3] The molecular organization at the nanoscale, becomes even crucial when using short peptides, to which a stable ligation yet a sufficient mobility should be conferred upon immobilization to optimize the recognition with the specific biological receptor. Several approaches have been explored to govern ligand positioning, including conjugation mediated by affinity tags inserted in the protein primary sequence, oriented immobilization on MNP mediated by recombinant protein linkers, and site-specific chemoselective ligation.[4] Recently, we have proposed a novel approach relying on engineering proteins consisting of a receptor-targeting domain genetically fused with a nanoparticle-capture domain, in which the capture module should be an enzyme capable of irreversibly cross-reacting with a suicide inhibitor covalently anchored on MNP [5]. In principle, this bimodular orthogonal bioreaction could present two important advantages when the homing ligand is a short peptide (i.e., 5-30 aa), which would drastically improve the targeting efficiency and selectivity: 1) the peptide is separated from direct contact with the nanoparticle surface by a protein spacer, which prevents undesired interactions thus optimizing the ligand availability for molecular recognition; 2) introducing globular proteins enhances the whole solubility of the nanoconstruct. We designed a bimodular genetic fusion (HALO-U11) comprising a small peptide of 11 amino acids (U11) with high affinity for urokinase plasminogen activator receptor (uPAR), found overexpressed in several metastasizing tumors, as a targeting module, and HALO, a Haloalkane Dehalogenase which can be exploited for the formation of an irreversible stable

bond with an alkyl conjugate, as a capture module. Therefore, we reasoned that a chloroalkane linker could be a good candidate to mediate the covalent, properly oriented immobilization on MNP of homing peptides genetically fused with HALO. We demonstrate the utility of this method for peptide nanoconjugation and cellular imaging by evaluating the capability of MNP covalently bound with HALO-U11 to specifically recognize uPAR-positive cancer cells.

[1] K. Park, et Al., Adv. Funct. mater. 2009, 19, 1553-1566
[2] D. Simberg, et Al., Proc. Natl. Acad. Sci. USA 2010, 104, 932-936.
[3] E. Occhipinti, et Al., Nanoscale 2011, 3, 387-390.
[4] S. Mazzucchelli, et Al., ACS Nano 2010, 10, 5693-5702; M. Colombo, et Al. Angew. Chem. Int. Ed. 2012, 51, 496-499.
[5] M. Colombo, et Al., Small 2012, 8, 1492-1497.

SOLVOPHOBIC FORCES DRIVEN AGGREGATION OF PEGYLATED LIPOSOMES

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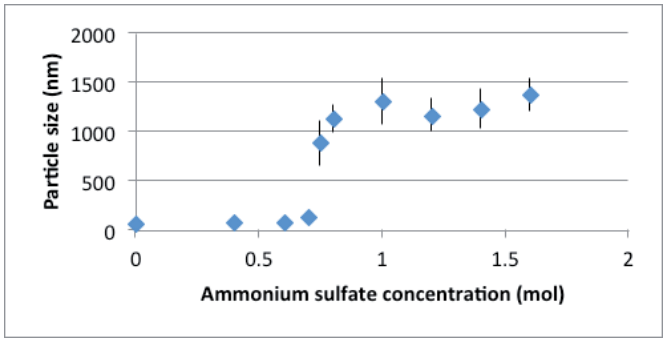
Aggregation in a drug product is a historical formulation and stability concern. The aggregation phenomena turned particularly important as the biopharmaceutical industry started to produce larger, more complex and more complicated drug products. Aggregation of proteins became the industry's dire concern, sometimes with life-threatening consequences. Lately, the aggregation of liposomes of medicinal interest came into the center of attention. There are evidences that unwanted side effects may be connected to the aggregation of the liposomal drug. Protein aggregates are well described as the formation of dimers or higher "mers" of an original molecule. In case of liposomes we use the word aggregates assuming a similar "polymerization" of the liposomes. It will take more studies to identify the nature of the formed particles created by solvophobic forces.
Traditional liposomes have charged surfaces and prior studies concluded that the aggregates were generated in the presence or absence of ionic compounds which could be also polymer ions, like heparin. The ionic type aggregation could be prevented by shielding the surface charges by media additives or by preparing liposomes with non-ionic and hydrophilic moieties like polyethylene glycols (PEG).
Here, we report a technique to produce aggregates in a reproducible and stable fashion from PEGylated liposomes. The aggregate formation was measured by DLS since the generated liposomes represented various particle sizes. The aggregates were formed by hydrophobic interactions of the solvophobic effect of the increasing salt concentration (see Figure).



The application of the technique and the exploration of parameters effecting the particle formation could provide an intimate insight into the aggregation mechanism of certain class of liposomes.

AGGREGATE FORMATION IN LIPOSOMES MEASURED BY FLOW CYTOMETRY: EFFECT OF PAYLOAD

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SUMMARY

Vesicle aggregation is one of the stability issues that liposomal drug developers need to consider. Taking liposomal doxorubicin (Doxil/Caelyx) as an example, we developed earlier a flow cytometric method to quantitate the amounts of aggregates in a liposomal batch. In the present study, using this method, we attempt to assess the effect of payload (doxorubicin) on aggregation of drug-free liposomes whose structure exactly corresponds to Doxil/Caelyx (called Doxebo). Incubation of Doxebo with free doxorubicin at a concentration 5-10 % of the total encapsulated drug led to a 2-3-fold increase of particles ≥ 500 nm compared to control (Doxebo without added doxorubicin), suggesting that the payload drug may have a significant influence on vesicle aggregation above a certain threshold concentration. These findings may help to understand the factors underlying aggregation, an omnipresent potential problem with nanomedicines and protein-based biologicals.

INTRODUCTION

Supravesicular structure (SVS) formation, such as aggregation or fusion, is a known property of liposomes (Bailey et al.; Ahl et al.). It has been proposed previously that such larger than normal particles in pegylated liposomal formulations could induce complement activation which leads to an allergic reaction (CARPA) after i.v. liposome administration (Szebeni et al.). In a previous study we proposed the use of flow cytometry for quantifying SVS (Milosevits et al.), and here we focus on those experiments which were carried out to unveil the possible contribution of free doxorubicin, leaked out from Doxil, to aggregate formation. According to product label, minor fraction (up to 10%) of free, unencapsulated drug is allowed in commercial Doxil/Caelyx, guiding us to test the effects of 5 and 10% free doxorubicin (relative to the total, 2 mg/mL). For these studies we used Doxebo, an empty liposome structurally equivalent to Doxil/Caelyx, without doxorubicin. We incubated Doxebo with free doxorubicin and measured supravesicual structure (SVS) formation by flow cytometry.

MATERIALS AND METHODS

Materials

1. Doxorubicin HCl
2. Doxebo
- hydrogenated soy lecithin (HSPC, 9.58 mg/mL)
- cholesterol (Chol, 3.19 mg/mL)
- N-Carbamyl-poly(ethylene glycol methyl ether)-1,2-distearoyl-sng-lycerol- 3-phospho-ethanol-amine triethyl ammonium salt with a polyethylene glycol (PEG) moiety of 2000 Da (2K-PEG-DSPE), 3.19 mg/mL
- ammonium sulfate, ≈0.2 g/mL
- histidine, 10 mM (pH 6.5);
- sucrose, 10%

3. Fluoresbrite® carboxylated polystyrene latex-containing size ranging kit

Methods

1. Preparation of doxorubicin-free Caelyx-equivalent liposomes (Doxebo)
The (phospho)lipids found in Doxil/Caelyx, in corresponding amounts and ratios, were dissolved in an equivolume mixture of isopropanol and 96% ethanol at 70 ◦C.
The lipid solution was then admixed to pre-warmed Salsol A and the preparation was extruded 4 times via two superposed 80 nm polycarbonate filters, using a 10-mL extruder barrel from Northern Lipids (Vancouver, British Columbia, Canada).

The extruded liposomes were finally dialyzed against Salsol-A.
2. FACS measurements
FACS measurements were done using a Becton & Dickinson FACScan and were started by running of backgrounds (3 mL filtered PBS), followed by the measurement of liposomal solution with given free, unencapsulated drug content.
Calibration was done with Fluoresbrite® kit, containing fluorescence-labeled latex beads of known sizes (100, 200, 500, 750 and 1019 nm).
Particle detection continued for 1 min, or until a set number of events (e.g., 20,000) were reached. The counts and forward (FSC) vs. side scatter (SSC) plots (on log scales) were registered at room temperature.

RESULTS

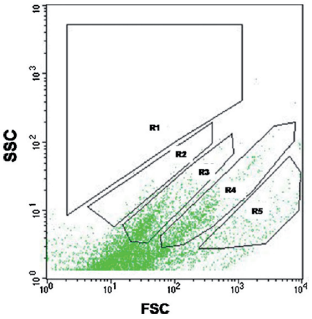

Figure 1. *Gating of the SSC vs FSC scatterplot in SVS measurements conducted by flow cytometry analysis.*

Free doxorubicin (mg/mL)	GATES				
	R1	R2	R3	R4	R5
0	0.9	27,8	**0.8**	**4.8**	0.1
0.1	0.9	24,7	**2.2**	**8.8**	0.2
0.2	0.9	24.1	**1.8**	**7.1**	0.2
0.4	1	25.9	**1.1**	**5.8**	0.1

Table 1. *Effect of free doxorubicin on aggregate formation in doxorubicin-free liposomes.*

Entries are % of events (dots) detected in the gated areas (R1–5) shown in Fig. 1.
Doxebo was incubated with doxorubicin for 30 min either at room temperature. Free doxorubicin values are final concentrations.
Light scattering patterns of particles in given solutions were analyzed by flow cytometry. According to size calibration conducted with latex beads, the size detection limit of particles registered as events in scattergams, such as shown in Figure 1., is 500nm, which implies that every event that was registered during the experiments signals structures larger than 500nm. Considering that Doxil/Caelyx contains 2 mg/mL doxorubicin and the fraction of free doxorubicin in it is within 10%, we incubated free doxorubicin with Doxil/Caelyx-mimicking, doxorubicin-free liposomes (Doxebo) in concentration ratios corresponding to that in Doxil/Caelyx, i.e., 0.1–0.4 mg/mL doxorubicin and 13 mM phospholipid. Incubations were carried out at 4 ◦C and at room temperature and the gating windows, upon FACS analysis, were the same as shown in Fig. 1. The data (Table 1) indicated 2–3 fold increases of events in the R3 and R4 regions following incubation with >0.1 mg/mL doxorubicin at room temperature, while the number of dots were not changed in the other regions, which are most easily rationalized by doxorubicin-induced aggregation of liposomes showing in R3 and R4.

CONCLUSION

These observations imply that free doxorubicin may have a significant impact on liposome structure, and, hence, in vitro stability of Doxil. The described FACS analysis can be utilized for qualitative and quantitative characterization of liposomes for the presence of aggregates,homogenicity and stability.

REFERENCES

• Ahl PL, Bhatia Sk, Meers P, Roberts P, Stevens R, Dausen R, Perkins WR, Janoff AS. 1997. Enhancement of the in vivo circulation lifetime of l-α-distearoylphosphatidylcholine liposomes: importance of liposomal aggregation versus complement opsonization, Biochim. Biophys. Acta 1997 ; 1329(2):370-82
• Bailey, A.L., Cullis, P.R., 1997. Liposome fusion. Curr. Top. Membr. 44, 359–373.
• Doxil, 2010. Package Insert., http://www.Doxil.com.
• Durand, R.E. Calibration of flow cytometry detector systems. Cytometry 2, 192–193.
J • akubowski, H.M., Penas, M., Saunders, K., 1994. The study of lipid aggregates in aqueous solution: formation and properties of liposomes with an encapsulated metallochromic dye. J. Chem. Educ. 71, 347.
• Milosevits G. et al. 2012. Flow cytometric analysis of supravesicular structures in doxorubicin-containing pegylated liposomes. Chemistry and Physics of Lipids 165 (2012) 482– 487
• Robert Brasseur, R., de Kruijff, B., Ruysschaert, J.-M., 1984. Mode of organization of lipid aggregates: a conformational analysis. Biosci. Rep. 4, 259–267.
• Szebeni, J., Barenholz, Y., 2012. Complement activation, immunogenicity, and immune suppression as potential side effects of liposomes. In: Peer, D. (Ed.), Handbook of Harnessing Biomaterials in Nanomedicine: Preparation, Toxicity, and Applications. Pan Stanford Publishing Pte. Ltd., pp. 311–335.
• Szebeni, J., Bedőcs, P., Rozsnyay, Z., Weiszhár, Z., Rosivall, L., Cohen, R., Garbuzenko, O., Báthori, G., Tóth, M., Bünger, R., Barenholz, Y., 2012. Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and Ambisome. Nanomed. Nanotechnol. Biol. Med. 8, 176–184.
• Szebeni, J., Muggia, F., Gabizon, G., Barenholz, Y., 2011. Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: prediction and prevention. Adv. Drug Deliv. Rev. 63, 1020–1030.
• Vemuri, S., Rhodes, C.T., 1995. Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm. Acta Helv. 70, 95–111.

SUPERPARAMAGNETIC NANOPARTICLES AND ANTICANCER AGENTS FOR LOCAL THERMO-CHEMOTHERAPY TREATMENT OF BONE TUMORS

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INTRODUCTION

Bone metastases might be efficiently treated using intraosseous implants[1]. In this view, we propose novel formulations that, once injected intratumorally, form a solid implant. Poly(methylmethacrylate) cements are relevant formulations already used in vertebroplasty. They can be loaded with both an anticancer agent and superparamagnetic beads (SSB) for combining chemotherapy and hyperthermia, the latter being an effective adjuvant in cancer therapy[2].

EXPERIMENTAL METHODS

Cements were prepared through an exothermic polymerization reaction. SSB at 24% or 30% (w/w) and doxorubicin (DOX) at 2.5% (w/w) were loaded within the cement. Heating capacity was assessed by exposing cements to an external alternating magnetic field (3 or 6 mT and 140 kHz). In vitro DOX release was carried out in a saline media at 37°C and the DOX was analyzed by spectrophotometry at 479 nm. In vitro bioactivity of the eluted DOX on PC3 cells was tested using XTT proliferation assay. Young modulus was determined by compression of Ø6x7 mm cylinders. Finally, the

cements were injected within human vertebra and the radiopacity was measured using a micro CT-scan.

RESULTS AND DISCUSSION

PMMA cements were able to generate heat in the hyperthermic range (Fig.1) and displayed sustained release of DOX over at least 10 days (Fig. 2).

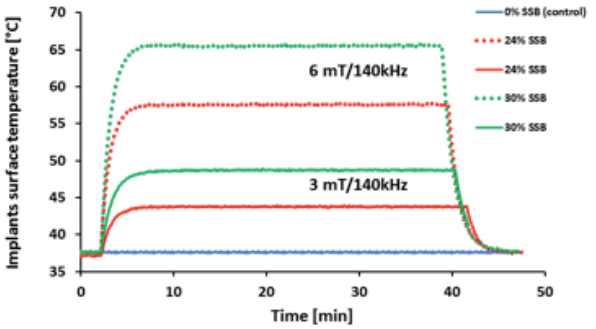

Figure 1 *Heat generation of SSB-loaded cements under two magnetic field amplitudes 3 and 6 mT (n=3)*

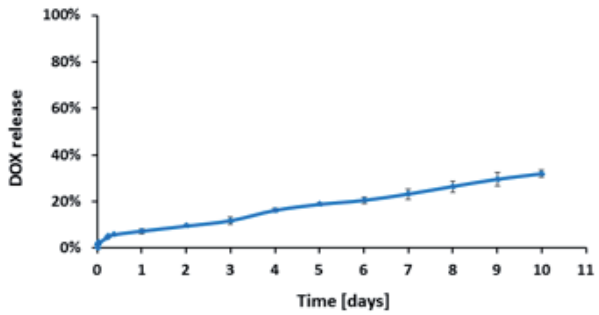

Figure 2 *DOX release profile of PMMA cement loaded with 24% SSB (n=3).*

Addition of SSB kept working time within clinically acceptable values and ensured safe non-necrotic polymerization. The heating power of the implants, called specific power loss (SPL), indicates the potential for hyperthermia-induced antitumoral effect (Table 1). Cements might provide some mechanical support to the weakened bone as the Young compression moduli are in the range of cancellous bone. In comparison to cancellous bone elasticity (100 to 400 MPa), the results for our formulations show that cements are suitable for intraosseous injection as they provide mechanical support to bone (Fig. 3)

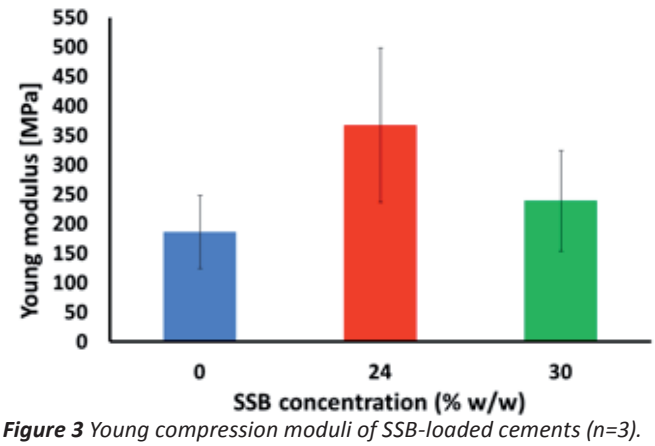

Figure 3 *Young compression moduli of SSB-loaded cements (n=3).*

In vitro bioactivity of eluted DOX shows preserved drug cytotoxicity compared to fresh DOX solution (98.9±1.8%).

Table I *Characteristics of the cements*

	SPL* [W/g of implant]	Y [MPa]
SSB 24%	0.33	368±130
SSB 30%	0.45	239±85

*measured at 6mT/140kHz

Finally, the ex vivo injection showed that the SSB acts as radiopacifier for the cements.

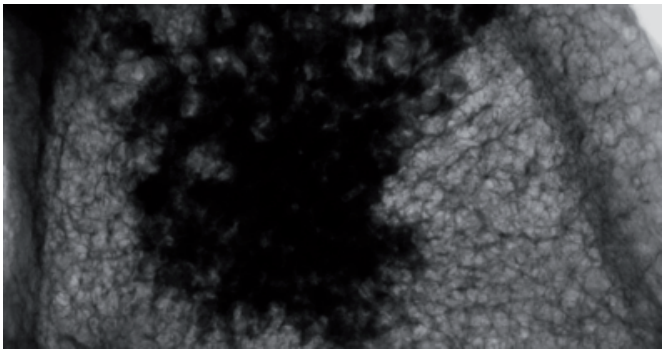

Figure 4 *Micro CT-scan of a human vertebra injected with cement*

CONCLUSION

Acrylic cement was successfully loaded with doxorubicin and superparamagnetic beads, providing a sustained anticancer agent delivery and potential cytotoxic temperature. These data show within clinically acceptable parameters the feasibility of combining locally hyperthermia with anticancer agent release.

REFERENCES

1. Mohamed M. et al., JDDST (2012),22:393-408
2. Le Renard P.E. et al., JH (2009),25:229-239

ACKNOWLEDGMENTS

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CYTOTOXICITY OF FUNCTIONALIZED CARBON NANOTUBES IN J774A MACROPHAGES

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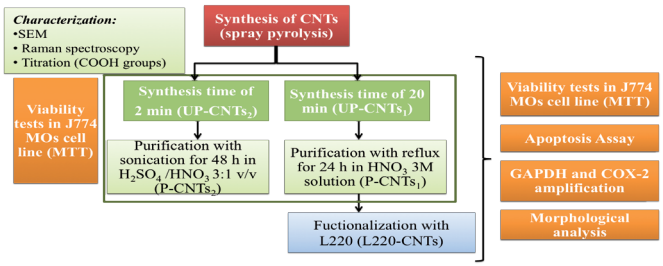
A potential use for f-CNTs is nanovaccine fabrication; however, information about their potential cytotoxicity and the immunological response that these particles induce is contradictory. The goal of this research is to formulate a nanovaccine against Entamoeba histolytica, a parasite that is responsible for intestinal amebiasis. In this work we evaluated the cytotoxic effect of unpurified (UP-CNTs), purified (P-CNTs) obtained by different methods: 1) reflux with 3M HNO3 (P-CNTs1), and 2) sonication in H2SO4/HNO3 (P-CNTs2), and Entamoeba histolytica 220-kDa lectin–functionalized CNTs (L220-CNTs) in J774A macrophage (MOs) cell line. Viability test was performed with MTT assays at 24 h of exposure with all nanoparticles. Aditionally apoptosis, morphological test and COX-2 amplification were performed for P-CNTs1 and L220-CNTs. Characterization and COOH content of CNTs was performed using scanning electron microscopy, raman spectroscopy and titration with NaHCO3. P-CNTs1 had lengths >100 µm and 2.76% COOH content, while P-CNTs2 had lenghts < 1µm and 7% COOH content. This particle showed a lower toxic effect. MOs interacted with L220-CNTs showed an increase in cell viability without signs of apoptosis. These results suggest that the length, COOH content and functionalization decreases toxicity, and that L220-CNTs may be an excellent candidate for the production of a nanovaccine against amebiasis.

INTRODUCTION

Carbon nanotubes (CNTs) are cylindrical nanoparticles, which can be funcionalized with substances and may be used as carriers in biological system. A particular use for CNTs is for nanovaccine pro-

duction, nevertheless, information about their potential cytotoxicity and the immunological response that these particles induce is contradictory. Some authors attribute the toxic effects to the hydrophobic nature of the CNTs, fabrication residues and high surface area or size. According to this last point, many works converge with the notion of the length and purity of CNTs are an additional factors for cellular toxicity. Moreover, studies carried out in cell cultures showed that functionalized CNTs (f-CNTs) do not exhibit cytotoxicity. In particular, it would be of interest to formulate a nanovaccine against Entamoeba histolytica, a parasite that is responsible for intestinal amebiasis. Among the virulence factors already reported for this parasite are 220 KDa lectin (L220). L220 is a highly immunogenic protein which has been shown to have peptides which have the ability to develop a TH1 response. The objective of this work is evaluate the cytotoxic effect of unpurified (UP-CNTs), purified (P-CNTs) obtained by different methods: 1) reflux with 3M HNO3 (P-CNTs1), and 2) sonication in H2SO4/HNO3 (P-CNTs2), and Entamoeba histolytica 220-kDa lectin–functionalized CNTs (L220-CNTs) in J774A macrophage (MOs) cell line.

METHODOLOGY



RESULTS

The UP-CNTs1 had 20 to 100 nm in diameter and 120 µm in length (Figure 1A and 1C). The P-CNTs1 obtained by reflux with HNO3 3M had similar length than for UP-CNTs1 (Figure 1E). UP-CNTs2 showed 20–40 nm in diameter and 30 µm in length (Figure 1B and 1D). P-CNTs2 obtained by sonication with H2SO4/HNO3 3:1 v/v showed a considerable decrease of their length from 30 µm to <1µm (Figure 1F). The analysis of elemental composition showed a decrease in Fe content in both, unpurified and purified samples. The P-CNTs2 had an increase of five fold of O content and a higher percentage of COOH groups (7%) than in P-CNTs1 (2.76%). In the raman spectra was observed that the structure quality was in the following order UP-CNTs2> P-CNTs1> UP-CNts1> P-CNTs2 (Data no shown). Viability results of MOs interacted with CNTs showed that UP-CNTs1 had a significant decrease of cell viability independent of the dose tested (Figure 2). The MOs interacted with UP-CNTs2, P-CNTs1 and P-CNTs2 had a dose-dependt toxic effect. In contrary the MOS interacted with L220-CNTs had a cell proliferation compared to control (Figure 2). These results indicate that length, quality and fuctionalization are important factors for toxicity of CNTs. The percentages of apoptosis showed at 48 h an increase in apoptotic cell number, and this was observed in almost all interaction conditions in MOs interacted with UP-CNTs1 and P-CNTs1. MOs that interacted with L220-CNTs had a significantly lower apoptosis. Amplification of the GAPDH mRNA was detected in all interaction conditions. The expressión of COX-2 mRNA was detected in MOs that interacted with L220-CNTs as well as in P-CNTs2 in all analyzed concentrations. Finally cell morphology was analyzed at 24 h of interaction. MOs that interacted with UP-CNTs1 and P-CNTs1 presented alterations characteristic of cell death. MOs that interacted with L220-CNTs exhibited normal morphology and formation of cellular aggregates, indicating a protein recognition by MOs.

CONCLUSION

Purification and functionalization decreases toxicity, and L220-CNTs may be an excellent candidate for the production of a nanovaccine against amebiasis.

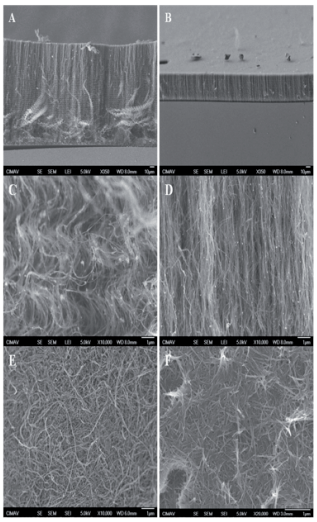


Figure 1. *Photomicrographs of CNTs obtained by SEM. (A) UP-CNTs1 length; (B) UP-CNTs2 length; (C) UP-CNTs1 diameter; (D) UP-CNTs2 diameter; (E) P-CNTs1; (F) P-CNTs2.*

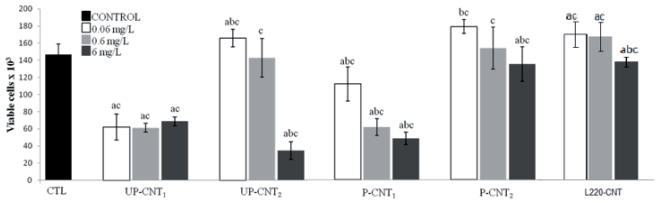


Figure 2. *Effect of interaction with different CNTs concentrations on MO viability in 24 h cultures. Each bar shows the median of 2 experiments triplicated (n=6). a) P< 0.01 difference in respect to control (SE). b) P<0.01 difference between CNTs concentration in same treatment. c) p<0.01 difference between different CNTs types.*

ULTRASOUND-MEDIATED DELIVERY OF A NOVEL NANOPARTICLE-MICROBUBBLE PLATFORM

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INTRODUCTION

The ability to create nanoparticles (NPs) incorporating multiple functionalities, such as imaging and therapy as well as cell targeting, opens new possibilities in the combination of diagnosis and therapy into so called theranostic NPs. However, using drug-carrying NPs in cancer treatment has been a challenge due to the low uptake and heterogeneous distribution of NPs in tumor tissue.

Combining multifunctional NPs with focused ultrasound (US) exposure may enable therapeutic molecules to reach their targets while limiting the exposure to normal tissue. US can enhance the delivery of encapsulated drugs in various ways depending on the frequency and intensity applied by inducing heating, radiation force or cavitation [1]. We have developed a novel multimodal, multifunctional drug delivery system consisting of microbubbles (MBs) stabilized by polymeric NPs to be used in US-mediated delivery of NPs. Miniemulsion polymerization was used to prepare PEGylated polymeric NPs in a single step. The NPs are composed of the widely used biocompatible and biodegradable poly(alkyl cyanoacrylate) (PACA) polymers [2]. MBs stabilized by PACA NPs were subsequently prepared in a second step. The aim of the present work was to study cellular uptake of these NPs and a hydrophobic model drug in vitro and the microdistribution of the NPs/model drug in tumor tissue in vivo.

EXPERIMENTAL METHODS

Nanoparticle synthesis and characterization: Oil-in-water emulsions were prepared by emulsifying a monomer phase, consisting of butyl- or octyl cyanoacrylate, co-stabilizer and hydrophobic model drugs (fluorescent dye nile red or miglyol), in an acidic aque-

ous medium containing surfactant. The initiation of the anionic polymerization was carried out by adding a polyetheramine (PPO/PEO copolymer) to the emulsion, resulting in PEGylated NPs. Particles were also fluorescently labeled by covalent binding of rhodamine 6G on the particle surface. Particle size, concentration and ζ-potential were determined using Zetasizer and Nanosight, whereas scanning electron microscopy was used to study NP morphology.

Microbubble preparation and characterization: Air-filled MBs stabilized by PACA NPs were prepared by mechanical stirring (ultra-turrax) of NP dispersions added with various proteins. The size and number of MBs was determined using Coulter Counter and their morphology was analyzed by light microscopy, confocal laser scanning microscopy (CLSM) and structured illumination microscopy (SIM).

In vitro cell uptake studies: Human prostate cancer cells (PC3) were incubated with fluorescently labeled (nile red or rhodamine 6G) NPs and the cellular uptake measured by flow cytometry CLSM.

Distribution of NPs and model drug in tumor: Balb/c nude mice bearing a subcutaneous tumor (xenograft of human PC3 prostate adenocarcinoma) in the leg were injected intravenously with NPs alone or NP-stabilized MBs. The particles circulated for 5 min or 24 h, before the tumors were exposed to US, thus the effect of US both on extravasation and penetration through the extracellular matrix could be studied. The tumors were exposed to a focused US beam at low (300 kHz or 1 MHz) or high (5 MHz) frequency, applying various intensities. The blood vessels were visualized by injection of FITC-tomato lectin 5 min before euthanizing the mice. The distribution of NPs was studied by CLSM, imaging frozen tumor sections along a radial track from the periphery of the tumor sections.

In vivo visualization of MBs: Rabbits were injected with PBCA NP-stabilized MBs while image acquisition using the GE Vivid E9 ultrasound system.

RESULTS AND DISCUSSION

Multifunctional nanoparticles

Stable PEGylated PACA submicron particles encapsulating hydrophobic model drugs were produced in one step. The particle diameter could be varied from 100 to 250 nm (PDI < 0.2). The particles were successfully labeled with different fluorescent probes either by encapsulation or by covalent binding to the particle surface.

Nile red, a hydrophobic fluorescent model drug, was encapsulated to study cellular uptake of a hydrophobic molecule in vitro. Nile red exhibits a blue shift of the fluorescent emission maxima which is proportional to the hydrophobicity of the environment [3]. Hence, nile red associated with more hydrophobic compounds (such as PACA NPs, intracellular lipid-droplets and vesicle membranes can be distinguished from nile red associated with various cytosolic proteins, the latter giving a diffuse general staining of the cytoplasm (Fig. 1). Our studies indicated that free nile red was internalized in cells by diffusion and that direct cell-NP contact was important for delivery of the model drug to cells.

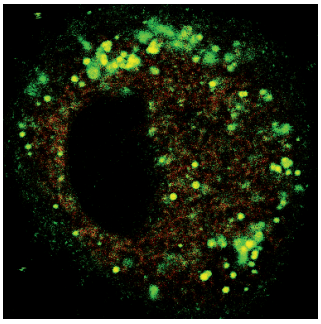
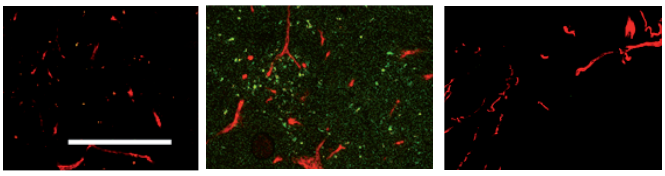


***Figure 1**: CLSM image of a human prostate cancer cell 3 h after PACA NP exposure showing nile red bound to lipids/hydrophobic molecules (green) and nile red bound to less hydrophobic molecules (red).*

In vivo, US enhanced the uptake and improved the distribution of PACA NPs in the extracellular matrix. In untreated tumors only small amounts of NPs were observed and they were located close to the blood vessels. In the US-exposed tumors, the uptake was enhanced and the NPs had penetrated further away from the blood vessels compared with unexposed tumors. US administered 5 min after NP-injection was more efficient than US given after 24 h (Fig. 2). This indicates that the effect of using US is more pronounced for NP extravasation from blood vessels compared to the effect on NP penetration/drug diffusion through the extracellular matrix.



No ultrasound | 5 MHz 5 min post NP injection | 5 MHz 24 h post NP injection

***Figure 2**: CLSM image through an equatorial section of a mouse tumor. Blood vessels are seen in red and PACA particles in green. Scale bar = 50 µm*

Nanoparticle-stabilized microbubbles

Microbubbles injected into the blood stream can be used both for US imaging of the tumor vasculature, and to facilitate sonoporation thus further improving tumor uptake of NPs and drugs. A method for producing air-filled MBs (diameter of 1-6 µm) stabilized by a shell of 1-2 PACA NP layers (Fig. 3) was developed. The bubbles were stable for a long period of time (> 8 months) in water.

***Figure 3**: Structured illumination microscopy showing 1-2 layers of nanoparticles surrounding the microbubbles (diameter approx. 2 µm).*

Nanoparticle-stabilized MBs were intravenously injected into rabbits and visualized using an US imaging system. The MBs were found to provide good contrast in small blood vessels, and could easily be destroyed by increasing the US power output. Preliminary in vivo studies of distribution of NPs and model drugs in tumors indicate that nanoparticle-stabilized MBs in combination with US treatment enhance NP/drug uptake and distribution.

CONCLUSION

A prerequisite for successful cancer therapy is that the cytotoxic drugs reach all the cancer cells. We have shown that stable, PEGylated biodegradable polymeric NPs can be formed in a single step. Further, NP-cell interaction is needed for efficient uptake of hydrophobic model drugs into cells. The NPs developed can further be successfully utilized for stabilization of microbubbles with ideal acoustic properties for both imaging and sonoporation. Hence, improved diagnosis can be combined with therapy by incorporating NPs containing the appropriate drugs into the microbubble surface.

REFERENCES

1. Frenkel, V. Adv. Drug Deliv. Rev. 60, 1193-1208, 2008
2. Nicolas, J. & Couvreur, P. Wiley Interdiscipl. Rev. Nanomed. Nanobiotechnol. 1, 111-127, 2009
3. Greenspan, P. & Fowler, S.D. J Lipid Res. 26 (7), 781-9, 1985

IG-004 – A NON-BIOLOGIC NANOEMULSION PACLITAXEL FOR THE TREATMENT OF SOLID TUMORS-COMPARISON TO ABRAXANE

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INTRODUCTION

nab-paclitaxel (Abraxane®)—a Cremophor-free, albumin-bound, nanoparticle form of paclitaxel—though a breakthrough in paclitaxel formulation, has inherent problems associated with any biologics. IG-004 is a cremophor-free, vitamin E-based paclitaxel emulsion incorporating a P-glycoprotein (Pgp) inhibitor and particle size-based tumor targeting. IG-004 has been developed to overcome a number of the limitations of the commercially available formulation of paclitaxel. The particle contains three components: The inner core consists of lipophilic material; d, l-alpha tocopherol. At the interface between the lipophilic emulsion particle and its aqueous environment, a number of surfactants are employed, including the p-glycoprotein (pgp) inhibitor alphatocopherol polyethylene glycol succinate (TPGS). The surfactants, along with the manufacturing conditions, define and stabilize the emulsion particle size.

Despite its proper construction, IG-004 was not superior to Taxol in a phase III head to head comparison; in contrast, Abraxane was. In this report- we examined the differences between Abraxane and IG-004 and define parameters for making IG-004 successful in the clinic.

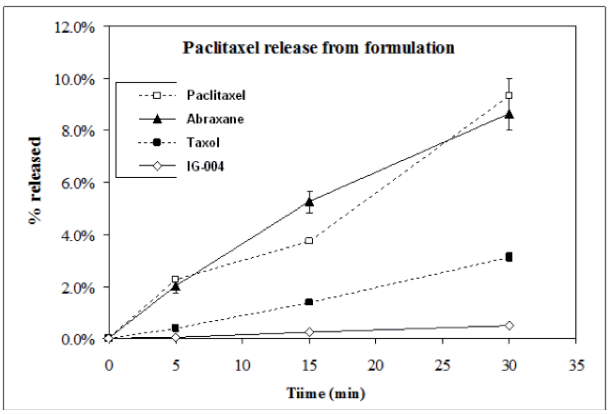
METHODS

The pharmacokinetic parameters were estimated by using the non-compartmental open model and the WinNonlin program (Pharsight, CA). Descriptive statistics were computed for PK assessment. Regression analysis of AUCinf vs. dose was performed to gain an appreciation of dose proportionality. Paclitaxel release from formulation was tested using equilibrium dialysis. Briefly, paclitaxel, IG-004, Taxol or reconstituted Abraxane was added to one side of the well and blank buffer to the other side. Sample was taken from the buffer side for the analysis of the appearance of free paclitaxel. Tumor distribution for IG-004, Taxol and Abraxane were compared in MDA-MB-435 xenograft model.

RESULTS

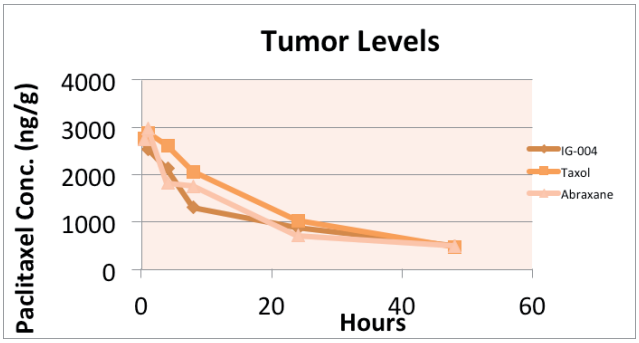
The release of paclitaxel from Taxol, Abraxane, and IG-004 was investigated by measuring the appearance of free paclitaxel in the receiver side of equilibrium dialysis unit over 30 minutes. The appearance of free drug reflects a combination of drug release/dissociation from the formulation and partitioning across the dialysis membrane. Figure 1shows the free drug concentration in the receiver side 30 minutes after paclitaxel, Taxol, Abraxane, or IG-004 was spiked in the donor side at nominal concentration of 10 ug/mL paclitaxel. The drug release profile from Abraxane appears similar to neat paclitaxel. Drug release is slowest for IG-004 (0.5% at 30 minutes, statistically significant versus the other three groups). This is showing that IG-004 was constructed correctly – to be a stable nanoparticle even down to 10 ug/mL; whereas, Abraxane was not. Stable nanoparticle is associated with slow release of paclitaxel. The rapid release of paclitaxel from an unstable nanoparticle such as Abraxane is probably responsible for its effective use of albumin-mediated transport.

Figure 1. *Paclitaxel release assay*



Tumor accumulation of paclitaxel was similar for all three formulations at dose level of 10mg/kg. This despite the lower AUC for both Abraxane and IG-004 in mice- therefore, tumor penetration was equally effective: Abraxane- probably via albumin mediated transport due to its rapid dissociation in vivo and IG-004 due to EPR effect.

Figure 2. *Tumor accumulation assay.*



We examined the PKs of Abraxane, IG-001- a polymeric micelles paclitaxel nanoparticle formulation with similar characteristics as Abraxane,Taxol and IG-004. IG-004 has a higher plasma AUC than Taxol; whereas IG-001 and Abraxane have lower plasma AUC than Taxol. IG-001 range for PK dose-proportionality is the most expanded of the four paclitaxel formulations examined (Taxol, Abraxane, IG-001, IG-004). The dose-proportionality of Abraxane and IG-001 clearly separated these formulations from Taxol and IG-004. The high level of paclitaxel accumulation in plasma for IG-004 is suggesting that it is not be transported out of the blood compartment and is opposite of the preclinical PK data in mice.

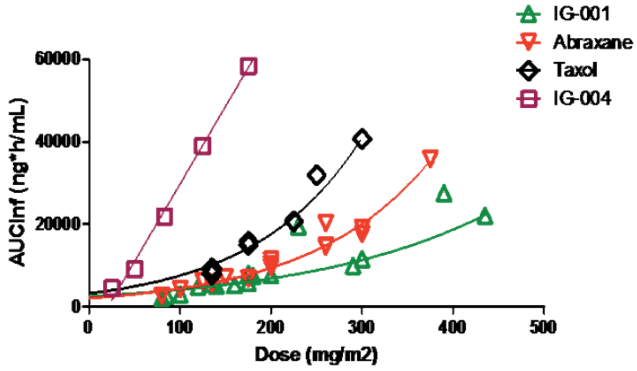


Figure 3. *Clinical PK dose-proportionality assay.*

CONCLUSIONS

A non-biologic nanoparticle formulation (IG-004) with stable nanoparticle utilizing EPR effect for tumor penetration was compared to Abraxane- an unstable nanoparticle formulation utilizing albumin mediated transport. In contrast to preclinical data, IG-004 did not perform as expected during phase III clinical trial whereas Abraxane did. This is suggesting that the EPR effect observed in mice was not a dominant factor in human. This information has allowed us to formulate parameters necessary for successful stable nanoparticle formulation in the clinic.

CYNVILOQTM/IG-001 – A NON-BIOLOGIC NANOPARTICLE PACLITAXEL FOR THE TREATMENT OF SOLID TUMORS

KOUROS MOTAMED, Catherine Cheng, Larn Hwang, Chao Hsiao, Vuong Trieu
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INTRODUCTION

nab-paclitaxel (Abraxane®)—a Cremophor-free, albumin-bound, nanoparticle form of paclitaxel—though a breakthrough in paclitaxel formulation, has inherent problems associated with any biologics. CynviloqTM/IG-001, utilizes biodegradable di-block copolymers composed of methoxy poly (ethylene glycol)-poly (lactide) to form nanoparticles with paclitaxel containing a hydrophobic core and a hydrophilic shell and is being developed as the next generation nanoparticle paclitaxel (Fig. 1). It is free of Cremophor-induced toxicities such as hypersensitivity reactions, prolonged or irreversible peripheral neuropathy, as well as altered lipoprotein patterns (refs. 1,2). Its target indications are difficult to perfuse hypoxic tumors such as pancreatic cancer, taking advantage of its ability to rapidly deliver paclitaxel to the targeted tissue via albumin-mediated transport, as previously described for nab-paclitaxel.

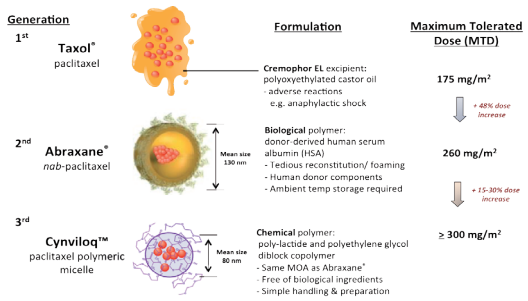

Fig. 1. *Evolution of Paclitaxel Therapy*

METHODS

Stability of CynviloqTM/IG-001 nanoparticles was evaluated in PBS and serum. Tissue pharmacokinetics (PK) and distribution in rodents were also evaluated. Clinical PK of the various formulations were also compared. The pharmacokinetic parameters were estimated by using the noncompartmental open model and the WinNonlin program (Pharsight, CA). Descriptive statistics were computed for PK assessment. Regression analysis of AUCinf vs. dose was performed to gain an appreciation of dose proportionality.
Results: The dissolution profile of CynviloqTM/IG-001was similar to that of nab-paclitaxel being controlled primarily by the solubility limit of paclitaxel. The dissolution profile confirmed that once injected into the circulation, both formulations quickly dissolved into paclitaxel complexed with endogenous circulating albumin (Fig. 2). Hence, both formulations appear to be equally capable of utilizing the natural albumin transport system.

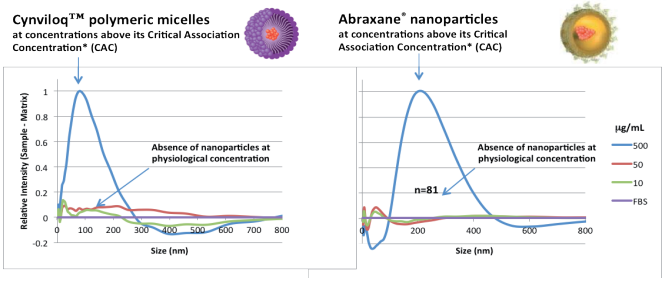

Fig. 2. CynviloqTM/IG-001 and Abraxane exhibit similar nanoparticle disintegration in serum

The PK profiles in human and rodents were similar between the two formulations with large Vss and low AUC/dose relative to the Cremophor-based formulation Taxol. Nab-paclitaxel and CynviloqTM/IG-001 exhibit comparable dose-proportionality demonstrating the ability to utilize albumin pathway to deliver paclitaxel to tissues unhindered by Cremophor micelles of Taxol. In human (Fig. 3), CynviloqTM/IG-001 exhibited the AUC/dose value of 0.06 uM*h/mg/m2 across the dose levels of 135-390 mg/m2; a dose-proportional characteristic of nab-paclitaxel. In tumor bearing mice, CynviloqTM/IG-001 showed AUC of 1.54 ug*hr/ml/mg/kg versus Taxol of 4.25 ug*h/ml/mg/kg. Yet tumor AUC of 74.28 ug*h/ml/mg/kg versus 107.00 ug*hr/ml/mg/kg. The ratio of tumor AUC/plasma AUC was 48.2 and 25.2 for CynviloqTM/IG-001and Taxol, respectively, with IG-001 having a 1.9-fold higher tumor penetration than Taxol. In comparison, the ratio of tumor AUC/plasma AUC was 5.1 and 2.6 for nab-paclitaxel and Taxol, respectively, with nab-paclitaxel having a 2-fold higher tumor penetration than Taxol (Fig. 4).

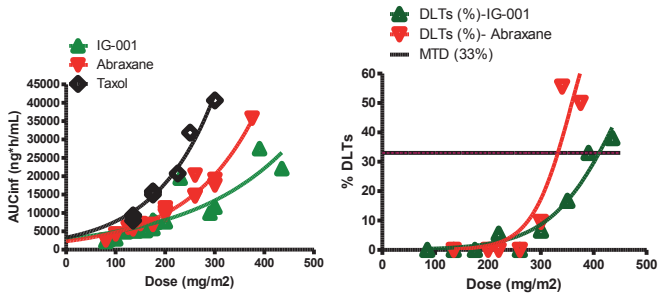

Fig. 3. Expanded dose-proportionality of Cynviloq™ vs. Abraxane® (top left). Gradual dose-limiting toxicity of Cynviloq™ vs. Abraxane® (top right).

Tumor	Agent	Dose (mg/kg)	AUC Tumor/ ug/ml/hr	AUC plasma/ ug/ml/hr	Tumor AUC/D	Plasma AUC/D	Tumor/ Plasma	Test/ Taxol
B16	Genexol	50.0	3714.28	77.0	74.3	1.5	48.3	1.9
	Genexol	20.0	1290.8	16.3	64.5	0.8	79.4	3.2
	Taxol	20.0	2139.41	85.0	107.0	4.3	25.2	
H460	Genexol	50.0	273.07	61.3	5.5	1.2	4.5	2.3
	Genexol	20.0	105.58	15.1	5.3	0.8	7.0	3.6
	Taxol	20.0	131.63	67.6	6.6	3.4	1.9	
MX-1	Abraxane	21.7	5869.0	1161.0	270.5	53.5	5.1	2.0
	Taxol	19.5	3716.0	1438.0	190.6	73.7	2.6	

Fig. 4. Consolidated Tumor Xenograft Distribution Data for 3 paclitaxel formulations

CONCLUSIONS

A non-albumin-based paclitaxel formulation (CynviloqTM/IG-001) was shown here to have all the properties of the albumin-based paclitaxel formulation (nab-paclitaxel), including dissolution in plasma, high tumor plasma ratio, and dose-proportional PK. These are consistent with CynviloqTM/IG-001 capable of utilizing the albumin-mediated transport described for nab-paclitaxel (Abraxane®). Moreover, CynviloqTM/IG-001 is ideal for personalized paclitaxel therapy due to its expanded dose-proportionality and more gradual toxicity profile versus the currently available nanoparticle formulation of paclitaxel—namely Abraxane. Personalized paclitaxel therapy should allow for increased dosing without undue toxicity and therefore enhanced clinical efficacy. Clinical development of CynviloqTM/IG-001 could give us the next generation paclitaxel nanoparticle formulation that can be more readily modified than nab-paclitaxel (Abraxane®).

REFERENCES

1) Nuhad K. Ibrahim, J Clin Oncol 2005;23:6019-6026.
2) William J. Gradishar, J Clin Oncol 2005;23:7794-7803.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL DRUG TARGETS FOR PERSONALIZED BREAST CANCER NANOMEDICINE

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Personalized cancer nanomedicine aims at the recovery of new nanodrugs that would match the molecular fingerprint of an individual patient`s tumor. Expression profiling and next generation-sequencing data represent rich resources for discovering new starting points for such approaches. Here, we selected a set of 140 genes, which have been proposed to show potentially relevant alterations in breast cancer by genome-wide next generation-sequencing. We constructed a panel of isogenic breast cancer cells lines and serially analyze the normal and the aberrant gene variants for their effects on breast cancer cells.
To this end, we have analyzed a total of 117 out of the 280 variants in an initial growth assay. This resulted in the identification of 12 new breast cancer growth modulators, of which we selected 2 promising candidates for further validation and functional analysis. We anticipate that our strategy will lead to the identification of novel drug targets for personalized cancer nanomedicine.

THE NANOSTRUCTURE OF THE ERYTHROCYTE MEMBRANE IN PATIENT WITH SEVERE TRAUMA

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INTRODUCTION

The red blood cells are nuclear-free disc-shaped cells. A special form of red blood cells contributes to the performance of his main function - the transfer of respiratory gases. Reduction of red blood cell caused by massive blood loss and hypoxia may lead to change in their shape and surface. This may be one of the causes of the critical conditions due to trauma. A perspective method for research of nano-surface cells membranes is atomic force microscopy (AFM). Goal: To study the changes of the nanostructure

erythrocyte membranes in patients with severe trauma (ST) with different volumes of blood loss. Materials and methods. A total of 17 patients (9 men, 8 women) aged 48±19 (27-79) years with ST and different volumes of blood loss 23,5 ± 15,9 ml/kg (3.3 to 46.1 ml / kg), and hemodynamic instability. All the patients were divided into three groups in depending on the amount of blood loss: 1st - blood loss <750 ml (<10.7 ml/kg - I degree of blood loss (dbl)) - 6 patients, 2nd - 750-1500 ml (10,7-21,4 ml / kg – II dbl) - 2 injured, 3rd -> 2000 mL (> 28.6 mL/kg – IV dbl) - 9 patients. Dynamics of clinical and laboratory parameters was performed at admission to the intensive care unit, at 3, 5, 7, and 15 day. The control group included five healthy volunteers, age was 26,4±2,7 (from 23 to 30) years. Red cells monolayers were prepared with a device «V-sampler» (Vision, Austria). Images of the nanostructure of erythrocyte membranes were obtained using by atomic force microscope («NTEGRAprima», NT-MDT, Russia) in the semi contact mode. Quantitative evaluations of changes nanostructure membranes were prepared with a spatial Fourier transformation. The resulted images of the surface of erythrocyte membranes was decomposed to three orders with spectral windows: the first order - the spatial period of L1 in the range of 600-1000 nm, the second order L2 - 80-210 nm, the third order of L3 - 30-60 nm. The heights (h) to each cell of the order of the selected fragment were measured. The parameters are chosen from the structural features of the erythrocyte membrane. Changes of h1 reflect the state and structural changes of the phospholipid bilayer and are a single-step of flickering, h2 - state and structural changes of the spectrin matrix, h3 - state and structural changes of protein clusters of cytoplasmic of the erythrocyte membrane. Statistical analyzes were conducted with software «Origin» (OriginLab Corporation, USA).

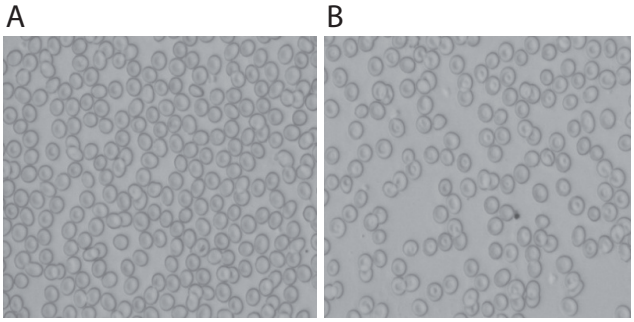
DESCRIPTION OF THE PATIENTS

All patients received a resuscitation department in a state of traumatic shock, accompanied by hemodynamic disturbances (BP average <45 mm Hg), coma (<8 points on the Glasgow Coma Scale), decreased partial pressure of oxygen in the artery capillary blood (59.4 ± 21,8 mm Hg), change of pH - acidosis (7,3 ± 0,05), basis deficit (from -11,2 to -2,4), decrease of hemoglobin (102,1 ± 36,9 g / l, and from 65,2 to 139 g/l), decrease of hematocrit (30% ± 11%, and from 19 to 41%), the number of erythrocytes (3, 4 ± 1,3 × 1012, and from 2,1 to 4,7), increase the lactate (5,8 ± 4,3 mmol/l, and from 1,5 to 10,1), reduction of 8-hydroxy-2-deoxy-guanosine (1,3 ± 0 7 pg / ml, and from 0,6 to 2,0 pg/ml). In the group of dead patients and in the patients with a group IV severity of bleeding, homeostasis changes are more expressed than in the other groups. At the first day after the injury to fill the volume of circulating blood (hypovolemia) and the elimination of hemodynamic disorders, patients were administered crystalloid and colloid solutions and blood loss greater than 40% of blood volume – transfusion of red blood cells and fresh frozen plasma. At the 3rd and 5th day after trauma, all the patients respectively showed a significant decrease in hemoglobin (77,5 ± 19,3 and 88,3 ± 13,0, p <0,05 against the first day of 102,1 ± 36.9 g / l), hematocrit (23% ± 6% and 25% ± 3%, p <0,05 with against the first day 30% ± 11%) and red blood cell count (2,6 ± 0,6x1012 and 3,0 ± 0,4x1012, p <0,05 against the first day 3,4 ± 1,3 × 1012), which is due to hemodilution, by conducting massive transfusion and infusion therapy. During the therapy noticed the normalization of pH to 7,43 ± 0,11 (third day), and 7,46 ± 0,11 (day 5), partial oxygen pressure (96,7 ± 38,5 mmHg, from 58,2 to 135,2 (third day), and 93,6 ± 48,3, from 45,3 to 141,9 mmHg (day 5)) due to the provide of mechanical ventilation. The correction of base deficit (BE = -1 , 8 ± 6,0, and from 4,2 to 7,8 (third day) and -0,7 ± 5,5, and from 4,8 to 6,2 (Day 5)). At the 7th day increase hemoglobin (93,2 ± 14,2 g / l), hematocrit (27% ± 5%), count of red blood cells (3,2 ± 0,5 × 1012), decrease of blood glucose levels to 6.9 ± 2,1. At the 7th day were marked the decrease the partial pressure of oxygen to 77,1 ± 38,3, from 38,8 to 115,4 mm Hg, and most likely due to the development of nosocomial pneumonia, and at the 15th day (69,6 ± 17,8, from 51,8 to 87,4 mmHg) - transfer of patients to spontaneous breathing. By the 15th day was a stabilization of the homeostasis parameters, increase of hemoglobin levels (105,3 ± 21,7 g / l), count of red blood cells (3,5 ± 0,7 × 1012), the normalization of pH (7,4 ± 0,05) and BE of plasma (0,8 ± 3,5, from 2,7 to 4,3). At the 1st day was found the significant differences between the groups of lactate in patients with I d. of blood loss (3,53 ± 2,18, and from 1,35 to 5,71 mmol / l) and IV d. of blood loss (7,78 ± 7,68, and from 0,1 to 15,6 mmol / L, p <0.05). The concentration of lactate in the first two weeks of observation and gradually decreases to the 15th day reaches normal values - 1,9 ± 0,5 mmol / l. At the 3rd and 5th day in patients were marked increase in the level of white blood cells (11,3 ± 3,1 x109), p / shift (9,5 ± 2,5), according to X-ray examination of the chest were marked infiltrates in the lungs. The content of 8-hydroxy, 2-deoxyguanosine in serum during the observation period tends to increase.

RESULTS AND DISCUSSION

Control group, light-optical microscopy and AFM. Number of normocytes in a field containing 300 cells, was 71,6±3,4% and was significantly different from the number of normocytes, that were counted in field of 1000 (63,8±4,0%, p<0,05) and 200 (61,6±4,5%, p<0,05) cells. Number of macrocytes in field of 1000 cells was 19±4,6% and 300 cells - 20,0±3,0% and was significantly different from the number of macrocytes, that were counted in field of 200 (29,2±3,8%, p<0.05) and 100 (27,0±9,9%, p<0,05) cells. Number of microcytes in field of 1000 cells was 16,8±3,9% and was significantly different from the number of microcytes, that were counted in field of 300 (7,8±1,3%, p<0,05) and 200 (8,6±1,1%, p<0,05) cells. Significant differences in the calculation of normocytes (60,0-73,5%), macrocytes (18,3-30,8%) and microcytes (6,3-8,3%) the same blood samples from the control group with AFM in a field of 100, 200, 300 and 1000 cells were not found. The distribution of the size of red blood cells were counted by light-optical microscopy and AFM, respectively, were: normocytes - 63,8%±4,1% and 60,0%±2,4%, macrocytes - 19,0%±4,6% and 30,7%±1,7%, p<0.05, microcytes - 16,8%±3,8% and 8,3%±0,9%, p<0.05. Group of patients, light-optical microscopy and AFM. In the group of patients with ST in field of AFM were founded 7,5±2,5% (I dbl), 7,9±4,3% (II dbl), and 8.3±4,2% (IV dbl) deep (deepening is 1944 nm, against 288 nm in the control group) diskocytes with outgrowth in the center.

LIGHT AND OPTIC MICROSCOPY



ATOMIC FORCE MICROSCOPY

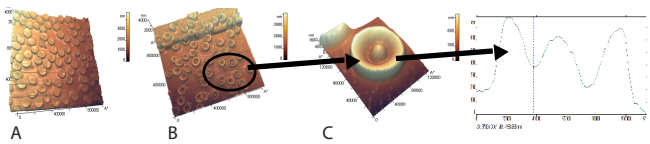


Fig. 1. *Image of a healthy red blood cell donor (A) and suffered from severe combined trauma (B) used by light-optical and atomic force microscopy in 3D in the 100x100 mm. The structure of a single red blood cell (C) in the field of 10x10 mm suffered from severe combined trauma and its graphic profile obtained using atomic force microscopy.*

At the first days after trauma in the patients group were obtained by using light-optical microscopy and AFM, the number of normocytes is reduced (46,2±4,5% and 44,8±18,3%) with compare to control (63,6±9,2 and 73,5±3,1); the number of macrocytes is increased (34±11,3% and 33,4±19,2%) with compare to control (27,0±9,9 and 18,2±2,2) and the number of microcytes (19,2±10,1% and 25,6±15,2%) with compare to control (9,2±3,8 and 7,0±1,8). Significant differences in comparison to the control group were not

revealed. By the 15th day of observation is observed the increase in the number of macrocytes to 40,5±7,8% in field of light-optical microscopy and 45,3±23,7% in field of ACM; and reduction of microcytes to 13,5±4,9% (light-optical microscopy), 12,7±8,0% (AFM). Changes of anisocytosis, were found using by AFM, depended on the degree of blood loss: the number of normocytes in the I dbl was 51,3±19,9%, and in the IV dbl - 37,6±19,3%; the number of macrocytes in the I dbl was 36,7±28,0%, and in IV dbl - 33,5±20,3%; the number of microcytes of I dbl was 12±10,5%, and in IV dbl - 35,6±32,5%. At the admission patients, which suffered from various trauma, there are significant changes in the erythrocyte membrane nanostructures: the average of the first order in all affected groups increases as compared with the control of almost 9 times, the height of the second order - 1.5 times, the height of the third order - in 3 times. At the 1st day after the injury, there was a trend to an increase in the values of the heights of the first and third orders with an increase in blood volume: h1 - 6,7 ± 5,0 (from 1,7 to 11,7) nm for I dbl; 9,9 ± 2,6 nm II dbl; 10,8 ± 6,0 (from 4,8 to 16,8) nm IV dbl; h3 - 0,39 ± 0,26 (from 0,13 to 0,65) nm for I dbl; 0,45 ± 0,35 (from 0,1 to 0,8) nm II dbl; 0,63 ± 0,47 (from 0,16 to 1,1) nm IV dbl. The height of the second order does not depend on the severity of blood loss and is at I dbl 1,45 ± 0,48 (from 0,97 to1,93) nm, with II dbl - 1,28 ± 0,02 (from 1,26 to 1,3) nm, with IV dbl - 1,50 ± 0,56 (from 0,94 to 2,06) nm.

In the first two weeks after the trauma was marked tend of decrease of all three orders. At the 15th day of the height of second order reaches normal values - 0,98 ± 0,09 nm. The height of the 1st and 3rd order to the 15th day after the injury decrease: 7,2 ± 6,2 nm and 0,46 ± 0,18 nm, respectively.

CONCLUSION

In the fields of light-optical microscopy and AFM indicated that as a result of blood loss at severe trauma, the anisocytosis becomes more expressed in comparison with the control. At the first day after severe trauma reduced the number of normocytes and increased the number of macro-and microcytes. About 8% of diskocytes had abnormal the deepening with outgrowths in the center. The changes of anisocytosis depended on the degree of blood loss. At 15 day was increased the number of macrocytes, associated with intensification of erythropoiesis. Quantified the degree of change nano-surface membranes of red blood cells in the dynamics in patients with severe trauma and varying degrees of blood loss were evaluated. The significant changes were revealed in the nanostructure of erythrocyte membranes in patients at the first day after the trauma: the average of height of the first order (h1) increased almost 9 times, the second-order (h2) ~ 1.5 times, the third-order (h3) ~ 3 times. Changes in the value of h1, h2 and h3 depend on the amount of blood loss and have trend in the first two weeks after trauma.

TRANSPORT AND TOXIC EFFECTS OF SILICA NANOPARTICLES IN HUMAN TROPHOBLASTS: PLACENTAL BARRIER MATTERS?

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INTRODUCTION

Rapidly developing nanotechnologies provide novel approaches for various industrial and research fields, as well as for diagnostics and medicine due to unique properties of nano-scaled materials. However growing environmental exposure to ultrafine particles has raised concerns among toxicologists and “nanotoxicology” has been developing as a separate branch to study adverse health effects of nanoparticles (NPs). Reproductive nanotoxicology, however, is still in its infancy and almost no data exists on NP interaction with germ cells, placenta and embryonic development especially in humans. Due to the species-specific structure of the placental barrier and its unique physiology, animal data can hardly be transferred to the human situation. Therefore choosing an adequate research model is of special importance. The human placental barrier consists of trophoblast cells, which basically form 2 layers, the cytotrophoblasts and the syncytium, a continuous multinucleated layer covering all the placental villi, facing maternal plasma, and from 2nd trimester of pregnancy on, maternal blood. The morphology and the function of the barrier changes with gestational age. Embryonic development is known to be more sensitive to teratogens in early gestation, therefore we have been focusing on NP interactions with first trimester human placenta.

MATERIALS AND METHODS

We used potentially toxic silica NPs of different types and sizes for our experiments: 5nm crystalline (Min-U-Sil5, unlabeled), 30nm and 100nm amorphous silica NPs (Micromold, labeled). Human trophoblast cell line BeWo has been cultured and exposed to NPs for 6, 12, 24, 48h. Placental explants were prepared from early placental samples, obtained after elective pregnancy termination and cultured under hypoxic conditions (3% oxygen, physiological hypoxia, specific for early stages of pregnancy) and exposed to NPs for 6, 12, 18, 24, 48h. Electron microscopy with element analysis, as well as light and fluorescent microscopy were performed to localize NPs in cells and tissue. LDH concentration in culture medium were measured by ELISA. Apoptosis was analysed by automatic image analysis in placental explants, immuno- stained for M30.

RESULTS

5nm Silica NPs tend to form aggregates about 0.3-0.5 µm in culture medium that could be seen in the light microscope (Fig.1A) and in the transmission electron microscope (Fig.1B). Element analysis has been performed to confirm silica nature of observed particles (Fig.1C). These aggregates were localized close to apical membrane of the syncytiotropholast, outside the membrane. We did not find crystalline silica NPs inside placental tissue. Amorphous silica NPs have been found inside syncytiotrophoblast layer, as small agglomerates, but no agglomerates were localized in the cytotrophoblast or close to fetal capillaries (Fig.1D,E). However BeWo cells showed massive accumulation of NPs inside the cell which were localized in the nuclear and perinuclear area of the cell (Fig.2A-D). In placental explant`s culture medium no difference in LDH concentration were found between crystalline silica NP- incubated and non-incubated explants (Fig.3, left panel) while BeWo cells showed dose-dependent cytotoxicity of crystalline silica NPs (Fig3, right panel). Placental explants did also not show significant differences in trophoblast apoptosis events between NP- exposed and unexposed.

CONCLUSIONS

Trophoblast layer of placental explants exposed to silica NP showed different pattern of NPs localization and cytotoxicity compared to BeWo trophoblast cell line. Simple cell culture experiments using standard trophoblastic cell lines like BeWo might therefore be misleading when used to assess placental nanotoxicity. Placental barrier is seemed to provide better protection against NPs compared to single trophoblast cells, what should be considered by further studies on NPs interaction with human placenta.

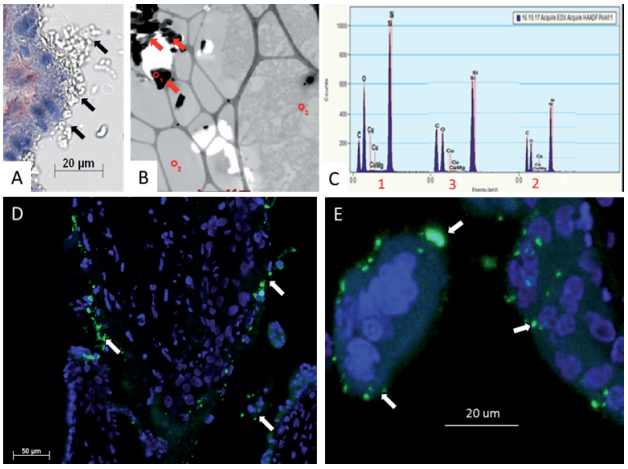

Figure 1

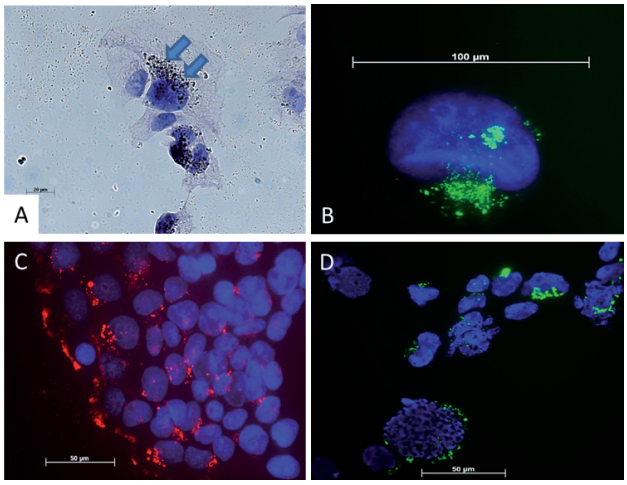

Figure 2

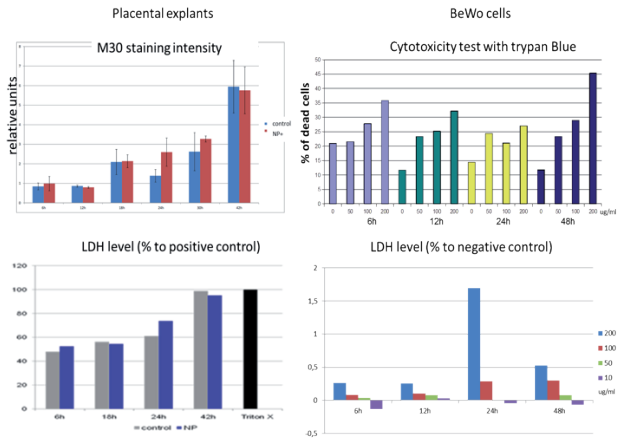

Figure 3

DEVELOPMENT OF METHODOLOGY FOR THE SEPARATION AND QUANTIFICATION OF LIPOSOME-BOUND AND FREE DOXORUBICIN IN PLASMA

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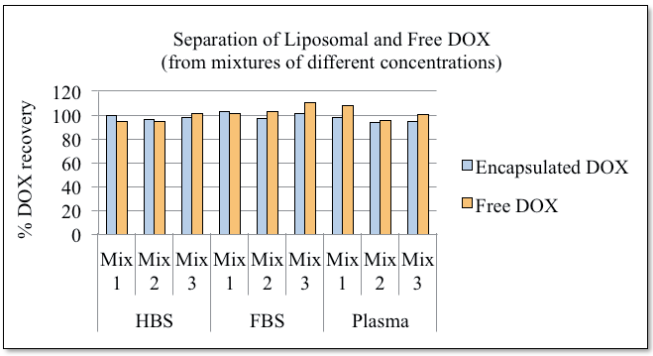
Magnetic Resonance Imaging - Guided High Intensity Focused Ultrasound (MR-HIFU) is an emerging technology that allows non-invasive thermal therapy with real-time monitoring. It can be used for controlled thermal ablation of tumor tissue, but also holds great potential for improving the efficacy of chemotherapy through the triggered local release of anticancer agents from targeted nanomedicines. In the HIFU-CHEM project, the clinical application of MR-HIFU for the treatment of liver metastases and bone metastases in combination with a thermosensitive liposomal doxorubicin formulation is addressed. Thermosensitive liposomal doxorubicin (ThermoDox) is a novel nanomedicine formulation that is stable at room temperature and body temperature (37°C) but rapidly releases its cytotoxic content at temperatures raised slightly above body temperature (42°C). For the pharmacokinetic part of the clinical study, we report here on a bioanalytical method that has been developed successfully for the determination of liposome-bound and free (i.e. not bound to liposomes) doxorubicin in human plasma samples.
This research was supported by the Center for Translational Molecular Medicine (HIFU-CHEM).



"NANOTECHNOLOGY APPLICATION TO A NOVEL CELL BASED PERSONALIZED IN VITRO MODEL OF NEURODEGENERATIVE DISEASES"

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Niemann Pick type C (NPC) disease is an autosomal recessive neurodegenerative lysosomal storage disorder, caused by the abnormal function of NPC1 or NPC2 protein. Both proteins are involved in the intracellular trafficking of cholesterol and other lipids. The deficiency of either of them leads to the accumulation of the endocytosed unesterified cholesterol, gangliosides and other glycosphingolipids (GSLs) within the lysosomes [1].
Clinically, NPC disease presents a highly variable phenotype, with a time of presentation ranging from fetal to adult age. Despite the recent progress in characterizing the biochemical and genetic defects in NPC disease, the underlying patho-physiological mechanisms of this disorder are not clear and the currently available therapeutic interventions are limited. In particular, the study of the molecular pathways linking the lipid accumulation to the neurodegeneration has been hindered by the limited availability of neuronal models of the disease. In this regard, the possibility to isolate stem cells from accessible cell sources and to commit them to a neuronal lineage could be of great utility in dissecting the molecular pathway leading to the neurodegeneration in NPC patients.
Taking advantage of the method previously established in our laboratory for the isolation of stem cells from different human adult tissue[2,3], we developed a human neuronal model of NPC disease by inducing the neuronal differentiation of Multipotent Adult Stem Cells (MASC) isolated from skin biopsies.
MASC cultures were successfully grown from skin biopsies of five NPC patients and two healthy donors. All cell lines expressed the pluripotent stem cells markers OCT-4, while only cultured cells obtained from patients were positive for Filipin staining of free cholesterol.
The differentiation was monitored both by evaluating morphological parameters and revealing the presence of neural markers such as nestin, MAP2, O4 and GFAP. After two weeks of neural induction, a large fraction of cells (≈50%) acquired a neuron like morphology and stained positive for neuronal markers such as nestin and MAP2, suggesting that cells obtained from skin biopsies retained the ability to differentiate toward a neural phenotype. No GFAP, O4 and OCT4 positive cells were detected at the end of differentiation.
Morphological analysis of neuronal cells were performed by Atomic Force Microscopy (AFM), a powerful biophysical technique able to probe the structure and the mechanical properties of cells under physiological concentration. Our AFM combines optical and atomic force microscopy in order to reveal structural details of nanoscale dimensions difficult to resolve with conventional microscopy, such as the precise measurements of invaginations, growth cones, lamellipodia[4].
Performing this analysis we found that, once differentiated, cells obtained from patients presented a morphology clearly different from normal cells: NPC differentiated cells developed significantly

more abundant and longer neurites than the healthy counterparts. Importantly, distortion of neuron shape, ectopic dendrites and meganeurite formation are the main features described in NPC brain cells.
These results indicate that it is possible to isolate cells with stem cell properties from skin biopsies of NPC-patients and that these cultures retain the ability to differentiate toward a neuronal lineage, able to recapitulate the NPC features present in vivo.
The in vitro model we developed will be useful to evaluate the impact of different mutations on the pathological phenotype and might represent a powerful tool to perform drug screening on cells obtained from NPC patients presenting different genotypes.

1. Patterson M, Vanier MT, Suzuki K, et al. Niemann Pick disease type C: a lipid trafficking disorder. The metabolic and molecular basis of inherited disease. Mc Graw-Hill. 2001:611-634.
2. Beltrami A.P, Cesselli D., Bergamin N., et al. Multipotent calls can be generated in vitro from several adult human organs (heart, liver and bone marrow). Blood. 2007;110:3438-3446.
3. Cesselli D, Beltrami AP, Rigo S, et al. Multipotent Progenitor Cells are present in human peripheral blood. Circulation Research. 2009;23.
4. Efremov Yu M., Dzyubenko E.V. et al. Atomic Force Microscopy Study of the Arrangement and Mechanical Properties of Astrocytic Cytoskeleton in Growth Medium. Acta Naturae 2011

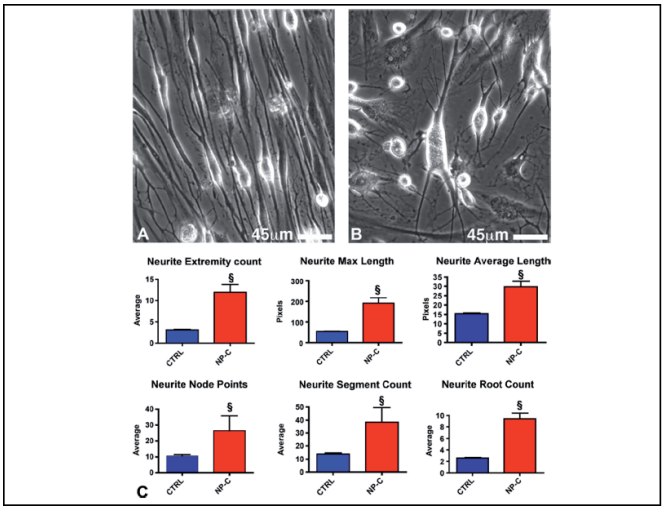

Figure 1. *Morphometric analysis of differentiated cells obtained from hSKIN-MASC of healthy donors or NPC patients. (A- B): Phase contrast image showing the morphological differences between differentiated cells (5 days in N2 medium + 48 h in N3 medium, see methods) of a healthy donor (A) and a NPC patient (B). Specifically, NPC differentiated cells were characterized by distortion of neuron shape, ectopic neurites and meganeurite formation. (C): Quantitative analysis of neuronal morphological parameters linked to neurite outgrowth as evaluated by a specific Image analysis software on MAP-2 stained cells. Data are presented as mean ± SD of 3 independent experiments; §, p<0.01 vs healthy donors (CTRL). Bergamin et al. Orphanet Journal of Rare Diseases 2013, 8:34*

IN VITRO EVALUATION OF SILVER NANOPARTICLES OF DIFFERENT SIZES IN ASSAYS FOR CYTOTOXICITY, INFLAMMATION AND DEVELOPMENTAL TOXICITY

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Silver nanoparticles are currently listed as the most commonly used nanomaterials in consumer products, but their antibacterial properties are also of high interest for medical applications. In light of the anticipated increased human exposure to silver nanoparticles, evaluation of their potential adverse effects is necessary. We have studied well-characterized silver nanoparticles of 20, 80 and 113 nm in vitro, using assays for cytotoxicity, inflammation and developmental toxicity.
Silver nanoparticles were cytotoxic to both fibroblast and macrophage cell lines, and induced a variety of cytokines. Furthermore, silver nanoparticles of all sizes inhibited the differentiation of embryonic stem cells into cardiomyocytes, but only at cytotoxic concentrations. At equal mass concentrations, the 20 nm nanoparticles appeared to be most toxic for all toxicity endpoints considered. In macrophages, the toxic potential of the silver nanoparticles was lower than that of silver ions, as determined from studies using silver nitrate.
Our results demonstrate that safety evaluation of silver nanoparticles may need to be considered separately from other forms of silver. In addition, health based exposure limits for silver nanoparticles should not be expressed in mass concentration units.

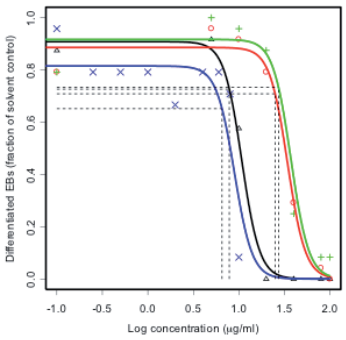

Figure 1. *Silver nanoparticles and silver nitrate inhibited D3 stem cell differentiation.*

ROLES OF MAC-1 AND GLYCOPROTEIN IIB/IIIA INTEGRINS IN LEUKOCYTE–PLATELET AGGREGATE FORMATION: STABILIZATION BY MAC-1 AND INHIBITION BY GPIIB/IIIA BLOCKERS

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Hypersensitivity reaction is a potentially life threatening side-effect of administering nano drugs. As cell aggregation is a leading cause of pulmonary complications accompanying hypersensitivity, interruption of aggregate building and reduction of cell-aggregate survival may be of great importance in improving clinical outcomes. Platelet–leukocyte heteroaggregates are built following cell activation. The association involves the receptor families of selectins and integrins. We investigated the role of CD11b/CD18 integrin (Mac-1) in hetero-aggregate formation and searched for a counter-receptor on platelets ready to interact with Mac-1. As a model of leukocytes, Mac-1 presenting Chinese hamster ovary (CHO) cells were used to evaluate the role of Mac-1 in hetero-aggregate formation. The amount of CHO cell-bound active and inactive platelets was measured by flow cytometry, while the counter-receptors on platelets were identified via using blocking antibodies. We observed significant platelet adhesion on Mac-1-bearing cells when platelet-rich plasma or activated platelets were present. Inactive platelets did not adhere to Mac-1-bearing cells. Addition of fibrinogen, a ligand of Mac-1 significantly increased platelet binding. CD40L was demonstrated to act similarly on Mac-1. Inhibition of platelet GpIIb/IIIa completely abolished CHO cell–platelet aggregation. In our study, we have shown for the first time that Mac-1 mediates the formation of hetero-aggregates without selectin tethering when Mac-1 ligands such as fibrinogen or CD40L are present and blockers of platelet GpIIb/IIIa are able to diminish this interaction.

MULTIFUNTIONAL POLYGLUCOSAMINE NANOCAPSULES USED FOR VACCINATION AGAINST HEPATITIS B

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Vaccination has been one of the greatest achievements in the history of medicine as it prevents a large number of infectious diseases globally. However, there is a necessity to develop new, safer and more effective vaccines. In this context, Nanomedicine can importantly contribute to this aim by allowing the incorporation of immunostimulants, adjuvants and antigens within the same nanostructure. Additionally nanocarriers are well known to protect antigens from degradation and to deliver them to antigen presenting cells (APCs).

OBJECTIVE

Design a polymeric nanostructure (polyglucosamine nanocapsules) which allows the combination of the recombinant hepatitis B surface antigen (rHBsAg), the immunostimulant agent imiquimod and the adjuvant squalene, in order to induce protective levels of specific antibodies after a single intramuscular shot in mice.

MATERIALS AND METHODS

We performed a comparative analysis of polyglucosamine nanocapsules containing or not imiquimod, associating the rHBsAg as a model antigen (Table 1). For this purpose, we carried out several in vitro studies: viability test, cytokine production in peritoneal macrophages and cell internalization. Additionally, these prototypes were evaluated in vivo after a single intramuscular shot in female BALB/c mice. The serum levels of total IgG and IgG1/IgG2a subclasses were measured at different time slots for six months in order to determine the long-term specific response and the type of immune response induced (Th1 or Th2).

RESULTS

In vitro studies showed that both prototypes reduced the cell viability in a dose dependent manner (Figure 1). Studies with macrophages showed a high rate of internalization (Figure 2) but only nanocapsules containing imiquimod were able to induce the production of a mixture pattern of pro and anti-inflammatory cytokines (IL-1, IL-6, TNF-α and IL-10, respectively) (Figure 3).

In vivo, both nanocapsule prototypes showed the ability to raise the specific IgG levels over 100 mUI/mL (seroprotective level in humans). Moreover, equivalent levels to two doses of the positive control (HB-alum) were achieved by the end of the study ($p>0.05$). The ratio IgG1/IgG2a observed after the administration of the prototype containing imiquimod showed a balanced response involving both cellular (Th1) and humoral (Th2) immune responses (Figure 4) against the associated antigen, supporting the results observed in the cytokine pattern in vitro.

Table 1. *Physicochemical characterization of the different prototypes of PG nanocapsules with different core composition (squalene or squalene+imiquimod)*

Shell composition	Core composition	Abbreviation	Associated Ag	Size (nm)	PdI	ζ potential (mV)	Ag A.E. (%)	Imiquimod A.E. (%)
Poly Glucosamine (PG)	Squalene	PG NC	--	172 ± 3	< 0.2	+66 ± 1	-	-
			HB	230 ± 13	< 0.2	+60 ± 3	72 ± 8	-
	Squalene + Imiquimod	I-PG NC	--	198 ± 5	< 0.2	+63 ± 1	-	40 ± 2
			HB	299 ± 11	< 0.2	+60 ± 4	78 ± 6	40 ± 2

PdI: polydispersity index; A.E.: association efficiency; Ag: antigen.

Figure 1. *Real-time monitoring of cell growth in the presence of PG nanocapsules (upper graph) or imiquimod-PG-nanocapsules (bottom graph). Growth was evaluated by the xCELLigence system.*

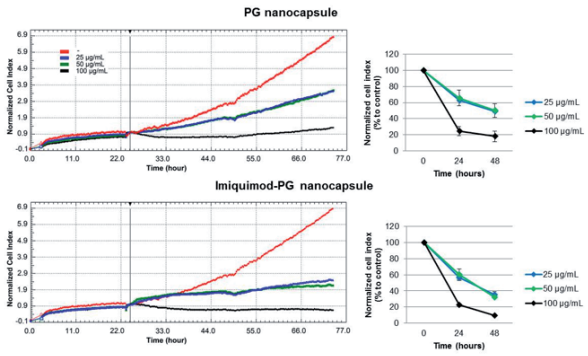


Figure 2. *Internalization of fluorescent PG nanocapsules (at 50 µg/mL) by macrophages after 30 minutes of incubation. It was analyzed by optical fluorescence microscopy (A) and flow cytometry (B)*

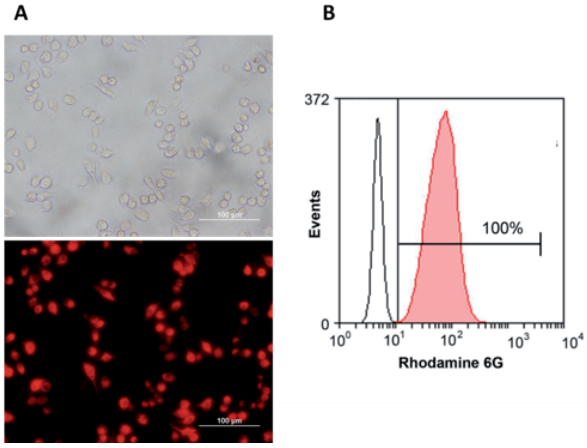


Figure 3. *Cytokine production by peritoneal macrophages incubated with PG nanocapsules (PG NC) and imiquimod-PG nanocapsules (I-PG NC) at two different doses (10 and 100 µg/mL) for 24 hours.*

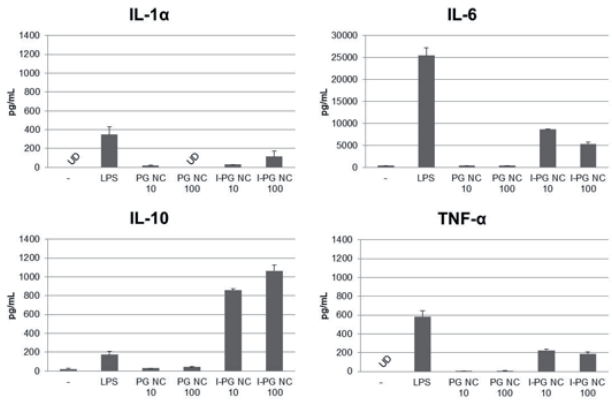
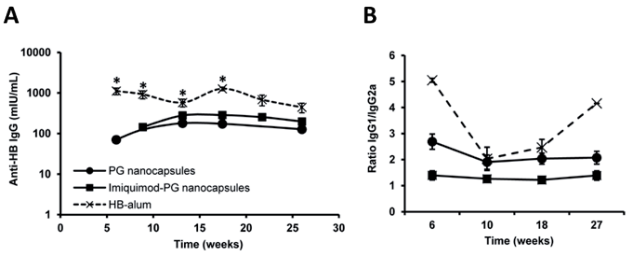


Figure 4. *A) Immune response elicited with PG and imiquimod-PG nanocapsules associating hepatitis B antigen (HB) and B) ratio of specific anti-HB IgG1/IgG2a antibodies. Mice (10 mice per group) were immunized with a single dose of PG or imiquimod PG-nanocapsules at day 0. Control group (HB-alum) received two immunizations (at days 0 and 30) with the conventional hepatitis B vaccine in the presence of alum as adjuvant.*



CONCLUSIONS

The polymeric nanocarrier which incorporates within the same structure an immunostimulant (imiquimod), an adjuvant (squalene) and the model antigen (rHBsAg) showed the capacity of prolonging and modulating specific immune response after a single-shot immunization regimen. Therefore, the polyglucosamine nanocapsules have proved to have very interesting features as a multifunctional immunoacting nanocarrier.

This work has been supported by two projects from the Spanish Ministries of Science and Innovation (PI080751) and of Economy and Competitivity (SAF2011-30337-C02-02). We also thank to the EU [FP7/REGPOT-2012-2013.1] under grant agreement BIO-CAPS-316265. MP and SV acknowledge a PhD fellowship (FPU, Spanish Ministry of Education)

THE NANOHYDROXYAPATITE-COMPOSITES MATERIALS FOR REGENERATIVE IMPLANTS

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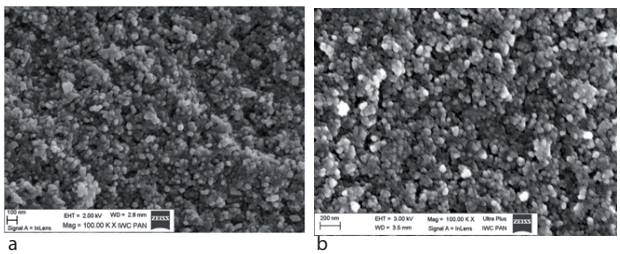
There is an ever-expanding requirement for hard tissue replacement such as bone, caused by aging population. Engineered artificial biomaterial scaffolds with appropriate mechanical properties and surface topography are in great demand for enhancing cell attachment, cell growth and tissue formation at such defective sites. One of the most widely used biomaterial in bone tissue engineering substitutes is hydroxyapatite (HAP). Natural bone contains around 70% HAP (by weight), collagen and cells.

The Institute of High Pressure Physics of the Polish Academy of Sciences (IHPP) has developed technologies of the nanohydroxyapatite powder synthesis (called GoHAP), obtained by microwave reactor and the high pressure consolidation technology for ceramic material. Those techniques aim to obtain materials close to the natural structure and mechanical properties of the bone tissues. Obtained GoHAP ideally mimics the natural hydroxyapatite found in human body. The prepared powder has plate and crystalline structure and is characterized by grain size in the range 5-25 nm (it can be controlled); specific surface area is 237m2/g,. The resorbable powder was investigated according to norm ISO 10993-14. In collaboration with Riga Technical University composite granules GoHAP- polylactide (GoHAP-PLA) were created in composition from 10 to 50% PLA by weight.

Our work shows mechanical properties of consolidated HAP-PLA composite. The high pressure consolidation technology permits to obtain dense ceramic with mechanical properties close to the natural bone. The process was carried out in extreme pressure 1GPa and temperature under 200°C. Thanks to this, the GoHAP nanopowder structure and biological properties are preserved in composite (SEM observation of topography GoHAP powder and consolidated composite- Fig. 1). The mechanical properties of this nanoceramic were investigated and the compressive strength reach assign value above 130 MPa (natural bone is in range from 100 to 200 MPa).



a b

Fig.1 *(a) GoHAP powder topography. (b) Consolidated composite topography*

Acknowledgement: This work is being realized in part in the projects Sonosca and CePT. The authors acknowledge support from The Faculty of Materials Science and Engineering, Warsaw University of Technology.

DESIGN OF A LUNG TARGETING HYDROGEL MICROPARTICLES WITH EMBEDDED NANOPARTICLES FOR THE TREATMENT OF LUNG CANCER

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Non-small cell lung cancer (NSCLC), the most common type of lung cancer, has a poor prognosis with only a 15% 5-year survival rate for combined cancer stages according to NCI. Challenges facing the current chemotherapy drugs include, hydrophobicity of therapeutic agents, excessive systemic toxicity and inability to target lung tumors. The hydrogel-based delivery vehicle described herein aims to overcome these challenges by selectively targeting the lung to deliver the hydrophobic, anti-cancer drug camptothecin (CPT).

The first layer of the delivery system is a degradable poly(ethylene glycol) based microgel particle (MGP) designed to take advantage of the venous lung filtration pathway and passively accumulate in the lungs after intravenous injection (figure 1.). The size and modulus of the MGPs affect the targeting, accumulation and clearance from the lungs. Using microfluidics, we are able to create nearly monodispered MGPs ranging in size from 15 to 50µm. We use Michael addition chemistry to create stable gels with encapsulated nanoparticles (NP) (Figure 1d.). The gelation process can be tuned via pH (Figure 2a.) The gel modulus is controlled by the reaction conditions (Figure 2b.). The degradation time for these gels is on the order of two weeks (Figure 2c.).

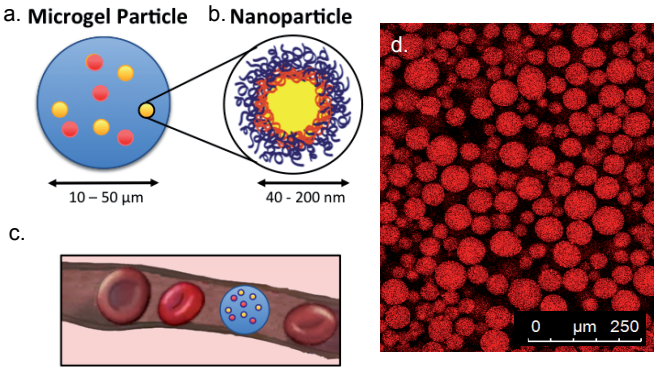

Figure 1. *Schematic of the Microgel Particle: (a.) The MGP (blue) has NPs (yellow and red) embedded within the gel matrix. (b.) The NPs have a core-shell structure with a hydrophobic drug loaded core (yellow) and a polymer stabilizing layer (blue). (c.) After IV injection, the MGPs lodge in the lung capillaries, release their payload and degrade away. (d.) A confocal image of MGPs with encapsulated fluorescent NPs.*

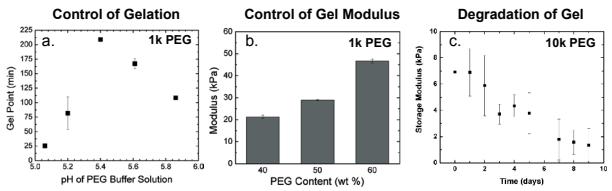

Figure 2. *(a.) The gelation of a 1k PEG triacrylate solution as a function of pH. (b.) Tuning the hydrogel modulus by the PEG composition in solution. (c.) The degradation of a PEG gel in PBS buffer at 37oC.*

The second layer of the delivery system consists of 60 to 200nm nanoparticles (NP) formed by Flash NanoPrecipitation (Figure 3.), which are embedded within the MGP matrix. The NPs are loaded with a CPT prodrug with a hydrolytically unstable ester linker. By varying the hydrophobicity of the CPT prodrug, the release rate can be tuned. The NP loading within the gel is correlated to the molecular weight of stabilizing PEG corona on the NPs.

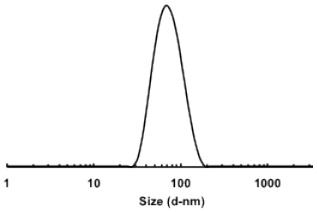

Figure 3. *The size intensity distribution of nanoparticles loaded with camptothecin produg.*

In a preliminary study, we investigated the effect of CPT in MGP form on tumor size in an orthotopic NSCLC mouse model. Previous work has shown that α-lipoic acid (ALA) is a chemopotentiator for CPT and as such was administered in conjunction with the MGPs in one test group. The mice treated with CPT MGPs showed significant tumor regression in comparison to the control group as determined by MRI and CT. The CPT MGP and ALA group had the largest tumor regression indicating the potential for combined therapy.

CELLULAR EVENTS IN FOUR B16 MELANOMA CELL LINES REVEALED BY SERS

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Cellular imaging techniques are crucial in life science research based in part on the notion that "seeing is believing." Among various imaging techniques, Raman spectroscopy based on the inelastic scattering of coherent light on molecules, provide a unique approach within a non-destructive manner. Because of the low scattering efficiency of about a million fold lower than the laser intensity used for excitation, surface enhanced Raman scattering (SERS) with noble metal nanoparticles .providing 106 to 1014 enhancement of the scattered signal, is widely used in prospecting the biological processes at cellular and molecular level. As a response to the increasing demand to develop new, sensitive, noninvasive and accurate methods in early diagnostic and to track the molecular changes in early stage of malignancy or in the therapy monitoring, an interdisciplinary approach exploiting the surface plasmon resonance [1] of the pure nanoparticles and ultrasensitive Raman spectroscopic techniques are employed here to get insight into the cellular events in the case of melanoma cells. Due to its unique ability to provide ultrasensitive detection limits, SERS has been used to provide rich information in medical nanodiagnostic [2]. Concepts like SERS active needle, in vivo SERS reporters, SERS tumor targeting, SERS molecular diagnostic, SERS cellular imaging, are currently expanding in the nanomedicine field. As recently summarized, [2, 3] SERS is currently a mature, powerful vibrational spectroscopic technique, that gained increasing interest in medical diagnostic over the last decade and the number of applications in medical studies is rapidly increasing. The molecular species located in the close vicinity of the uptaken nanoparticles exhibit strong Raman scattering which represents the SERS signal with high spatial resolution, enabling unprecedent capability in cells or tissue studies. However, a complete understanding of the intracellular uptake, transport, metabolism and subcellular distribution of nanostructured materials remains limited. Nevertheless, overall findings reported that live cells trap NPs of proper dimension in vesicles, hampering them to get into nucleus, although the SERS signals from cells were assigned mostly to nucleus components vibrational modes. Various nanoarchitectures from a noble metal or silica core covered with Raman reporter molecules or different linkers have been reported, aiming to provide good stability, biocompatibility and reproductive SERS signal for specific biological molecule, however, such architectures so far are not reported in live cell uptake, probably because of the difficulty in cell incubation. Linkers or specific molecular tags could indeed provide unambiguous SERS signal but could as well interact with various compounds while penetrating cell membrane. The lack of reproducibility in SERS signal was rather judged as a need to optimise such nanoarchitectures rather than taking into account the "live" system, i.e live cell, where biological processes are rolling on.

We investigated this process inside the live cells in the case of four different B16 melanoma cell lines, B164A5, B16F10, B16GMCSF, and B16FLT3 respectively, using Ag nanoparticles (AgNPs), a confocal Raman microscope Bruker Senterra and a near infrared (NIR) laser line at 785 nm, for noninvasive excitation of the cells. The incidemt laser power on the cell was about 3 mW. We evaluated the nanoparticles uptake ability of the cells using transmission electron microscopy (TEM) in conjunction with the SERS measurements. The nanoparticles exhibited both spheroidal (40 nm average diameter) and rod-like shapes with lengths ranging from 100 to 200 nm and about 30-40 nm rod diameter. Their optical properties, TEM image and SERS behavior in melanoma tissue from animal model are described in our previous study [4]. B164A5, B16F10, B16GMCSF, B16FLT3 are adherent cells and have a spindle shaped morphology, showing fibroblast-like characteristics. B164A5 cell line produces melanin. The complete growth medium for this cells is Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal calf serum (FCS) and 1% Penicillin/Streptomycin mixture (Pen/Strep, 10,000 IU/ml) and 2% HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. The cells were cultured by incubation at 37ºC in 5% CO2 atmosphere .When the confluence was 70-80% (every two or three days) the cells were passed using 0.25% Trypsin-1 mM EDTA solution followed by centrifugation (5 minutes, 1200 rpm) and replated in T75 culture flasks at a subcultivation ratio of 1:10 to ensure optimal proliferation. Incubation with Ag NPs was achieved by adding the colloid in the culture medium and the SERS measurements have been performed 24 h later on 30 different cells from each line. TEM revealed NPs accumulation as an endocytosis mechanism involving the vesicle internalization, as shown in Fig. 1. The NPs were able to penetrate the cells membrane in different stage of the cell development but not into nucleus. SERS measurements have been conducted point by point over the adherent cells, by collecting three times signal from each point, both from nucleus area, cytoplasm regions and membranes. The SERS signal exhibited specific spectral shape from one point to another over one cell, which is consistent with the distance from the NPs accumulations. The molecular species located in the close vicinity of NPs are generally responsible for the signal. However, in the optical image prior laser focus on Raman microscope, these nanoaggregates in vesicles are not visible. Since laser spot size, depending on the objective magnification (i. e. 50x, N.A. 0.45) comprises about 3 µm diameter in the collecting cone of the objective, we may have both region with NPs and regions free of NPs. An interesting feature is the signal change within the same focus point upon multiple acquisitions. The changes are illustrated in the Fig. 2 in the case of two B16FLT3 cells, where in 5 seconds, in the same collecting point we noted spectral changes in the 1700-1500 cm-1 range, where the amide I band of proteins as well as characteristic C=C and C=N stretching modes exhibited clear differences, which can be assigned to specific biochemical changes due to cell proliferation. We compared the raw SERS signal collected from different cells of the same line and between different cell lines. A common characteristic is the presence of the huge band at 236 cm-1, which is well known SERS band attributable to Ag-N bond for the N-containing adsorbed species. For B64A5 cells the SERS signal variation as well as the strongest signal among the four lines has been obtained, suggesting the best NPs uptake efficiency. Cellular membranes and nucleus generate low signal (lack of nanoparticles) while the cytoplasm area revealed highest SERS signal. Strong bands were observed at 1619, 1524, 1383, 1320, 1188, 985, 921, 820, 706, 478 and 236 cm-1. An interesting feature is the presence of the melanin-like concentration spots over the cell, which exhibited characteristic SERS bands at 1452, 1287 1198, 712, 411 and 236 cm-1. We compared the data with those from SERS of melanoma tissue in mice models [4]. The tissue characteristic reproducible SERS bands located at 1618, 1455, 1359, 1283, 1119, 976, 899, 798, 752 and 236 cm-1. According to Kneipp et al. [6] such bands are characteristic for the native chemical constituents in the cell nucleus and cytoplasm, like DNA, RNA, phenylalanine, tyrosine. Although the spectra collected from random points exhibit small band shape differences due to the

tissue inhomogeneity, this seems to be different from cell inhomogeneity. Zhu et al [5] demonstarted that the live breast cancer cells uptake gold NPs and isolate them into vesicles in cytoplasm. The nucleus penetration was not observed. Evaluating about 200 spectra from 30 cells suspended in PBS buffer, they found the SERS intensity variation on the Raman mapped area of 10 µm x 10 µm. However, their SERS signal reported revealed the same spectral shape as signal characteristic to phenylalanine adsorbed on gold NPs (1004 and 1030 cm-1). This signal was not observed here, in all the evaluated cells. The same passive fluid-phase uptake was accounted for the absorption of gold NPs into cultured eukaryotic cells [6], where different SERS bands were also reported. Since the Ag NPs provide a good reproducibility, the differences are supposed to come from different cellular identity. Other results show that the cellular uptake of gold NPs is dependent on their size and surface properties [7]. The NPs were transported in vesicles of 300–500 nm diameter within the cytoplasm. The average velocity and diffusion coefficient of the vesicles containing NPs were 10.2 (±1.8) µm/hr and 3.33 (±0.52) µm2/hr, respectively. These results are consistent with the present experiments Analysis of the time-dependent intracellular spatial distribution of the NPs demonstrated that they reside in lysosomes (final degrading organelles) within 40 minutes of incubation. SERS measurements within one hour from incubation revealed no signal. These findings can be used to tailor nanoscale devices for effective cell targeting and delivery[7]. Another illustrative cellular event revealed by SERS is shown in the Fig. 2, in the case of cell 2, among two cells from B16 FLT3 line. The presence of the band at 735 cm-1, characteristic the DNA base (adenine) is clearly revealed, suggesting that the 5 seconds SERS measurement surprised a cell division event, where at least one of the divided nuclei were close enough to the NPs to generate SERS specific signal. This process is accompanied by the protein band decrease (Fig. 2).
Concluding, the four B16 melanoma cells provided specific endocytosis mechanism against Ag nanoparticles as revealed by specific SERS signal. The observed SERS bands are different from one cell line to another, revealing different NPs uptake ability. Within the same cell line, every cell exhibits time dependent signal that could be correlated with the cellular events like division, proteins consuming, melanin appearance, or even die, when the SERS signal revealed reproducible feature.
These results provide better ingisht into the complex cellular events in the presence of nanoparticles and open new challenges in addressing therapy monitoring using new drug-functionalized nanoparticles to cancer cells.

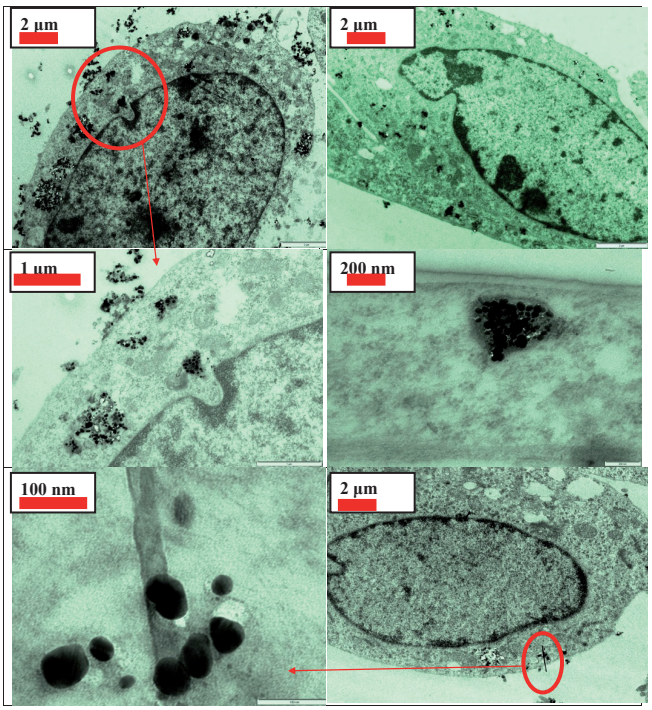

Fig. 1. *TEM images of the Ag nanoparticles inside the cells, revealing the inhomogeneous distribution in cytoplasm and their concentration in vesicles: upper- two distinct B164A5 cells; middle: concentration of nanoparticles in vesicles-like shapes, suggesting specific endocytosis mechanism against Ag nanoparticles; lower: detailed view of the nanoparticles in a cytoplasm area marked with arrow.*

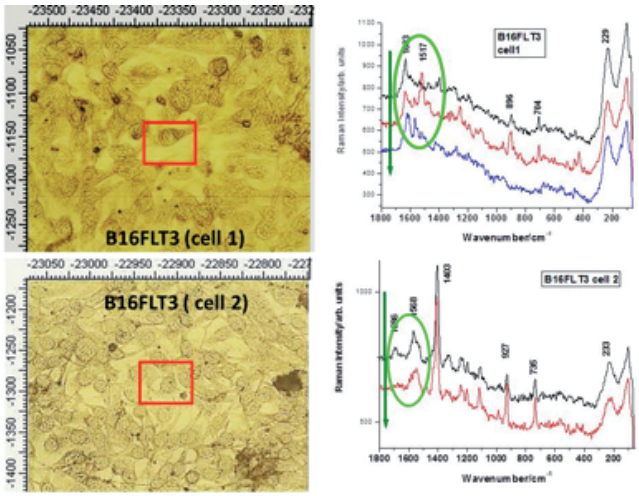

Fig. 2. *Left: Optical microscopy image of two B16FLT3 cells provided by the Raman microscope and the SERS signal (right) collected from the spotted cell under the same experimental conditions. The sequence of spectra is collected from 5 to 5 seconds. Laser line: 785 nm.*

ACKNOWLEDGEMENTS

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REFERENCES

[1]. H/ Kato, Traking nanoparticles inside cells, Nat. Nanotechnol, 6, 139. 2011.
[2]. W. Xie and S. Schlucker, Medical applications of surface-enhanced Raman scattering, Phys.Chem. Chem. Phys. ,15, 5329, 2013.
[3]. Y/ Wang and S. Schlucker, Rational design and synthesis of SERS labels, Analyst, 138, 2224-38. 2013
[4]. S. Cinta Pinzaru., A. Falamas, C. A. Dehelean, Molecular conformation changes along the malignancy revealed by optical nanosensors, J. Cell. Mol. Med., 17: 277–286, 2013.
[5]. J. Zhu, J. Zhou, J. Guo, W. Cai, Bo Liu, Z. Wang and Z. Sun, SERS investigation on human breast cancer cells, Chem. Central J. 2013, 7:37 doi:10.1186/1752-153X-7-37.
[6]. Kneipp J, Kneipp H, Wittig B et al. Novel optical nanosensors for probing and imaging live cells, Nanomedicine: Nanotechnol., Biol. Med; 6: 214–26, 2010.
[7]. B. Devika Chithrani, James Stewart, Christine Allen, David A. Jaffray, Intracellular uptake, transport, and processing of nanostructures in cancer cells, Nanomedicine: Nanotechnol., Biol. Med. , 5, 2 , 118-127, 2009.

NANOZAPPED! DNA, SIRNA, AND THEIR DENDRITIC NANOVECTORS: A COMBINED IN SILICO/IN VITRO/IN VIVO APPROACH

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Due to the relative easy synthesis and commercial availability, nanovectors based on dendrimers and dendrons are among the most utilized non-viral vectors for gene transfer. Contextually, recent advances in molecular simulations and computer architectures not only allow for accurate predictions of many structural, energetical, and eventual self-assembly features of these nanocarriers per se, but are able to yield vital (and perhaps otherwise unattainable) molecular information about the interactions of these nanovectors with their nucleic acid cargoes. In this presentation, we aim

at reviewing our own efforts in the field of multiscale molecular modeling of these interesting materials (see Fig.1). In particular, our originally developed computational recipes will be introduced, and the link between simulations and experiments will be described and discussed in detail. The work is prepared by computational scientists for experimental scientists, with the specific purpose of illustrating the potentiality of these methodologies and the usefulness of multiscale molecular modeling as an innovative and complementary tool in their current research.

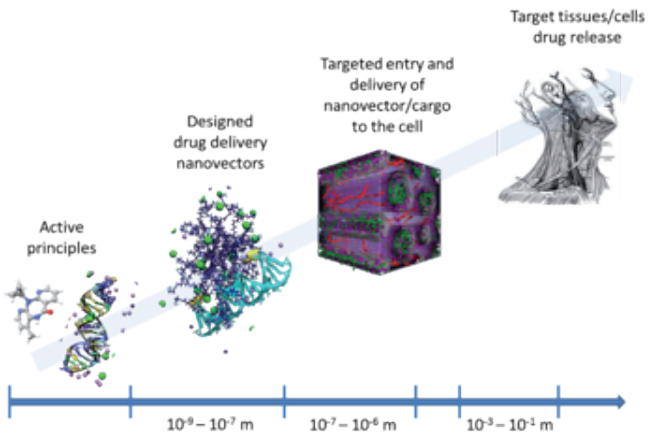

Figure 1 The (multi)length scale of a nanovector-assisted drug delivery process.

Given the space limitation, this work will summarize the story of how dendrimers and dendrons designed and synthesized by the jointed efforts of our international collaboration are exploited for gene delivery [1-4]. In particular, we will show and discuss how chemistry and physics are being used as natural tools to increase efficiency and probe structure-activity relationship in vitro. One example of nanovector development, from design to in vivo application, will also be presented.

Get flexible, get better!

We recently characterized the structural flexibility of the TEA-core PAMAM dendrimers by NMR experiments and computer modeling in details, and analyzed the corresponding DNA/dendrimer complexes using gel electrophoresis, transmission electron microscopy and dynamic light scattering techniques. Cell uptake of DNA/dendrimer nanoparticles via various endocytotic pathways was examined using live-cell confocal microscopy and specific inhibitors. Furthermore, the excellent DNA transfection efficiency of these nanovectors was confirmed both in vitro, by cell-based experiments, and most spectacularly, in vivo, by direct intrathymic injection in mice. The results which are reported here provide the first experimental evidence that the structurally flexible PAPAM dendrimers are effective nanocarriers for DNA delivery and gene transfer in mouse thymus. This may open new perspectives to immune gene therapy via intrathymic dendrimer-mediated DNA delivery (see Fig. 2).

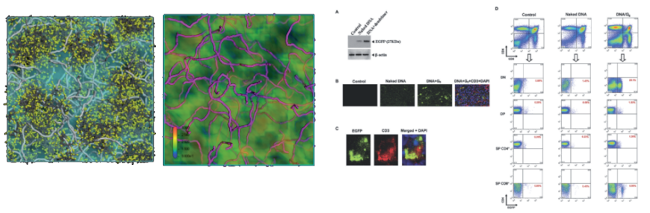

Figure 1. *(Left) Mesoscale morphologies of the assembled systems between TEA-core dendrimers G6 and DNA. The dendrimers are represented as dark grey and yellow sticks while the DNA is shown as light grey sticks. Water is portrayed as a light grey field. (Middle) DNA chains (purple) and water molecules distribution within the architectures of the TEA-core DNA/G6 nanoscopic assembly. In this case, water is represented as a colored density field: according to the scale reported in the lower left corners of the panels, low density values are blue, while high density values are red. (Right) In vivo EGFP expression in thymus was assessed by Western blot (A), immunofluorescence (B and C) and flow cytometry (D).*

Multivalency in action – less is more!

We performed multiscale modeling of spermine-functionalized dendrons designed to bind DNA and deliver it into cells, thus providing for the first time an insight into the mode of self-assembly of the dendrons, in particular aggregate charge density and shape, and hence suggests explanations for some of the unexpected experimental observations. Indeed, effective self-assembly of the first generation dendron leads to a high charge density assembled structure – more effective than the larger number of ligands on the second generation dendron – i.e., less is more (see Fig. 2). The modeling also suggests an alternative shape of self-assembly for the system with two hydrophobic cholesterol units (rather than one) at the dendron focal point – this may help explain why this system shows much enhanced gene delivery in vitro.

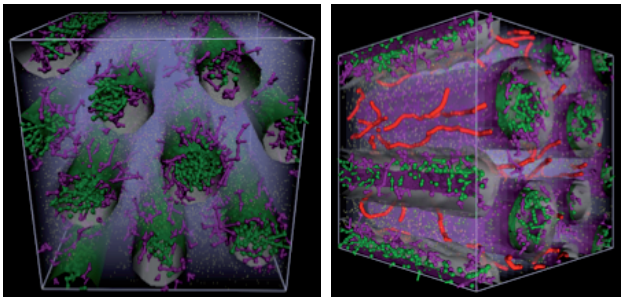

Figure 2. *Mesoscopic simulations of cholesterol-modified spermine-based G1 dendron cylindrical micelles alone (left) and in complex with DNA (left).*

What a difference a tail makes!

We recently developed a structure-activity relationship for a set of different dendritic vectors with DNA binding spermine surface groups. The easy synthetic tunability of these dendron structures can therefore be readily exploited to maximize their biological activity. Multiscale simulations demonstrated that these systems were better able to pack into tighter micellar aggregates (see Table 1), which have higher surface charge density and as such, show enhanced electrostatic interactions with DNA (see Fig. 3, left and middle panels). Furthermore, the differences in DNA binding affinity appeared to correlate with the transfection ability of the dendrons (see Fig. 3, right panel).

Table 1. *Values of the micellar diameter Dm (nm), aggregation number Nagg, and micelle surface charge density sm (e/nm2) for different amphiphilic dendrons as obtained from mesoscale simulations.*

Compounds	D_m	N_{agg}	s_m
Chol-G1-SP	3.4 ± 0.1	21	5.2
C_{12}Lys-G1-SP	4.0 ± 0.2	24	4.3
D2Gly-G1-SP	4.9 ± 0.2	32	3.8
C_{12}-G1-SP	4.0 ± 0.1	16	2.8
D1Gly-G1-SP	4.0 ± 0.2	12	2.1

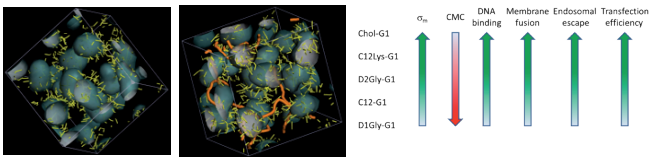

Figure 3. *Mesoscopic simulations of hydrophobically modified spermine-based G1 dendron spherical micelles alone (left) and in complex with DNA (middle). (right) Qualitative structure-activity relationship (SAR) of the main properties of the self-assembled dendron systems and their performance as gene delivery nanovectors. The green color denotes a positive effect while the red colour a negative one. The direction of the arrow head indicates an increase (upward direction) or a decrease (downward direction) of the relevant property/performance.*

REFERENCES

1) a) Bromfield SM, Posocco P, Fermeglia M, Pricl S, Rodríguez-López J, Smith DK. Chem Commun. 2013;49(42):4830-2; b) Welsh DJ, Posocco P, Pricl S, Smith DK. Org Biomol Chem. 2013;11(19):3177-86; c) Bromfield SM, Barnard A, Posocco P, Fermeglia M, Pricl S, Smith DK. J Am Chem Soc. 2013;135(8):2911-4; d) Barnard A, Posocco P, Pricl S, Calderon M, Haag R, Hwang ME, Shum VW, Pack DW, Smith DK. J Am Chem Soc. 2011;133(50):20288-300; e) Jones SP, Gabrielson NP, Wong CH, Chow HF, Pack DW, Posocco P, Fermeglia M, Pricl S, Smith DK. Mol Pharm. 2011;8(2):416-29; Posocco P, Pricl S, Jones SP, Barnard A, Smith DK. Chem Sci. 2010;1, 393-404 and references therein.

2) a) Liu X, Liu C, Laurini E, Posocco P, Pricl S, Qu F, Rocchi P, Peng L. Mol Pharm. 2012;9(3):470-81; b) Karatasos K, Posocco P, Laurini E, Pricl S. Macromol Biosci. 2012;12(2):225-40; c) Liu X, Wu J, Yammine M, Zhou J, Posocco P, Viel S, Liu C, Ziarelli F, Fermeglia M, Pricl S, Victorero G, Nguyen C, Erbacher P, Behr JP, Peng L. Bioconjug Chem. 2011;22(12):2461-73 and references therein.

3) Posocco P, Laurini E, Dal Col V, Marson D, Karatasos K, Fermeglia M, Pricl S. Curr Med Chem. 2012;19(29):5062-87 and references therein.

4) Posocco P, Gentilini C, Bidoggia S, Pace A, Franchi P, Lucarini M, Fermeglia M, Pricl S, Pasquato L. ACS Nano. 2012;6(8):7243-53.

VIRTUAL PHYSIOLOGICAL HUMAN: IN SILICO IMMUNOGENICITY PREDICTION FOR PATIENT STRATIFICATION

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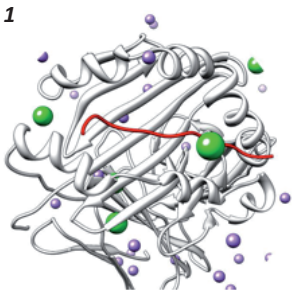
Immunogenicity is defined as the immune response (i.e., the production of specific antibodies) triggered by an exogenous (non-self) substance once it enters the human body. In the case of protein drugs (BioPharmaceutical Drugs or BPD), this phenomenon leads to the production of Anti-Drug Antibodies (ADA) that can compromise the efficacy of the drug itself. Even worse, it can lead to serious safety consequences because of possible occurrence of anaphylaxis, immune system disorders and complications derived from the interaction of the BPD with endogenous proteins.

It is now well established that all BPDs, once administered, generate an immunogenic response to some extent. As a matter of fact, one of the major issues of therapeutic proteins, in particular of engineered monoclonal antibodies (mAbs), stems from the substantial modifications that developers introduce in the protein with the intent to improve the performance of their candidate biomacromolecules. Such alterations are usually conceived with the objective of optimizing one or more properties of the protein (binding affinity, stability, effector functions, etc.). At the same time, however, they carry the risk of harmful side effects by producing molecules that are less human-like which in turn correlates with higher risk of immunogenicity.

Notwithstanding these inherent dangers, it is undeniable that BPDs will play a substantial role in the treatment of many fundamental human pathologies that cannot be treated with current/alternative therapies. In parallel with a significant increase of R&D efforts toward the development of new BPDs, however, it is apparent that the risk of unwanted immunogenicity constitutes one of the main hurdles for this type of drug to become a major clinical realty. Therefore an optimal, prediction-based management of immunogenicity risk is quickly becoming a key objective for the scientific community and the bio-pharmaceutical industry.

Aim of the present work is the development of computer-based models (EpiDyne technology) that are able to predict BPD-triggered immunogenic response using a combination of machine learning (ML) approaches together with structure-based molecular simulations (1-10).



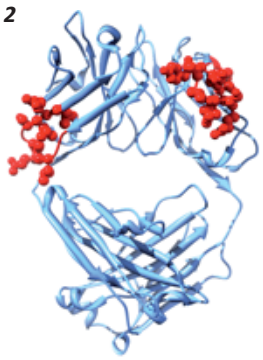


Figure 1. *Interaction of a MHCII protein with a BPD-derived peptide*

Figure 2. *Identification of hot-spots on the mAb by EpiDyne T-screen technology.*

Specifically, these methods are applied to the prediction of T-cell dependent (Td) immunogenicity. It is in fact recognized that one of the key events that triggers Td immunogenicity is the strength of T-cell epitope binding to MHC molecules (Fig. 1), where BPD-derived peptides with higher affinity to MHC class II proteins are more likely to be presented on the surface of APC (Antigen-Presenting-Cells).

To achieve maximum efficiency and optimal prediction power of our in silico methodology, we adopted a two-step approach: the first steps requires the use of ML-based methods for the quick and efficient screening of a BPD sequence that will identify the so called hot-spots, i.e., locations within the BPD at elevated Td immunogenicity risk (Fig. 2). The second step relies on a novel and proprietary structure-based, database-independent approach that is able to determine the binding of peptide-MHC molecules with very high accuracy.

The effectiveness of a computer-based approach strongly relies on the availability of accurate information about MHC alleles of a targeted patient (i.e. his/her HLA haplotype). It is in fact well know that the immune system repertoire is highly diverse from person to person, and this is the greatest limiting factor in developing efficient and generally applicable predictive solutions to BPD immunogenicity. This extent of diversity present in the human immune system is mainly achieved by the presence of an exceptional level of variability of the genes of the MHC. In the current research project, carried out in tandem with clinicians and biologists we aim at achieving a true personalized solution by applying state-of-the-art genomics technologies, like Next-Generation-Sequencing (NGS) to precisely determine the molecular makeup the MHC genes in a patient-specific fashion. The integration of computational models with genotype information obtained at patient level will ultimately be used to generate what we define Virtual Predictive Biomarkers (VPB) that are able to predict the response of (bio)therapeutic interventions.

Our EpiDyne computational methodology workflow is cheaper than in vitro and in vivo studies, is suitable for high throughput analysis in screening phases, and provides insights for the design of safer candidate drugs, new BPDs and the eventual reconsideration of the so-called fallen angels.

A prototypical EpiDyne technology workflow is shown in Figure 3. On any given mAb, the T-screen technology quickly identifies possible sequences at elevated Td immunogenicity risk. These sequences then are analyzed and scored for affinity and interactions with all alleles of MCHII in the T-mode phase. Lastly, the T-design technology leads to new, safe re-engineered mAb..

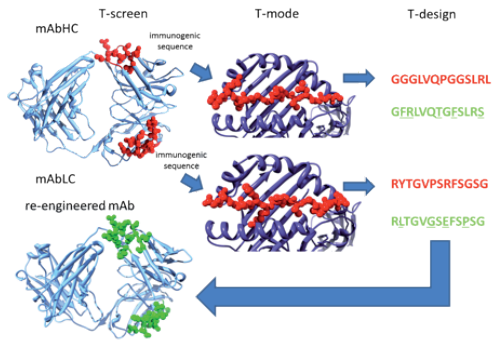


Figure 3. *EpyDyne computational technology workflow.*

Figures 4 and 5 illustrate just two examples out of the whole plethora of information afforded by our EpiDyne technology platform. In detail, Figure 4 shows an excerpt from the entire data set used to assess the prediction power of T-screen/T-design methodologies. Specifically, the graph presents the predicted affinity of a small subset 42 peptides for MHCII allele DRB1*01:01 vs. their experimental value: data correlation is 90% (note that the interval in experimental affinity is 1 nM £ IC50 £ 30000 nM). Figure 5 is a case of characterization of promiscuous T-cell epitopes.

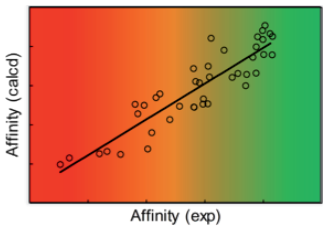


Figure 4. *Comparison between experimental and calculated affinity of a subset of 42 peptides towards MHCII allele DRB1*01:01. Data correlation is 90%.*

Figure 5 exemplifies the capacity of the proposed computational ansatz to predict the affinity of a given peptide (PKYVKQNTLKLAT) with respect to MHCII alleles RDB1*01:01 and RDB*04:01. In the case of the allele 01:01, the predicted IC50 is 3 nM (IC50,exp = 2-10 nM) while in the case of the alternative allele the estimated IC50 is 32 nM ((C50,exp = 28-45 nM). Note that the two alleles share 92.1% sequence identity and 95.4% sequence similarity.

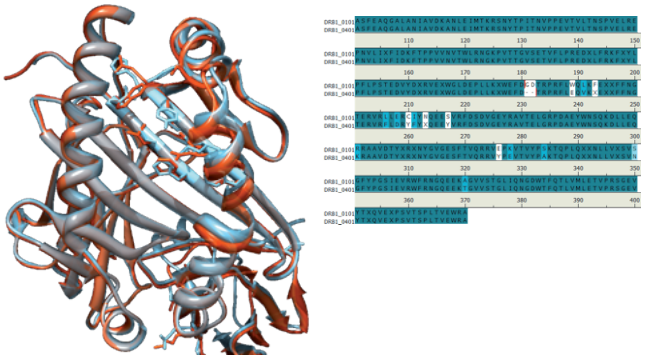


Figure 5. *(left) Superposition of MHCII RDB1*01:01 (orange) and RDB1*14:01 (sky blue) with the peptide sequence PKYVKQNTLKLAT.*

REFERENCES

1. See, for instance: a) Conca E, Miranda C, Col VD, Fumagalli E, Pelosi G, Mazzoni M, Fermeglia M, Laurini E, Pierotti MA, Pilotti S, Greco A, Pricl S, Tamborini E. Mol. Oncol. 2013, asap online http://dx.doi.org/10.1016/j.molonc.2013.02.019; b) Gibbons DL, Pricl S, Kantarjian H, Cortes J, Quintás-Cardama A. Cancer. 2012,118(2):293-9; c) Pierotti MA, Tamborini E, Negri T, Pricl S, Pilotti S. Nat Rev Clin Oncol. 2011,8(3):161-70; d) Dileo P, Pricl S, Tamborini E, Negri T, Stacchiotti S, Gronchi A, Posocco P, Laurini E, Coco P, Fumagalli E, Casali PG, Pilotti S. Int J Cancer. 2011,128(4):983-90; e) Pierotti MA, Negri T, Tamborini E, Perrone F, Pricl S, Pilotti S. Mol Oncol. 2010,4(1):19-37; f) Conca E, Negri T, Gronchi A, Fumagalli E, Tamborini E, Pavan GM, Fermeglia M, Pierotti MA, Pricl S, Pilotti S. Mol Cancer Ther. 2009,8(9):2491-5; g) Woodman SE, Trent JC, Stemke-Hale K, Lazar AJ, Pricl S, Pavan GM, Fermeglia M, Gopal YN, Yang D, Podoloff DA, Ivan D, Kim KB, Papadopoulos N, Hwu P, Mills GB, Davies MA. Mol Cancer Ther. 2009,8(8):2079-85; h) Negri T, Pavan GM, Virdis E, Greco A, Fermeglia M, Sandri M, Pricl S, Pierotti MA, Pilotti S, Tamborini E. J Natl Cancer Inst. 2009,4;101(3):194-204; i) McAuliffe JC, Wang WL, Pavan GM, Pricl S, Yang D, Chen SS, Lazar AJ, Pollock RE, Trent JC. Mol Oncol. 2008,2(2):161-3; j) Ferrone M, Perrone F, Tamborini E, Paneni MS, Fermeglia M, Suardi S, Pastore E, Delia D, Pierotti MA, Pricl S, Pilotti S. Mol Cancer Ther. 2006;5(6):1467-73; k) Tamborini E, Pricl S, Negri T, Lagonigro MS, Miselli F, Greco A, Gronchi A, Casali PG, Ferrone M, Fermeglia M, Carbone A, Pierotti MA, Pilotti S. Oncogene. 2006,25(45):6140-6, and references therein.
2. See also: a) Amadio M, Pascale A, Govoni S, Laurini E, Pricl S, Gaggeri R, Rossi D, Collina S. Chem Biol Drug Des. 2013, asap online, doi:10.1111/cbdd.12117; b) Laurini E, Da Col V, Wünsch B, Pricl S. Bioorg Med Chem Lett. 2013,23(10):2868-71; c) Rossi D, Marra A, Picconi P, Serra M, Catenacci L, Sorrenti M, Laurini E, Fermeglia M, Pricl S, Brambilla S, Almirante N, Peviani M, Curti D, Collina S. Bioorg Med Chem. 2013, May 1;21(9):2577-86; d) Bromfield SM, Barnard A, Posocco P, Fermeglia M, Pricl S, Smith DK. J Am Chem Soc. 2013,135(8):2911-4; e) Meyer C, Schepmann D, Yanagisawa S, Yamaguchi J, Dal Col V, Laurini E, Itami K, Pricl S, Wünsch B. J Med Chem. 2012;5(18):8047-65; f) Laurini E, Marson D, Dal Col V, Fermeglia M, Mamolo MG, Zampieri D, Vio L, Pricl S. Mol Pharm. 2012,9(11):3107-26, and references therein.

STABILITY EVALUATION OF SELF ASSEMBLED PEPTIDE NANOPARTICLES IN PHYSIOLOGICAL FLUIDS

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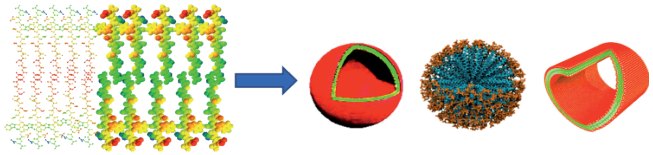
Amphiphilic peptides (Aps) have gained increasing attention as versatile molecules to generate diverse supramolecular structures, such as hydrogels, nanofibres, micelles and vesicles. Amphiphilic peptides that self-assemble to generate structures at the nanoscale may find application as drug delivery vehicles. SA2 (Ac-AAVVLLLWEE) is such an amphiphilic peptide developed in our group(1) that assembles into vesicles of approx. 100 nm in size with a net negative surface charge. We have recently shown proof-of-concept that these SA2 peptide vesicles can be used to deliver hydrophobic drugs into cells in culture. However stability in vivo is an important criterion for drug delivery. Peptide vesicles may be destabilized upon dilution in the blood compartment or by interaction with components in the blood.



The aim of this study was to measure SA2 peptide vesicle stability and concentration in serum and whole blood by using Nano Tracking Analysis (NTA).NTA detects and visualizes populations of nanoparticles in liquids down to 30nm, dependent on material, and measures the size of each particle from direct observations of diffusion. This particle-by-particle methodology goes beyond traditional light scattering and other ensemble techniques in providing high-resolution particle size distributions. Additionally, by using fluorescent labels, distinct populations of nanoparticles in a mixture of other nanoparticles can be discerned and sized. To monitor stability of SA2 vesicles in serum, SA2 peptides were fluorescently labeled with amino- modified Rhodamine 6G using EDC/NHS coupling chemistry. Particle stability and concentration was measured in PBS and serum. The results demonstrate that SA2 vesicles can be detected even in the presence of full serum, containing high concentrations of biological nanoparticles. More importantly, SA2 vesicles remain stable for at least 12hrs in the presence of serum.

REFERENCES

1. van Hell AJ, Costa CI, Flesch FM, Sutter M, Jiskoot W, Crommelin DJ, et al. Self-assembly of recombinant amphiphilic oligopeptides into vesicles. Biomacromolecules. 2007 Sep;8(9):2753-61.

FORMULATION AND CHARACTERIZATION OF SUNITINIB SYNBIOSYS MICROSPHERES AS A PLATFORM FOR INTRATUMORAL DELIVERY OF KINASE INHIBITORS

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One of the main obstacles in treating brain tumors is the blood brain barrier, a protective barrier which prevents drugs from entering the brain (1). There is an urgent need for delivery platforms which can localize the activity of antitumor agents in tumors. Moreover, local sustained treatment can eliminate the need of daily administration and prolong treatment efficiency. In this study we therefore designed polymeric particles for intratumoral delivery of kinase inhibitors. Amongst the family of biodegradable polymers, amphiphilic block copolymers hold a great promise for sustained delivery of drugs (2).
In the current investigation we utilized a class of multi-block copolymers, SynBiosys (Fig 1), consisting of L-lactide, DL-lactide, and polyethylene glycol (PEG) with different molecular compositions. Sunitinib (Fig 2) was successfully encapsulated into SynBiosys polymeric microspheres by an oil/water emulsification solvent evaporation method. High encapsulation efficiency was obtained when the pH increased during the solidification time of the particles, favoring the partitioning of the drug into the hydrophobic phase. The drug release profile was tailored using SynBiosys co-polymers with different percentage of soft and hard blocks, which affect properties such as swelling and degradation rate of the particles. It was found that particles prepared with a relatively high percentage of hydrophilic block rapidly released the drug, while sustained release over 7 months was achieved with SynBiosys containing 10% of the soft hydrophilic block. Further studies will address the therapeutic benefit of sunitinib microspheres as drug depot in the treatment of cancer.

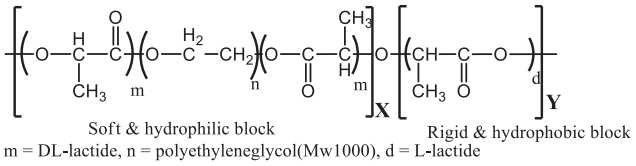

Fig. 1. *SynBiosys polymer with different ratios of soft blocks (X) and rigid blocks (Y).*
Polymer A (10%X, 90%Y), polymer B (20%X, 80%Y) and polymer C (30%X, 70%Y).

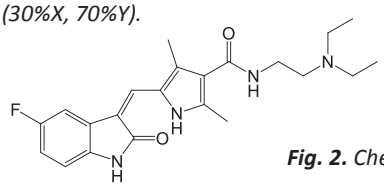

Fig. 2. *Chemical structure of sunitinib.*

REFERENCES

(1) Ohka F, Natsume A, Wakabayashi T. Current trends in targeted therapies for glioblastoma multiforme. Neurol Res Int 2012: 878425.
(2) Gillissen M, Steendam R, van der Laan A, Tijsma E. Development of doxycycline-eluting delivery systems based on SynBiosys™ biodegradable multi-block copolymers. J Controlled Release 2006;116(2):e90-e92.

TARGETED FUNCTIONAL GENOMICS SCREEN FOR METASTASIS AND EMT MODULATORS IN BREAST CANCER

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Metastatic spread still represents the major cause of death among women diagnosed with breast cancer, so that suppression of metastasis formation or causing selective lethality to metastatic breast cancer cells would represent a desirable goal. siRNAs can selectively address both corresponding target genes and miRNAs, thus represent a favorable option for the next generation of anticancer nanodrugs. The knowledge of targets to be addressed by this approach, however, is still limited.
To recover novel starting points for therapeutic approaches, we selected a set of 100 candidate genes and miRNAs from available molecular profiles of metastatic breast cancer and subjected these to systematic functional studies regarding their impact on breast cancer growth and invasion as well as their ability to cause the so-called epithelial-mesenchymal transition (EMT). According to current concepts EMT is tightly linked to both metastatic potential and the breast cancer stem cell (BCSC) phenotype. For this purpose, we utilize a unique system, which allows for the rapid creation of a large number of stable and standardized cancer cell lines. Out of the 100 analyzed candidates, we identified seven novel breast cancer growth modulators and four novel invasion modulators. Remarkably, the data set suggests that EMT is neither sufficient nor necessary for increasing the invasive properties of MCF-7 breast cancer cells in vitro. Downstream analyses of one novel growth and invasion suppressor showed that this candidate surprisingly is able to directly induce BCSCs. Moreover, its expression induces a hypoxia-like profile, which is commonly also associated with the emergence of BCSCs. However, in vivo analyses confirmed that the gene functions as tumor suppressor. To our knowledge, this represents the first example for a tumor-suppressive effect of a breast cancer stem cell inducer.

SUPPORT TOOLS AND ACTIVITIES FOR PROACTIVELY HANDLING SOCIO-ECONOMIC AND ETHICAL ASPECTS IN EMERGING R&D PROJECTS

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There is increasing pressure on both researchers and product developers to consider the socio-economic impacts and ethical aspects of their activities. For public funded research, this has translated into requirement of funding programmes to include this in their research activities, for firms it has translated into pressures to explore societal impacts and include this intelligence in the design process itself.
In parallel to this pressure, there is a move by research scientists and firms to proactively handle socio-economic and ethical aspects by engaging in debates, developing elements of corporate social responsibility, interacting with social science scholars to make sense of debates and tensions in and around new areas of science, technology and innovation.
Amidst this pressure to move and willingness to act, there is a need for tools and approaches that are robust and useable by researcher scientists and product developers. This is particularly so for emerging fields of technology and innovation, where there is high uncer-

tainty and considerable (and often uncontrolled) speculation on potential benefits and risks.
This poster presents a structured look at support tools and approaches for handling socio-economic and ethical aspects of new technologies. It pays particular attention to the theme of this meeting, targeted delivery and nanomedicine.
It also gives an indication of a number of tools and approaches that are part of the activities of TEQNODE Limited, a Paris-based company with an expertise in robust and usable tools for application in the world of research and innovation.

TEQNODE provides strategic intelligence and future-oriented analysis on emerging science and technology fields through contract research and consultancy. With a focus on early stage and emerging fields, where high uncertainty reigns and expectations about the promising technology are abundant, TEQNODE combines innovation models to inform analysis and future-oriented assessments of potential innovations to characterise opportunities, challenges and projections of emerging technologies and their embedment in markets and society.

TEQNODE also supports researchers and firms in new and emerging fields of technology and innovation with societal aspects, ethics, governance and Product development aspects, effectively the elements that allow the effective translation of research into applications in society.

A RESEARCH PROFILE FOR A PROMISING EMERGING INDUSTRY– NANO-ENABLED DRUG DELIVERY

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With the global emphasis on the development of nanotechnology ("nano"), Nano-Enabled Drug Delivery ("NEDD") systems are rapidly emerging as a key nano application area. NEDD offers promise in addressing pharmaceutical industry challenges concerning solubility, cost-reduction, cell and sub-cellular targeting, and patent lifecycle extension. A combination of factors promotes nanoparticle-enhanced and other nano-facilitated drug and gene delivery systems.
To get a grip of this vast, varied and highly promising area of nano application, we have devised a multi-component search strategy, detailed here, to generate an NEDD dataset from the Web of Science (WOS). We then analyze these scientific and biomedical research publication records to profile R&D activity, gauge evolving research foci, and forecast NEDD research trends. Such "Tech Mining" can position one to address a wide array of further technology management questions concerning this important biomedical arena. Our aim is to build a family of analytical tools to facilitate the forecasting of innovation pathways.
The variety of publication and patent databases available today provide a reservoir of information on research activities which can be tapped. The challenge is smart extraction of what is relevant, both for the players in the field and for those wishing to make policy decisions.
In this project, we have been exploring and developing a process to allow for smart extraction of information from these databases in a logical way, so that the route from database to final analysis is both transparent and has a clear logic. Such technical intelligence can inform the private sector and public agency decision making reagrding investment in fundamental R&D, on which topic and allows identification of potential collaborators or competitors.

This research draws on support from the National Science Foundation (NSF) Science of Science Policy Program – "Revealing Innovation Pathways" (Award No. 1064146) to Georgia Tech, and also NSF support through the Center for Nanotechnology in Society (Arizona State University; Award No. 0531194). The findings and observations contained in this paper are those of the authors and do not necessarily reflect the views of the National Science Foundation.

EXPERIMENTAL EVALUATION OF COMPUTATIONALLY DESIGNED PEPTIDES WITH HIGH AFFINITY FOR A TARGET PROTEIN

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Proteins are involved in every biological process, and they represent both major pharmacological targets and biomarkers1. Therefore a key step for the development of selective drugs and new probes for the development of new diagnostic tools is the identification of novel ligands for protein detection.
So far the antibodies are the protein probes with highest affinity and selectivity, but their production and optimization are both long and expensive, requiring both in vivo (lab animal immunization) and in vitro procedures (cell line culture and monoclonal screening)2. Here we propose short-peptides as alternative probes, which represent suitable small molecules for in silico design, and exhibit other advantages such as automatable and cost-effective synthesis.
A set of decapeptides have been computationally designed to bind with high affinity the ligand site of a model protein: the maltose binding protein (MBP) which is the prototype for the study of protein-ligand recognition3. The algorithm is based on a combination of molecular dynamics, semi-flexible docking using Autodock Vina4, and replica exchange Monte Carlo4.
To evaluate the binding affinity of the optimized peptides high-throughput format fluorescence emission assays were performed by recording the emission fluorescence intensity (480/520) of each fluorescein-labeled peptides upon addition of increasing amount of protein. It has been found that the emission fluorescence intensity of the dye-labeled peptides increases upon binding to MBP in a dose-dependent manner (figure 1), and the determined dissociation constants were in a low nanomolar range, as predicted from the theoretical values of binding energy.
This is the first step towards the design of a bidendate binder (figure 2) as a new selective drugs prototype consisting of two peptidic binding motifs for two different sites of a target protein and a flexible linker. With this design we expect enhanced selectivity and affinity with respect to a single binder alone.

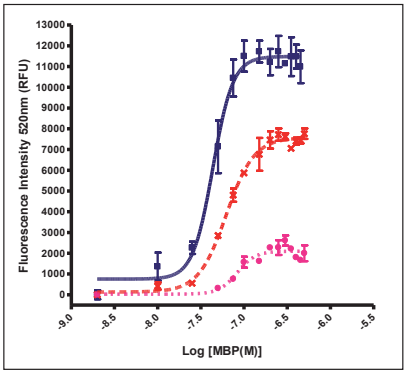

Figure 1. *Binding curve of the peptides 1 (blu solid), 2 (red dashed) and 3 (pink dotted). The fluorescence emission intensity at 520nm was plotted against increasing concentration of protein. The curve were corrected for blank (fluorescence emission of peptide).*

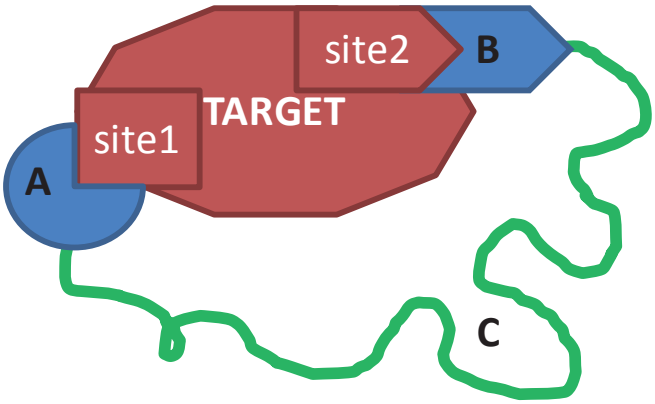

Figure 2. *A schematic representation of bidendate binder. Target: protein. Site 1: ligand site. A, B: two peptidic binding motifs. C: flexible linker.*

- (a) Balgkouranidou, I. et al, Biomarkers in Medicine 2013, 7 (1), 49-58; (b) Henry, M. S. et al. Int. J. Geriatr. Psychiatr. 2013, 28 (4), 331-340.
- Harlow, E. and Lane, D., Antibodies: A Laboratory Manual (CSHL Press, NY, 1988)
- Quiocho, F. A.; Ledvina, P. S., Molecular Microbiology 1996, 20 (1), 17-25.
- Enriquez, R. P. H. et al.; Journal of Chemical Theory and Computation, 2012, 8 (3), 1121-1128.

ANTIBACTERIAL PROPERTIES OF QUATERNARY N-ALKYL CHITOSAN DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND STRUCTURE-ACTIVITY RELATIONSHIP

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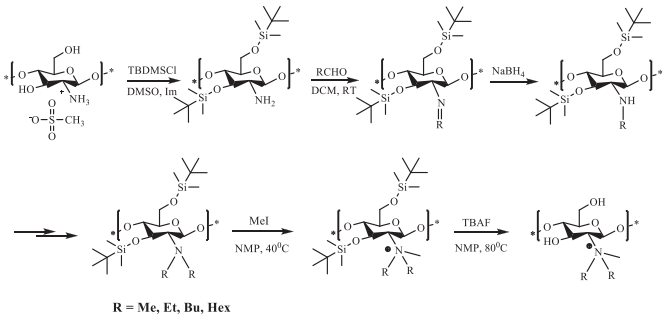
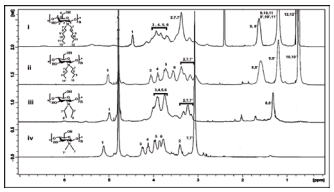
Quaternary N-alkyl chitosan derivatives with varying chain lengths were synthesized with the aid of TBDMS protected chitosan to obtain highly quaternised products having high antibacterial activity. A highly efficient method for regioselective modification of chitosan biopolymer using a simple reductive amination procedure to yield N,N-dialkyl chitosan derivatives was developed. Four different dialkyl derivatives namely di-methyl, di-ethyl, di-butyl and di-hexyl were synthesized using the 3,6-O-di-TBDMS chitosan as a precursor. The reaction involves treatment of the protected chitosan with the aldehyde forming the imine followed by reduction to get the mono alkyl derivative, which was then subjected to similar treatment once again to have the dialkylated product. The use of the TBDMS protected chitosan enabled the reaction to be performed in organic solvent simplifying the method and resulting in 100% substitution.



Scheme 1: *Synthetic route for preparation of N,N,-dialkyl-N-methyl chitosan derivatives. Reagents and conditions : (i) methanesulfonic acid, water, 10°C; (ii) TBDMS chloride, imidazole, DMSO, N2, room temperature; (iii) aldehyde, NaBH4, DCM, room temperature, (iv) TBAF (1 M), NMP, 50 °C. (v) MeI, NMP, 40°C. Note : Chitosan has 5% N-acetylation that has been omitted for clarity.*

Dialkylation reaction in absence of the protecting groups i,e. on native chitosan (unmodified chitosan) was performed under acidic conditions which resulted in lower degree of substitution. N-methylation and quaternisation of the dialkyl chitosan derivatives was attempted under different reagents and conditions. With the protected dialkyl compound only a very low degree of quaternisation was obtained due to the steric hindrance caused by the highly bulky tertiarybutyldimethylsilyl groups. However, a high degree of quaternisation of these derivatives could be achieved by using MeI as reagent and NMP as solvent after the removal of the protecting groups. The quaternisation reaction yielded compounds which carried permanent positive charges on the polymer backbone and showed good aqueous solubility, making them suitable for antimicrobial testing. The degree of substitution calculated from the integrals of 1H-NMR spectrum was found to be 1 for N-alkylation and ~ 0.75 for quaternisation. All the derivatives were well characterized using 1H-NMR, IR and COSY spectrum.



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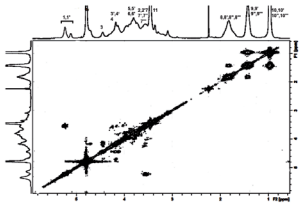

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Fig 1*: 1H-NMR spectra of (i) N,N-dihexyl chitosan, (ii) N,N-diethyl chitosan, (iii) N,N-dibutyl* ***Fig 2****: 1H-1H-COSY spectra of N,N-dibutyl-N-methyl chitosan. chitosan and (iv) N,N-dihexyl chitosan.*

These well-defined derivatives were then investigated for antibacterial activity against four different bacterial species, gram-positive S.aureus, E.faecalis and gram-negative E.coli and P. aeruginosa. TMC , N-ethyl-N,N-dimethyl and N-butyl-N,N-dimethyl derivatives showed high activity against S.aureus, while increasing the hydrophobicity further resulted in a decrease in activity. N,N-diethyl chitosan showed the highest activity against S.aureus with an MIC of 4 µg/ml, which is comparable to the activity of the antimicrobial peptide Melitin and much higher than than that of human Cathelicidin LL-37. Against the other gram-positive bacteria, E.faecalis the activity was seen to increase as the length of the mono-alkyl chain increased from methyl to hexyl. The N-hexyl derivative showed the highest activity of 16 µg/ml, which is only one dilution less than the MIC value of Melitin, but lower than the MIC of benzalkonium chloride and cetyl pyridinium chloride (≤0.5). However as the hydrophobicity further increased with the di-alkyl groups the derivatives became less active. A similar trend in activity with mono-alkyl and di-alkyl compounds was observed when treated with gram-negative E.coli. Increase in chain length was proportional to the activity in both the series of compounds. The enhancement in activity with hydrophobicity could be explained by the difference in the cell wall composition of gram-negative bacteria as compared to that of gram-positive bacteria. N-hexyl and N,N-dihexyl derivatives showed the highest inhibition with an MIC value of 16 µg/ml. This value is similar to that of benzalkonium chloride, cetylpyridinium chloride as well as Melitin. In general the all alkyl chitosan derivatives showed the lowest activity against the gram negative bacteria P. aeruginosa.

Table: *MIC and MLC values of N-alkyl-N,N-dimethyl, N,N-dialkyl-N-methyl chitosan derivatives, along with benzalkonium chloride, cetyl pyridinium chloride, Melitin and LL-37 against four bacterial species.*

SL. NO.	COMPOUND	*S.aureus ATCC 29213*		*E.faecalis ATCC 29 212*		*E.coli ATCC 25 922*		*P.aeruginosa ATCC 27 853*	
		MIC (µg/ml)	MLC (µg/ml)	MIC (µg/ml)	MLC (µg/ml)	MIC (µg/ml)	MLC (µg/ml)	MIC (µg/ml)	MLC (µg/ml)
1.	N,N,N-trimethylamine	32	64	128	1024	64	128	512	512
2.	N-ethyl-N,N-dimethyl chitosan	4	16	128	128	64	64	512	512
3.	N-butyl-N,N-dimethyl chitosan	32	64	64	64	32	32	512	512
4.	N-hexyl-N,N-dimethyl chitosan	256	256	16	32	16	16	512	512
5.	N-methyl-N,N-diethyl chitosan	256	1024	512	1024	128	128	1024	2048
6.	N-methyl-N,N-dibutyl chitosan(R-2)	512	2048	128	128	32	32	512	512
7.	N-methyl-N,N-dihexyl chitosan	1024	2048	256	256	16	16	1024	2048
8.	Benzalkonium chloride	≤0.5	1	≤0.5	4	16	16	64	64
9.	Cetyl pyridinium chloride	≤0.5	2	≤0.5	2	16	16	256	256

In general, the quaternary derivatives showed increasing activity with increasing chain length from methyl to ethyl against S.aureus. While, the hexyl spacer showed highest activity against E.faecalis and E.coli. Against P.aeruginosa, the activity was similar for all compounds. Thus we can conclude that the effect of chain length on antibacterial activity of chitosan derivatives is dependent on the nature of the bacterial species.

REFERENCES

- Avadi, M.R.; Sadeghi, A.M.M.; Tahzibi, A.; Bayati, Kh.; Pouladzadeh, M.; Zohuriaan-Mehr, M.J.; Rafiee-Tehrani,M. Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects Eur Polym J 2004, 40, 1355-1361.
- Kim, C.H.; Choi, K. S. Synthesis and Antibacterial Activity of Quaternized Chitosan Derivatives Having Different Methylene Spacers J Ind Eng Chem, 8, 1,71-76.
- Benediktsdóttir, B.E.; Gaware, V. S.; Rúnarsson, Ö. V.; Jónsdóttir, S.; Jensen, K. J.; Másson, M. Synthesis of N,N,N-trimethy chitosan homopolymer and highly substituted N-alkyl-N,N-dimethyl chitosan derivatives with the aid of di-tert-butyldimethylsilyl chitosan Carbohyd Polym 2011, 86, 1451-1460.

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF GUANIDYLATED CHITOSAN DERIVATIVES

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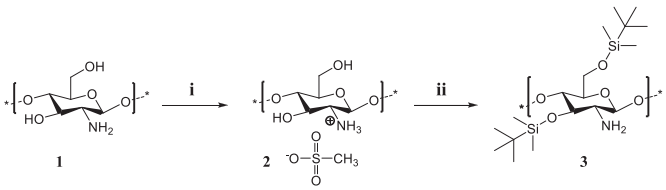
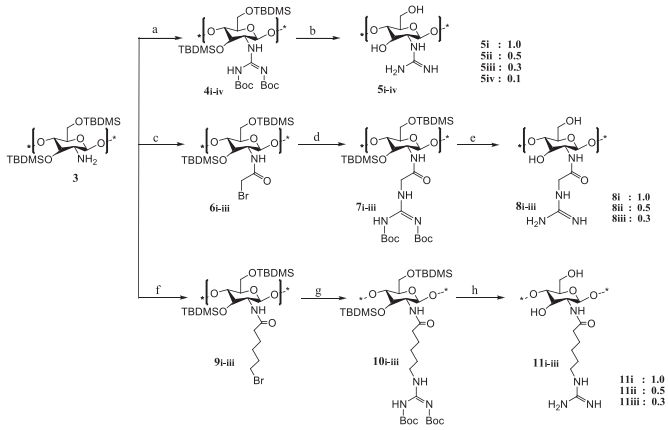
Guanidine derivatives have been known to have good antimicrobial activity and with this objective guanidylated chitosan derivatives were synthesized with the aid of TBDMS and Boc protection groups. Fully substituted guanidine derivatives could be obtained by this method in contrast to the previously reported acidic conditions which gave low substitution degree. Guanidylated derivatives of chitosan with varying degrees of substitution and different spacer lengths were synthesized with the aid of 3,6-di-O-TBDMS-protected chitosan. The guanidyl group was attached to the polymer backbone either directly or through acetyl and hexanoyl spacer. Boc-protected guanidine reagents were reacted with TBDMS-protected chitosan or chitosan derivatives under homogeneous conditions to give highly selective N- substituted product. The use of both the protection groups enabled the reaction to be performed in common organic solvent like DCM which gave a good control on the degree of substitution of the reaction. Another advantage was that both the protecting groups could be easily removed from the polymer in a single step using mild acidic conditions.

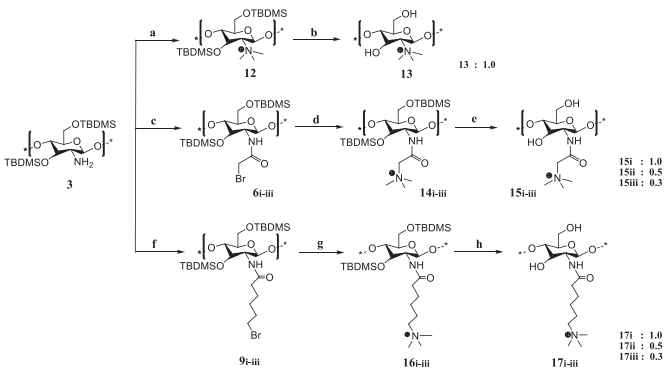


Scheme 1: *Synthetic route for preparation of 3,6-O-di-TBDMS-chitosan. Reagents and conditions : (i) methanesulfonic acid, water, 10°C; (ii) TBDMS chloride, imidazole, DMSO, N2, room temperature.*



Scheme 2: *Synthetic route for preparation of N-guanidine, N-acetyl guanidine and N-hexanoyl guanidine derivatives. Reagents and conditions : (a) N,N'-di-boc-N''-triflylguanidine, TEA, DCM, room temperature; (b) HCl / MeOH, room temperature; (c) Bromo-acetylbromide, TEA, DCM, -20°C; (d) 1,3-di-boc-guanidine, NaH, DMF, room temperature; (e) HCl / MeOH, room temperature; (f) Bromohexanoylchloride, TEA, DCM, -20°C; (g) 1,3-di-boc-guanidine, NaH, DMF, room temperature; (h) HCl / MeOH, room temperature.*

Trimethyl amine derivatives of chitosan with varying spacer length and degree of substitution were also synthesized. Fully substituted N,N,N-trimethyl chitosan as well as trimethylamine substitution having acetyl and hexanoyl spacers were synthesized according to previously developed procedures. However different degrees of substitution have been obtained in this study by using different ratios of the reagents. These derivatives were used as reference compounds during antibacterial tests. All the derivatives synthesized have been well characterized by 1H-NMR, IR and COSY spectra.



Scheme 3: *Synthetic route for preparation of N,N,N-trimethylamine, N-acetyl-N,N,N-trimethylamine and N-hexanoyl-N,N,N-trimethylamine chitosan derivatives. Reagents and conditions : (a) Cesium carbonate, MeI, NMP, 50°C; (b)1M TBAF-NMP, 50°C; (c) Bromo-acetylbromide, TEA, DCM, -20°C; (d) Trimethylamine, DCM, room temperature; (e) HCl / MeOH, room temperature; (f) Bromohexanoylchloride, TEA, DCM, -20°C; (g) Trimethylamine, DCM, room temperature; (h) HCl/MeOH, room temperature.*

The chitosan derivatives were investigated for antibacterial activity against gram-positive Staphylococcus aureus (S.aureus) ATCC 29213 and gram-negative Escherichia coli (E.coli) ATCC 25922. The N-guanidyl chitosan showed high activity against S.aureus with an MIC value of 32 µg/ml, while N-acetylguanidyl and N-hexanoyl guanidyl chitosan derivatives showed a decrease in activity with increasing spacer length. As seen in figure (A), the increase in activity is also seen to be proportional to the degree of substitution. Fully substituted N-guanidyl chitosan had the highest activity (MIC of 32 µg/ml) while DS of 0.1 was the least active with and MIC value of 8192 µg/ml against S.aureus. The hexanoyl guanidyl chitosan with lower degrees of substitution were inactive within the measured concentration range. Figure (C) shows a similar trend in activity with variation in spacer length and degree of substitution of the compounds when treated with gram-negative E.coli. The compounds in general exhibited higher MIC values i.e, they showed less inhibition towards E.coli as compared to S.aureus. The N-guanidyl chitosan showed the highest activity of 512 µg/ml, while the activity decreased when the length of the spacer between the guanidine group and the polymer backbone increased. All the N-hexanoyl derivatives showed no activity against E.coli. While the N-acetyl derivatives had intermediate activity.

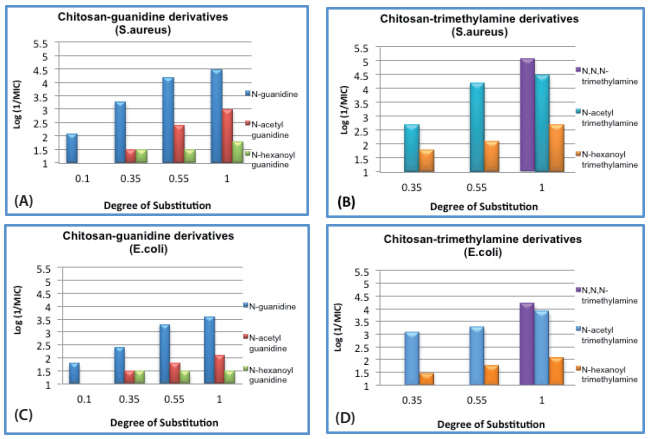


Fig: *Showing antibacterial activity of guanidine and trimethyl amine functionalized chitosan derivatives against S.aureus and E.coli.*

The trimethyl amine derivatives of chitosan were also tested against both the species of bacteria for comparison. In figure (B), we can see that N,N,N-trimethyl chitosan has the highest activity with an MIC value of 8 µg/ml while acetyl and hexanoyl derivatives have MIC of 16 µg/ml and 2048 µg/ml respectively for the highest degree of substitution. The antibacterial property also declines with decreasing degree of substitution. Like the guanidyl derivatives the trimethylamine derivatives also displayed higher activity against S.aureus as compared to E.coli.

Thus, in general we can conclude that all the derivatives showed higher activity against S.aureus. Their activity was proportional to the degree of substitution but inversely related to the distance of functional group from the polymer backbone. Also, the trimethylamine group was more active than the guanidyl group.

REFERENCES

- Hu, Y.; Du, Y.; Yang, J.; Kennedy, J. F.; Wang, X.; Wang, L. Synthesis, characterization and antibacterial activity of guanidylated chitosan Carbohyd Polym 2007, 67, 66-72.
- Zhai, X.; Sun, P.; Luo, Y.; Ma, C.; Xu, J.; Liu, W. Guanidylation: A Simple Way to Fabricate Cell Penetrating Analogue-Modified Chitosan Vector for Enhanced Gene Delivery J Appl Polym Sci, 2011, 121, 3569-3578.
- Tang, H.; Zhang, P.; Kieft, T. L.; Ryan, S. J.; Baker, S. M.; Wiesmann, W. P.; Rogelji, S. Antibacterial action of a novel functionalized chitosan-arginine against gram-negative bacteria Acta Biomater, 2010, 6 (7), 2562-2571.

TUMOR-HOMING PEPTIDE BOOSTS POROUS SILICON NANOVECTORS ACCUMULATION IN VIVO

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INTRODUCTION

Nanoparticulate delivery systems are designed to target anticancer drugs to tumors passively and/or actively.[1] These systems have been shown to increase the efficacy of anticancer drugs and reduce the side effects of chemotherapy.[2-4]

Porous silicon (PSi) particles are promising drug delivery vehicles due to their unique properties, such as high loading degree for different drug molecules and easy tuning of the particles' surface properties and pore size of the particles. These features enhance the drug dissolution behavior and/or allow the control of the drug release.[5-11]

The targeting moiety used in this study is a 9-amino acid long peptide (herein designated as CooP) that has been shown to home specifically to the intracranial murine brain tumor models of human glioma.[12]

Here, we aimed to prepare, characterize and test a PSi nanovector platform composed of THCPSi NPs functionalized with a tumor-homing peptide, CooP, and to evaluate their specificity to target the THCPSi NPs to MDGI-expressing MDA-MB-231 breast carcinoma cells in vitro and tumor xenografts in vivo. Flow cytometry and confocal microscopy were used to quantify the targeting efficiency and the uptake of the THCPSi-CooP NPs into MDGI-expressing MDA-MB-231 cells. Subsequently, we established subcutaneous MDGI-expressing tumors in nude mice and analyzed the biodistribution of the THCPSi-CooP NPs after intravenous injections. In addition, the homing efficiency and specificity of THCPSi-CooP into tumor site was evaluated. To our knowledge this is the first demonstration of the PSi-based materials biofunctionalized with a tumor-homing peptide to specifically target tumors in vivo.

EXPERIMENTAL SECTION

Preparation of THCPSi NPs: THCPSi NPs were prepared by the electrochemical anodization method as described elsewhere.[15] THCPSi NPs were labelled with tetramethylrhodamine-5-isothiocyanate (TRITC).

Preparation of THCPSi-CooP NPs: The particles were incubated in the peptide solution for 1-2 h under magnetic stirring at room temperature.

Physicochemical characterization of the THCPSi NPs: The structural properties (pore volume, specific surface area and pore diameter) of the THCPSi NPs were characterized by N2 sorption method and Attenuated Total Reflectance-Fourier transform infrared spectrometer (ATR-FTIR).

Stability of THCPSi-CooP NPs: The stability of the THCPSi NPs functionalized with CooP was studied in buffer (pH 7.4) at 37 ± 1 °C for 24 h and in human plasma after incubation at 37 ± 1 °C for 2 h.

Expression vectors, transfection and generation of stable MDGI-expressing MDA-MB-231 breast cell line: A stable MDA-MB-231 human breast carcinoma cells expressing MDGI as a c-Myc fusion protein (MDGI-c) were obtained by transfection with the pcDNA3-9E10 plasmid encoding the full-length MDGI protein.

Cell viability assay: MDA-MB-231 breast cancer cells were seeded in 96-well plates followed by addition of 50 µL of THCPSi and THCPSi-CooP NP suspensions (25, 50, and 100 µg mL–1). After 3 and

24 h incubations, the number of viable cells was assessed using the CellTiter-Glo™ reagent assay (Promega® Corporation, USA) according to the manufacturer's instructions.

Confocal microscopy: MDGI-GFP-expressing MDA-MB-231 cells were seeded in Lab-Tek™ 8-chamber glass slides and 200 µL of TRITC-labeled THCPSi and THCPSi-CooP NP suspensions (125 µg/mL) were added to the wells. After 3 h incubation, the particle containing suspensions were removed, fixed and analysed with Leica TCS SP2 AOBS confocal microscope.

Flow cytometry: Parental MDA-MB-231 and MDGI-expressing MDA-MB-231 cells were seeded in 12-well plates followed by the addition of 500 µL of THCPSi and THCPSi-CooP NP suspensions (75 and 125 µg/mL). After 3 h incubation, the cells were and analysed by flow cytometer (BD Biosciences, USA).

In vivo targeting studies: To establish a subcutaneous tumor xenograft derived from the MDA-MB-231 breast cancer cells expressing MDGI-c, athymic female Balb/c nu-nu mice were injected with 2 106 cells in PBS on the abdominal side of the mice. 50-days post-injections, after the tumors reached a volume of ~200–600 mm3, 150 µL of TRITC-labeled THCPSi and THCPSi-CooP NP suspensions (700 µg/mL) were injected into the tail vein of the tumor-bearing mice. Tumors and organs were collected, fixed and the tissue sections of tumors and organs were analyzed using an Axioplan epifluorescence microscope (Carl Zeiss AG, Germany).

RESULTS AND DISCUSSION

Characterization of THCPSi and THCPSi-CooP NPs: The specific surface area and the total pore volume for the THCPSi NPs were of 210 m2/g and 0.56 cm3/g, respectively. The average pore diameter of the THCPSI NPs was 10.7 nm and the Z-average particle size and ζ-potential obtained were 280 ± 18 nm and –35.9 ± 3.0 mV, respectively. After biofunctionalization with the CooP peptide, the Z-average particle size and ζ-potential values obtained were 181 ± 14 nm and –21.4 ± 1.2 mV, respectively.

Stability of THCPSi-CooP in buffer and human plasma: CooP biofunctionalization on the surface of THCPSi was stable for at least 24 h, and after several days no major detachment of CooP from the surface of the NPs was observed (data not shown).

Effect of NPs on viability of MDA-MB-231 cells in vitro: First, we studied the viability of the parental MDA-MB-231 cell line in the presence of THCPSi and THCPSi-CooP NPs. Either the THCPSi or THCPSi -CooP NPs did not induce toxicity to the MDA-MB-231 cells (results not shown).

Uptake of THCPSi-CooP NPs by the MDA-MB-231 cells: Confocal microscopy studies were performed with the THCPSi NPs labeled with TRITC and incubated with MDGI-GFP expressing MDA-MB-231 cells for 3 h. Confocal microscopy showed higher association of the THCPSi-CooP NPs with the MDGI-GFP expressing cells compared to the bare THCPSi NPs (Figure 1).

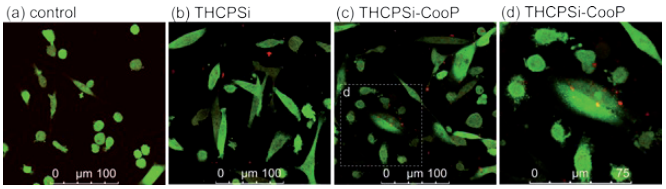

Figure 1. *(a) Confocal microscopy images of the MDGI-GFP-expressing MDA-MB-231 cells. (b) Cells were incubated with TRITC-labeled THCPSi NPs and (c) TRITC-labeled THCPSi-CooP NPs for 3 h at 37 °C. (d) Magnification of the selected region in (c), indicating association and internalization of THCPSi-CooP by the MDGI-GFP-expressing MDA-MB-231 cells.*

We next quantified the amount of THCPSi-CooP NPs internalized or associated with the MDGI-c expressing MDA-MB-231 cells by flow cytometry measurements. Comparison of the SSC-values showed that MDGI expression significantly ($p < 0.001$) increased the internalization of the THCPSi-CooP NPs in MDA-MB-231 cells (Figure 2) compared to the parental cells, which express negligible amounts of MDGI.

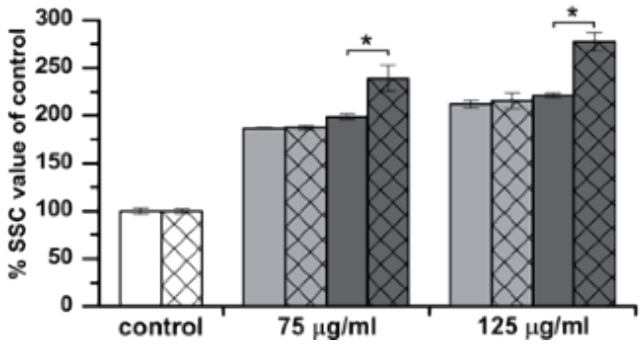

Figure 2. *SSC-values obtained from the flow cytometry dot plots of the treated cells were normalized to that of untreated cells (n ³ 4; mean ± s.d.). THCPSi-CooP NPs were taken up most efficiently by the MDGI-c expressing cells, which had significantly higher SSC-values compared to the control cells treated with the THCPSi-CooP NPs (*p < 0.001). There was no significant difference between the control and MDGI-expressing cells treated with the THCPSi NPs.*

Enhanced in vivo tumor targeting of THCPSi-CooP NP: To study the potential of the CooP peptide to target the THCPSi NPs to the tumors, we established an MDGI-c expressing MDA-MB-231 tumor cell line and first verified that the presence of MDGI increases CooP homing to these tumors. 105 µg of TRITC-labeled THCPSi or THCPSi-CooP NPs were administered intravenously via the tail vein into tumor-bearing mice and allowed to circulate for 1 h after which the animals were sacrificed. To study the tissue distribution of NPs, tumors and organs were collected and prepared for immunohistochemistry. Our results showed efficient homing of the THCPSi-CooP NPs to the tumor site (Figure 3a-b) when compared to the control THCPSi NPs (Figure 3c-d). Quantification of the accumulation of the NPs revealed ~3-fold ($p < 0.001$) more THCPSi-CooP NPs in the tumor tissue compared to the control THCPSi NPs (Figure 3e).

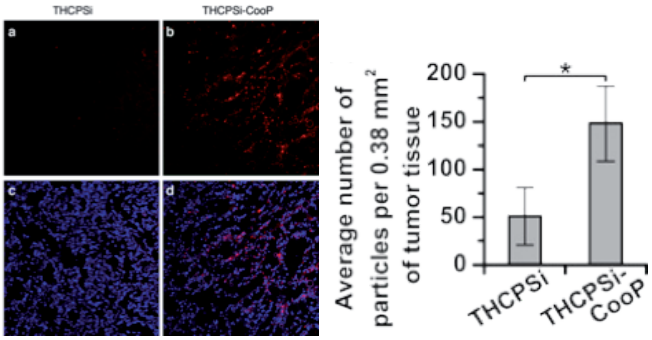

Figure 3. *Representative images of tumor tissue after intravenous injection of THCPSi (a and c) and THCPSi-CooP NPs (b and d). TRITC-labeled THCPSi and THCPSi-CooP NPs (red) were injected (105 µg) into the tail vein of tumor bearing mice (n ³ 2) and the accumulation of the NPs in the tumor tissue was assessed by examining the tissue sections under an epifluorescence microscope. The nuclei of the tumor tissues were visualized by using DAPI (blue in c and d). Scale bars are 200 µm. (e) Quantification of the average number of NPs per 0.38 mm2 of tumor tissue section (n ³ 4). The amount of accumulated THCPSi-CooP NPs in the tumor area was significantly higher compared to the control THCPSi NPs (*p < 0.001). Error bars represent s.d. (n ³ 2).*

In addition, biodistribution studies of THCPSi or THCPSi-CooP NPs were not detected in the kidneys after 1 h post-injection and the accumulation of the NPs in the lungs, heart) and brain was also negligible (results not shown).

CONCLUSIONS

We have demonstrated a simple and stable biofunctionalization of THCPSi NPs with a 9-amino acid long tumor-homing peptide (CooP), which targets the MDGI-expressing MDA-MB-231 breast carcinoma cells. The developed PSi nanovectors 181 nm in size were stable under physiological conditions in human plasma and did not cause any cytotoxic effects to the carcinoma cells. Expression of MDGI,

the interacting partner for the CooP peptide, enhanced the cellular uptake of the THCPSi-CooP NPs in MDA-MB-231 cells compared to the control THCPSi NPs. After intravenous injections into nude mice bearing MDGI-expressing tumors, THCPSi-CooP NPs homed specifically to the tumor site whereas no major accumulation to the vital organs was observed. Our results provide the first proof-of-principle of targeted delivery of PSi nanovectors biofunctionalized with a tumor-homing peptide in vivo. Next, the THCPSi-CooP NPs carrying cytostatic drugs to the tumor tissue and the potential of this drug delivery system to suppress tumor growth will be evaluated.

ACKNOWLEDGEMENTS

The Finnish National Graduate School in Nanoscience for a doctoral scholarship, the Finnish Cancer Organizations and the Helsinki Biomedical Graduate Program for funding, the Academy of Finland (decision nos. 131732, 252215 and 256394), the University of Helsinki and the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) grant no. 310892 are acknowledge for financial support.

REFERENCES

[1] F. Danhier, O. Feron, V. Préat, J. Control. Rel. 2010, 148, 135-46.
[2] L. Brannon-Peppas, J. O. Blanchette, Adv. Drug Deliv. Rev. 2012, 64, 206-212.
[3] S. E. Mcneil, WIRES Nanomed. Nanobi. 2009, 1, 264-271.
[4] A. Z. Wang, R. Langer, O. C. Farokhzad, Annu. Rev. Med. 2012, 63, 185-198.
[5] L. M. Bimbo, E. Mäkilä, J. Raula, T. Laaksonen, P. Laaksonen, K. Strommer, E. I. Kauppinen, J. Salonen, M. B. Linder, J. Hirvonen, H. A. Santos, Biomaterials 2011, 32, 9089-9099.
[6] P. Kinnari, E. Mäkilä, T. Heikkilä, J. Salonen, J. Hirvonen, H. A. Santos, Int. J. Pharm. 2011, 414, 148-56.
[7] D. Liu, E. Mäkilä, H. Zhang, B. Herranz, M. Kaasalainen, P. Kinnari, J. Salonen, J. Hirvonen, H. A. Santos, Adv. Funct. Mater. 2013, doi: 10.1002/adfm.201202491.
[8] H. A. Santos, L. M. Bimbo, V.-P. Lehto, A. J. Airaksinen, J. Salonen, J. Hirvonen, Curr. Drug Discov. Technol. 2011, 8, 228-249.
[9] M. P. Sarparanta, L. M. Bimbo, E. M. Mäkilä, J. J. Salonen, P. H. Laaksonen, A. M. K. Helariutta, M. B. Linder, J. T. Hirvonen, T. J. Laaksonen, H. A. Santos, A. J. Airaksinen, Biomaterials 2012, 33, 3353-3362.
[10] L. M. Bimbo, M. Sarparanta, H. A. Santos, A. J. Airaksinen, E. Mäkilä, T. Laaksonen, L. Peltonen, V.-P. Lehto, J. Hirvonen, J. Salonen, ACS Nano 2010, 4, 3023-32.
[11] J. Rytkönen, R. Miettinen, M. Kaasalainen, V.-P. Lehto, J. Salonen, A. Närvänen, J. Nanomater. 2012, 2012, 1-9.
[12] P. Laakkonen, E. Ruoslahti, G. Bergers, U.S. Patent #8188221, May 29, 2012.

A FINE BALANCE: ETHICAL CHALLENGES OF FIRST IN HUMAN TRIALS IN NANOMEDICINE

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BACKGROUND

Nanomedicine has tremendous potential for early and accurate disease diagnosis and targeted drug delivery, giving strong reasons to test these applications in first in human (FIH) trials. But the risk associated with this novel technology has to be accounted for at least at three levels: the nature of potential risks to trial participants, the probability of these risks manifesting, and the seriousness of any adverse effects in terms of magnitude and harm caused.

The decisions of research ethics committees are based on assessing risk-benefit balance but questions nonetheless remain. How can we assess risks and benefit using pre-clinical data and animal studies and extrapolate it to humans? How do we achieve an objective evaluation of both risks and benefits to ensure scientific and technological progress and alleviate human suffering without exposing human subjects to significant yet unforeseen harms?

Furthermore, it is also worth exploring what is at stake for the scientists and clinical trial coordinators while conceptualizing FIH trials with novel applications of nanotechnology in medicine. From obtaining ethics committee approvals to patient/participant recruitment, what challenges do they face, in facilitating informed consent and eventually gathering sufficient clinical data to improve the technology? This exploratory, empirical, multidisciplinary qualitative research aims to increase our understanding of the complex ethical challenges involved in FIH trials of medical applications of nanotechnology.

METHODOLOGY

Participants

Two main groups of stakeholders will participate in this study: scientists and physicians who coordinate FIH trials, and ethics committee members who evaluate the proposals for FIH trials of medical applications of nanotechnology. Participants will be recruited from the broader context of Switzerland and other countries such as Germany, the United Kingdom, the Netherlands and United States to see if and how national policies, regulations and differences in local ethical review processes influence the translation of medical applications of nanotechnology from the laboratory to FIH trials. Insights will be collected from 20 scientists and physicians and 20 ethics committee members in a series of semi structured interviews to gain a 'thick' description of current practices and challenges of FIH trials in medical applications of nanotechnology.

The respondents will be identified through a thorough search of scientific publications and through conferences such as CLINAM Basel, which brings together experts and scholars working in nanomedicine. Ethics committee members will be approached via the websites of local ethics committees and clinical trial Units (CTUs) in Switzerland and similar appropriate bodies in other countries.

Interviews and analysis

All the interviews will be conducted in person or on Skype and will be audiotaped after obtaining informed consent. The interviews will be transcribed and analysed inductively as well as deductively using software Atlas ti. Coding of the data will be done in a team to ensure the quality of the analysis and to improve the triangulation and reliability of emerging themes and patterns. We also intend to analyse available documents such as submitted research proposals of FIH trials to ethics committees and the minutes of discussions on ethics committees while evaluating submitted FIH trials; this aspect depends on having access to these documents.

EXPECTED OUTCOMES

Though there has already been a lot of theoretical and practical discussion on the ethics of nanotechnology and nanomedicine, we would like to enhance this debate in the particular context of FIH trials of nanotechnology. Also relevant here is the on-going discussion on FIH trials on stem cell therapy and gene therapy, which could provide valuable insight into FIH trials of nanomedicine, or be a stumbling block due to unfortunate events in the past and consequent negative publicity. We hope to establish how FIH trials with stem cell therapy or gene therapy have changed the global and European discussion in terms of nanotechnology. Have these other novel technologies made ethics committees more cautious, and more concerned about public outcry and opinions? The stem cell/gene therapy experience may also have influenced the willingness of national and international funding agencies to fund this research.

Furthermore, we aim to understand and highlight the key ethical and general challenges faced by each of these stake holders. These could range from funding constraints to difficulty in recruiting trial participants and facilitating informed consent. For ethics committee members, it may be challenging to apply the general umbrella guidance in existing national and international policy documents and guidelines, which may not take into account the specific issues involved in FIH trials of nanomedicine that they are expected to evaluate. At the end of this study, we hope to be able to provide a balanced and holistic picture of current practices, and identify any missing links or the perceived need for specific clarifications.

POLICY IMPLICATIONS

Insights generated through this research, (which to our knowledge is one of the first of this type of study) has the potential to significantly advance the debate regarding the ethics of FIH trials in nanomedicine. Identifying the concerns of different stake holders will contribute to the formulation of specific guidelines for FIH trials in nanomedicine.

THE NANOMECHANICAL PROPERTIES OF BREAST BIOPSIES AS DIAGNOSTIC AND PROGNOSTIC MARKERS IN BREAST CANCER

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According to conventional wisdom, tumors are stiffer than the surrounding normal tissue. This apparent stiffness provides the basis for the detection of tumors in breast palpation and elastography. Paradoxically, biophysical techniques reveal that single cancer cells are more compliant ('softer') than their healthy counterparts. Moreover, tumor progression has been shown to be associated with an increase in cell softening. Here we use indentation-type atomic force microscopy (IT-AFM) to measure at high sensitivity and spatial resolution the stiffness across native biopsies that were obtained from women with suspicious breast lesions. Based on correlative stiffness maps we could identify characteristic stiffness profiles for normal mammary gland tissue, benign tumors and carcinomas. Malignant tissues have a broad stiffness distribution resulting from tissue heterogeneity with a prominent low-stiffness peak representative of cancer cells whereas normal tissue and benign lesions show a uniform stiffness profile that are characterized by a single distinct peak. Similar nanomechanical 'fingerprints' are seen in specific stages of breast cancer in MMTV-PyMT transgenic mice. The detection of corresponding low stiffness values in metastatic lungs indicates that a soft phentotype of primary cancer cells is conducive to migration and metastatic spreading. Nanomechanical profiling by IT-AFM provides quantitative indicators in the clinical diagnostics of breast cancer. Moreover, the unique mechano-markers can be used to identify different cancer stages. The molecular and structural changes in the extracellular matrix and cellular architecture that are associated with tumor progression result in very soft and thus deformable cells. Our data indicate that the mechanical response of cells reflects their propensity to metastasize.

ABUNDANCE AND ORIENTATION OF MYELIN SHEATHS IN PARTS OF HUMAN BRAINS

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INTRODUCTION

The human brain is one of the most fascinating and important structures in nature. For investigations of the different structures on the cellular level and even below histology has been the gold standard so far. Using a light microscope the limitation of the spatial resolution is in the range of a fraction of a micrometer.
Reciprocal space techniques including small-angle X-ray scattering (SAXS) with an inverse relationship between the size of the inspected nanostructures and scattering angle can be used for the investigation of nanostructures within the human brain (e.g. myelin sheaths with a periodicity of 16.5 nm) [1]. However, it is impossible to relate the results to established histology because of the lack of localization. Combining SAXS with a spatial resolution of a few tens of micrometers in real space (scanning SAXS at cSAXS beamline, SLS, PSI, Switzerland [2]) provides key information such as abundance and orientation of the nanostructures present within the natural organization of the examined tissue [3]. Thus, the result is a more detailed understanding of the histological analysis of brain tissue slices.

METHODS

A human thalamus was extracted and was fixed in formalin. A 50 µm-thin slices was obtained by cryo-sectioning and transferred into a polyimide sachet to avoid further drying. The slice was measured at the cSAXS beamline using the photon energy of 11.2 keV and a specimen-detector distance of about 7.1 m. The X-ray beam was focused to 20 × 5 µm2 and scanned over the slice in 100 µm steps in the two orthogonal directions of the histological slice. The scattering signal was recorded using a PILATUS 2M detector [2].

RESULTS

The examination of the scattering signals provides local information on abundance, size, size distribution and preferential orientations of brain tissue nanostructures. The radial intensity profile shown in the figure exhibits two peaks. The first peak corresponds to the characteristic myelin periodicity of 16.5 nm. The second peak corresponding to 8.3 nm is the related second diffraction order signal. The image bottom right shows the results obtained from an unstained human thalamus slice in the range 8 to 9 nm. The color corresponds to the preferential orientations of the scattering signal perpendicular to the present nanostructures, (cp. color wheel), whereas the abundance of the latter is given by the brightness. The image shows no significant orientation inside the thalamus but in the adjacent regions. For comparison, the investigated slice was mounted on a microscope glass, myelin stained using black gold and visualized using an optical microscope. The image bottom left shows the slice where dark regions correspond to areas with a high amount of myelin in comparison to the bright regions.
The non-destructive spatially resolved X-ray scattering method bridges the gap between the real-space, optical techniques with micrometer resolution and large field of view and the reciprocal space scattering/diffraction techniques with nanometer resolution, but restricted field of view. In the future scanning SAXS and SAXS tomography will play a dominant role in the development of nanomedicine and related fields.

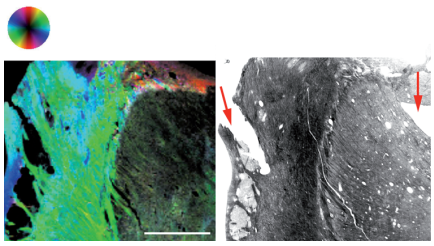

Figure 1. *The image on the left shows the orientation of the scattering signal in the ranges of 8 to 9 nm of an unstained slice placed in a polyimide sachet. The bar corresponds to 5 mm. Compared to myelin staining using black gold (image right) where only the concentration of myelin is visualized the cSAXS data shows additional information on the orientation of the nanostructures. Furthermore, the staining procedure induces deformations including global shrinkage and local strains within the anisotropic inhomogeneous brain tissue. The red arrows exemplary indicate regions with large distortions, which are not present in the cSAXS slice.*

REFERENCES

[1] De Felici M, Felici R, Ferrero C, Tartari A, Gambaccini M, Finet S. Structural characterization of the human cerebral myelin sheath by small angle x-ray scattering. Phys Med and Biol 2008;53:5675-88.
[2] Bunk O, Bech M, Jensen TH, Feidenhans'l R, Binderup T, Menzel A, Pfeiffer F. Multimodal x-ray scatter imaging. New J Phys 2009;11:123016.
[3] Müller B, Deyhle H, Bradley DA, Farquharson M, Schulz G, Müller-Gerbl M, Bunk O. Scanning X-ray scattering: Evaluating the nanostructure of human tissues. Eur J Nanomed 2010;3:30-3.

ALEXANDER - MUCUS PERMEATING NANOPARTICULATE DRUG DELIVERY SYSTEMS

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In 333 BC, while wintering at Gordium in Asia Minor, Alexander the Great opened the door to the East by cutting the 'Gordian knot'. Opening the door to the development of efficient and cost effective nano-delivery systems, from an industrial point of view, for macromolecular drugs necessitates the solution of another 'Gordian knot' problem, namely, that of enhanced permeation of nanoparticles through the mucus gel barrier. Apart from oral mucosal vac-

cinations where Peyer's patches, which are not coated by a mucus gel layer, are targeted, the development of mucosal nanoparticulate delivery systems for macromolecular drugs such as therapeutic peptides, oligo- and polysaccharides and DNA-based drugs only makes sense, if effective strategies are available to overcome the mucus gel barrier. Similarly to Alexander the Great, who simply cut the 'Gordian knot' into parts, the mucus gel layer barrier, consisting of mucus glycoproteins that are highly entangled and knotted, will have to be 'cut' by 'chemical swords' such as mucolytic agents in order to open the door to mucosally administered macromolecular drugs for systemic circulation. According to these considerations it is the mission of the large EU-project 'ALEXANDER - Mucus Permeating Nanoparticulate Drug Delivery Systems' to develop nanodelivery systems to overcome the various mucus gel barriers without destroying them. Based on results gained within this project fundamental knowledge how to overcome the mucus gel barrier in a highly efficient manner shall be provided leading to more potent mucosal drug delivery systems in general.

ALEXANDER is a large-scale collaborative research project within the 7th European Framework Programme for Research and Technological Development. Within ALEXANDER, 14 partners from industry and academia have joined forces, combing leading expertise in chemistry, biochemistry, pharmacy, chemical engineering, pharmaceutical technology, molecular physiology, biopharmaceutics, toxicology, drug development and project management.

CHANGES IN MEMBRANE OF RED BLOOD CELLS DURING STORAGE OF DONOR BLOOD

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INTRODUCTION

The purpose of storage and transfusion of blood is to maintain the gas transport function of the blood and in the restoration of circulating blood volume. Keeping blood requires a number of stringent technological and physiological regulations. Red blood cells are placed in an artificial environment of hemokonservant, as the storage, lose their functional abilities.

OBJECTIVE

To study the macro-and ultrastructure changes in the surface of the erythrocyte membrane during the storage of donor blood for 30 days and to assess the functional state of membranes during the storage period.

MATERIALS AND METHODS

In S.P. Botkin City Clinical Hospital, the blood samples were collected from four healthy donors from 27 to 35 years in containers with a preservative CFDA-1. During 30 days, the blood was stored at - 4 ° C in accordance with WHO recommendations. On the day of the experiment, the samples (10 ml) were collected. Blood samples were collected at 0, 2, 5, 6, 8, 10, 13, 16, 21, and 30 days of storage. Experiments were carried out at a temperature of 190 C.

Images of nanosurface membranes of red blood cells were obtained using an atomic force microscope NT-MDT «NTEGRA prima» (Russian Federation). Monolayers of erythrocytes was obtained by the device «V-sampler, Vision Microscopy» (Austria). Scanning was performed in the semi contact mode. We used cantilevers NSG01-A, with the resonance frequency 87-230 kHz. The radius of the tip - 10 nm. The number of scanning points 512 and 1024 of the scanning field 100x100 mm, 10x10 mm. The scanning area is displayed as a flat format, and 3D formats.

To measure the rigidity of erythrocyte membranes (Km) was used atomic force spectroscopy. Power curves were recorded in 6 points on the torus erythrocyte. The measurements were performed cantilever SD-R150-NCL-10. Zoned radius 150 nm, the resonance frequency 181 kHz, K = 36 N/m. Measurements were made at 6 cells per sample. Various different sections of cells and the erythrocyte membrane had Km dispersion. Therefore, in the study was estimated average local rigidity of the membranes to 360 cells.

Complete blood count was performed on the ADVIA 60 analyzer (Bayer, Germany). Erythrocyte count were measured, cell hemoglobin, hematocrit. At the biochemical analyzer Cobas Mira Plus (Roche, Switzerland) was measured bilirubin, glucose, total protein. With ionomer I-510 (Russia) analyzed the acid-base balance of the blood, hemoglobin spectrum (Apel PD-303, Japan). Blood parameters for all days of storage compared to benchmarks in the day of collection.

Results: The number of red blood cells decreased from 4,1 ± 0,9x1012L the first day to 3,1 ± 0,4 x1012L the thirtieth day of storage of blood (p <0,05). Cellular hemoglobin dropped to 12,1 ± 2,2 g /dL for the first storage to 9,2 ± 1,2 g /dL (p <0,05 compared with the first day of storage.) Concentration of the active intracellular glutathione during the entire period of storage of blood and fell to the 30 day storage fell by 18%. Potassium ion content increased from 3,8 ± 0,3 to 21 ± 8 mmol / l to 30 day. In one day, the pH was 6,9 ± 0,1. By the thirtieth day of the pH decreased to 6,5 ± 0,1. Nanosurface of membranes significantly changed for the 30 days of. The biggest changes are expressed in the parameters h2 (Fig. 1)

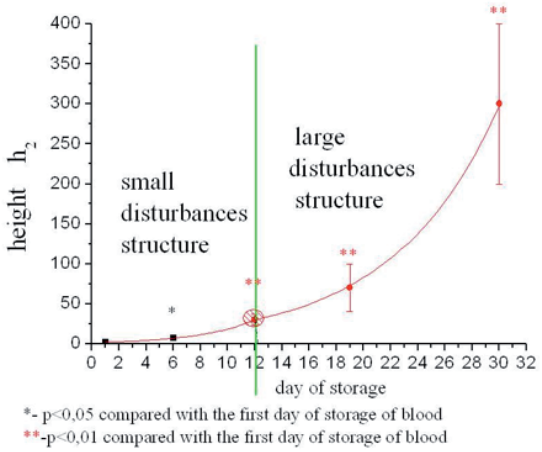
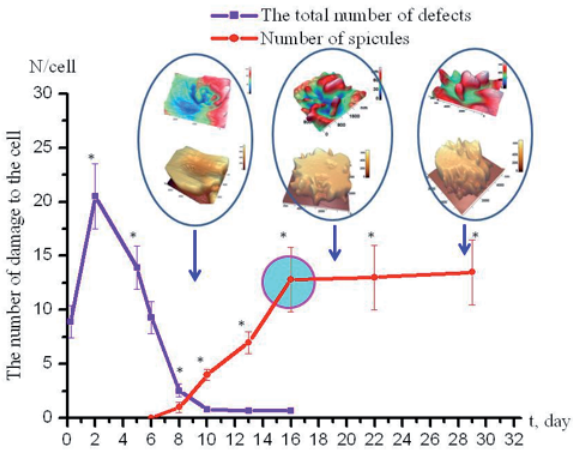


Fig.1 *Change in parameter h2 red blood cells membrane*

With the decrease in the number of small defects began to grow the number of large spicules on the observed surface membranes. The number of large defects (500 - 750 nm) has a maximum of 16 days of storage of blood. Medium and large defects on the sixth - eighth day turned into a multi-tiered and spicules on the tenth - fourteenth day formed echinocytes that to thirty days formed sferoehinotcytes (Fig. 2).



*p<0,05 compared with the first day of storage of blood

Fig. 2*. Dynamics of changes in membrane defects during storage of blood blue curve - the total number of small - defects red curve - major defects.*

Dynamics erythrocyte membrane rigidity during the storage of blood. During the entire period of storage of blood was measured average local rigidity of the membrane of red blood cells. Indicators average local rigidity erythrocyte membranes during storage of blood are shown in table 1.

***Table 1**: In the average local rigidity of the membrane of red blood cells during storage of blood*

days of storage	1	15c (70%)	15c (30%)	30c (84%)	30c (16%)
K (relative units)	4,3±2,6	5,0±1,8	8,4±1,07	9,2±1,5	5,1±1,6

The distribution of the membrane rigidity for 1, 15 and 30 days of storage are shown in Figure 3.
Stiffness in the first one day amounted to 5.3 rel. units, to the 15th day - it increased by 21% (70% of cells), and by day 30, it increased by 2.1 times. This increase in stiffness of the membrane of red blood cells may result in reduced deformability index ≈ 27-31%, deterioration of the rheological properties of the blood.

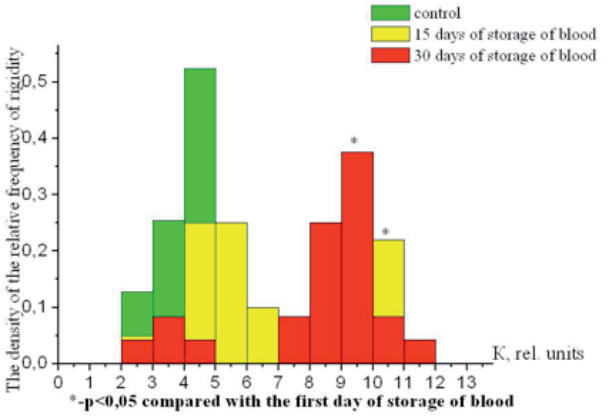


***Fig. 3** histograms rigidity of red blood cells in the blood 1, 15 and 30 days of storage*

CONCLUSION

Long before 30 days storage of whole blood at 4° C accompanied by changes in the major indices of erythrocyte membranes: pH plasma decreased from 6.9 to 6.5, the concentration of potassium ions in the plasma rose to 21 ± 8 mmol/l, the concentration of active reduced glutathione decreased 18%. As the storage of blood on the membranes appear irreversible defects that increase their size with increasing storage time, which later become the major outgrowths forming sferoehinocytes. As the storage of blood erythrocytes membrane rigidity increased in 1.8-2.1 times.

REFERENCES

- E.K. Kozlova, A.M. Chernysh, V.V. Moroz, A.N. Kuzovlev. Analysis of nanostructure of red blood cells membranes by space Fourier transform of AFM images. Micron. 2013;44:218-27.
- V. V. Moroz, A. M. Chernysh, E. K. Kozlova et.al. Comparison of red blood cell membrane microstructure after different physicochemical influences: Atomic force microscope research. Critical Care. 2010; 25(3):1-12.
- V. V. Moroz, A.M. Golubev, A.M. Chernysh, E.K. Kozlova, V.Yu. Vasilyev, O.E. Gudkova, V.A. Sergunova, M.S. Fedorova. Structural Changes in the Surface of Red Blood Cell Membranes during Long-Term Donor Blood Storage. General reanimatology. 2012; 8(1):5-12 (Article in Russia).

NANOTECHNOLOGICAL APPROACHES TO BIOMARKER DISCOVERY: ANTIBODY-ARRAY BIOCHIPS

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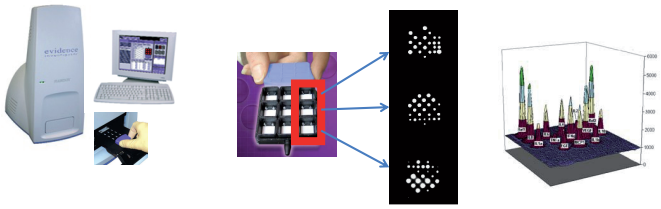
Protein biomarkers in bodily fluids, like the human plasma and in tissues are of outstanding interest as the ultimate biological function is associated with proteins not with genes.
Genetic code independent proteome variability is due to post-translational modification, protein interactions, folding variation and these multiply the genome dependent variability, splice variation, mutations etc.
Disease diagnosis thus needs detection of proteins and their genetic code dependent and independent variability. With precise detection and quantification of the multitude of protein variability, one expects detection of disease at non symptomatic stages as well as the collection of sufficiently granular information for optimal disease management.
Combination of affinity reagent driven protein biomarker detection with nanotechnology in the form antibody biochips promises a solution for the imminent need. At Biosystems International and in the Laboratory of Monoclonal Antibody Proteomics at the University of Debrecen, with patent protected technologies we prepared large monoclonal antibody libraries that detect independent epitops of the human plasma proteome. With the help of industrial partners and academic laboratories (Randox Ltd., Array-it Corp.) we developed micro and nano-arrays to detect and quantify dynamic changes of >1000 protein epitops in the human plasma in apparently healthy individuals and in disease conditions including various cancers, and chronic diseases (COPD, Obesity, OA).
The microarray application results indicate >95% accuracy (AUC in ROC analysis) for the detection of cancer and the results are promising in the other diseases. Preliminary analysis of nano scale application of arrays indicate good correlation with micro array results.
Our current focus includes bringing the micro array based biochips to the bedside in the form of qualified and validated diagnostic tools with our industrial partners and to focus on R&D activities to improve array performance with the nano scale applications.
The challenges include achieving printing consistency, developing a robust detection system and solving the analyte handling. Ultimately, we expect to deliver a plasma proteome profiling versatile nanochip for bedside use.



The Randox Evidence Investigator biochip technology with Biosystems' QuantiPlasma libraries is suitable for plasma proteome profiling

CANTILEVER BENDING FOR NANOSTRUCTURE CHARACTERIZATION OF ARTIFICIAL MUSCLES

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Incontinence is a common phenomenon in western countries with an increased prevalence in elderly people. An efficient treatment of severe cases is provided by inserting an artificial sphincter (AS). The presently available hydraulic AS, however, have some severe drawbacks, such as constant pressure on the surrounding tissues and a complicating handling. Some of these issues are thought to be eliminated by using a smart sphincter based on electrically activated polymers (EAP), which should respond to pressure pulses caused by coughing [1]. For clinical application, it is essential that the sphincter is already working at voltages below 24 V [1]. In order to investigate the EAP-actuators asymmetric microstructures are constructed. The approach is also known as microcantilever-bending. Such a bending structure consists of a few tens of micrometer-thick polydimethylsiloxane (PDMS) layer sandwiched between two 50 nm-thin silver layers on a 25 µm-thick polyetheretherketone (PEEK) substrate. Since the volume of the EAP is conserved an applied voltage between the two electrodes results in surface stresses on the PEEK layer (see Figure 1). Hence, the applied voltage leads to a bending of the whole structure. The bending is detected from a reflected laser using a position sensitive device (PSD). More precisely, a gold layer 28 nm thick is deposited on the other side of the PEEK layer to guarantee reasonable reflectivity for HeNe laser at a wavelength of 633 nm such that the reflected laser beam can be detected without any difficulty [2]. Therefore, the bending of the cantilever results in a dislocation of the laser beam on the PSD.

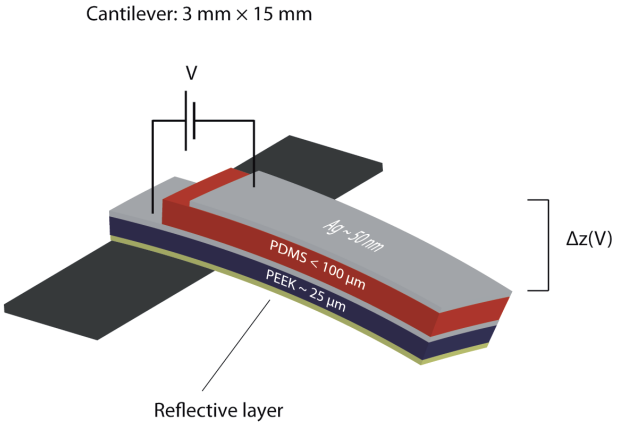

Figure 1. *Schematic of a cantilever with an applied voltage between the two electrodes. The deflection of the cantilever Δz is measured as a function of the applied voltage V.*

For our first measurements we restricted ourselves to a thickness of the PDMS layer of approximately 100 µm. Furthermore, the active area of the cantilever was approximately 3 mm × 15 mm. The measurements showed a linear dependence on the square of the applied voltage for higher voltages, but not for voltages below 400 V [3]. Hence, for higher voltages the deflection amplitude of the cantilever is proportional to the applied Maxwell pressure p which is defined as

$$p = \varepsilon_0 \varepsilon_r \left(\frac{V}{z}\right)^2$$

where V denotes the applied voltage, z the thickness of the elastomer, and ε_0 and ε_r the relative dielectric constant of the vacuum and the elastomer, respectively. Furthermore, the EAP-microstructure needed to be conditioned by running five cycles before an accurate reproduci-bility could be observed [3].

For application reasons, in future measurements the focus will be on the low-voltage region where we intend to measure EAP structures with nanometer thicknesses and thereby we in-tend to get a deeper insight on the bending behavior of EAP actuators such that the deflection of the cantilever per voltage can be optimized for the realization of actuators to be incorpo-rated into AS prototypes.

REFERENCES

[1] B. Müller, H. Deyhle., S. Mushkolaj et al., The challenges in artificial muscle research to treat incontinence. Swiss Medical Weekly, 139(2009), 591-595.

[2] X. Zhao, P. Urwyler, and B. Müller, Optimization of the optical readout of PEEK canti-levers. European Cells and Materials, 23(2012), 40.

[3] F.M. Weiss, X. Zhao, P. Thalmann, H. Deyhle, P. Urwyler, G.M. Kovacs, B. Müller. Measuring the bending of asymmetric planar EAP structures. Proc. SPIE, 8687 (2013), 86871X.

SILICA NANOPARTICLES FOR IMAGING AND DRUG DELIVERY

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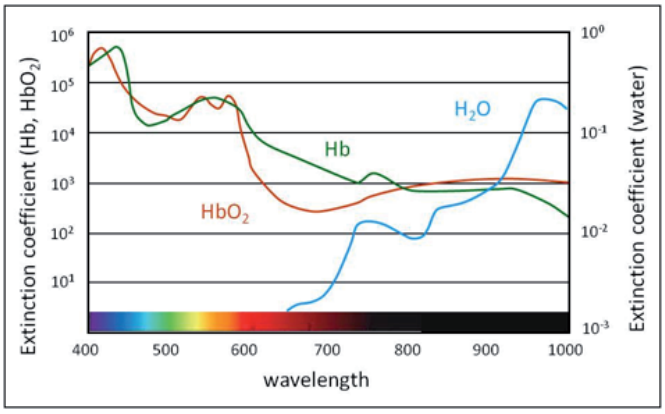
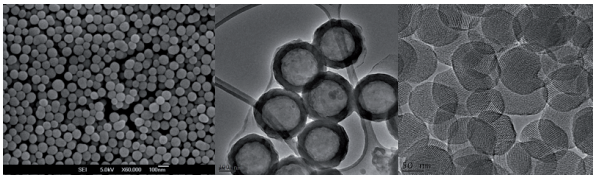
In vitro imaging in the visible light range is severely limited by the absorbance of light by the components of the blood. However, excitation in the Near Infrared (NIR) region shows minimal autofluorescence, minimal photodamage, and maximal tissue penetration.

There are a number of dyes which can be used in the NIR range, such as cypate, methylene blue, and the cyanine dyes. However, incorporation of the dyes in to the silica nanoparticle system can have a number of advantages: Nano-encapsulation of dyes makes the dyes more stable, a high intensity light can be achieved due to the large number of dye molecules within each particle, there is controlled quenching, and also good biocompatibility. These nanoparticulate systems can then also be used in conjunction with NIR imaging systems. We have prepared silica beads of between 20 and 420nm, with a predictable and narrow size distribution, with a variety of NIR dyes. The silica nanoparticles show a versatile surface chemistry which can be used for bio-conjugation through functional groups such as thiol, amino, and carboxyl groups.



Furthermore, mesoporous silica nanoparticles can be used to deliver pharmaceuticals. At present 95% of all new potential therapeutics have poor pharmacokinetics and biopharmaceutical properties. It is therefore desirable to deliver active drug molecules to the site of action, without affecting healthy organs and tissues. We have therefore synthesized silica nanoparticles with a variety of outer dimensions, pore structures, and shapes. Loading and unloading of the particles has been investigated. In addition, coatings have been added to the surface of the nanoparticles to control the release of the cargo.



CURCUEMULSOMES: NANOFORMULATION FOR ENHANCED SOLUBILITY AND DELIVERY OF CURCUMIN

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Curcumin is a natural polyphenolic compound with intrinsic anticancer properties. Its medical use remains limited due to its extremely low water solubility and bioavailability. The present study introduces novel curcumin-emulsome nanoformulations, so-called CurcuEmulsomes, where curcumin is encapsulated inside the lipid matrix of the nanocarrier. Incorporation of curcumin into emulsomes overcomes the bioavailability limitation and increases its solubility by up to 10,000-folds corresponding to a concentration of 0.11 mg/ml (Figure 1). CurcuEmulsomes are spherical, stable, mono-dispersed solid nanoparticles with an average size of 286 nm. Cell culture studies demonstrated that uptaken by the HepG2 human carcinoma cells CurcuEmulsomes release curcumin gradually inside the cell; thereby prolonging its physiological activity as evidenced by cytotoxicity and cell cycle profiles of HepG2. Like free curcumin, CurcuEmulsome induced G2/M cell cycle arrest and apoptosis of HepG2. The enhanced bioavailability, as well as the promising in vitro efficacy of CurcuEmulsomes highlights potential for in vivo therapeutic applications.

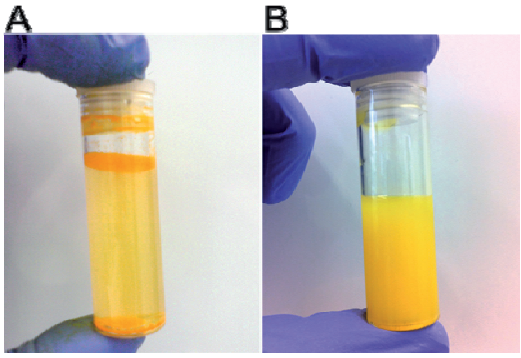

***Figure 1**. Photographic images of (A) curcumin in water and (B) CurcuEmulsome in water*

MOLECULAR DYNAMICS TO UNDERSTAND POLYENE ACTION – A STUDY ON AMPHOTERICIN B AND A NOVEL DERIVATIVE (A21)

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Molecular dynamics studies were performed to undersatnd the action of a novel derivative (A21) of Amphotericin B (AmB) with equal potency and increased safety compared to AmB. The state of aggregation of polyene molecules in aqueous solution is an important factor for the fungicide efficacy. In the present work the formation of dimers with different configurations is studied, as well as the analysis of the structural stability and interaction energy. Our results agree with the observed increase in solubility of A21 due to smaller dimerization energy. This suggest that A21 remains monomeric at concentrations where AmB does not, and thus has difficulty in penetrating the cholesterol membrane. This also agree with the finding that a large amount of A21 is required to observe activity in the cholesterol membrane.

TRANSMISSION OF TOPICAL SIO2 NANOPARTICLES THROUGH THE CORNEAL STROMA; A NEW HORIZON FOR MANAGEMENT OF CORNEAL AND CHOROIDAL NEOVASCULARIZATION

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INTRODUCTION

Corneal neovascularization (NV) is a crucial sight-threatening condition which can be the result of infections, degenerative disorders, inflammations, and other traumatic insults such as long-time contact lens wear. Corneal NV occurs when the balance between angiogenic and antiangiogenic factors is tipped toward angiogenic molecules. The abnormal vessels may result in the lack of corneal clarity, vision reduction, inflammation, and corneal scarring. While topical administration of corticosteroids and non-steroidal anti-inflammatory drug (NSAIDs) are clinically the primary treatments, in the cases which vessels have been established in cornea, they are ineffective [1].

Although new drugs have recently been developed within the field of ophthalmology, drugs administered whether systemic or topically have poor access to the inside of the eye because of the cornea, which is the main barrier against the drug penetrations due to complete surrounding and effective sealing of its superficial epithelial cells. The human cornea is normally an avascular, transparent connective tissue that consists of three layers; epithelium, stroma and endothelium, and a mechanical barrier to inhibit transport of exogenous substances into the eye [2]. The stroma is composed of an extracellular matrix of a lamellar arrangement of collagen fibrils. Topically administered drugs have poor access to retina and corneal stroma due to the annular tight junctions, which made cornea as a major ocular barrier [3]. Nanomedicine uses nano-scale technology for the treatment and prevention of disease that can pave the way for novel ophthalmologic therapeutic application with an ultimate goal of improving quality of vision and finally the quality of life. Therefore, many nano-structured systems have been employed for ocular drug delivery and yielded some promising results. Nanoparticles (NPs) of various molecules, such as gold and silver, have been reported to have anti-angiogenic effect on pathological neovascularization [4, 5]. Silicate NPs (SiNPs) have been used in drug delivery, gene therapy, and biolabeling [6]. Moreover, recently the intravitreal injected SiNPs have indicated the ability of inhibiting retinal NV through suppression of VEGFR-2 phosphorylation. Aqueous suspensibility of SiNPs as well as being cost effective and non toxic on ocular tissues, prompted us to prepare topical form of nano-suspension of SiNPs intended for inhibition of corneal NV. Although the intravitreal injection of silicate nanoparticles has been safely used for management of choroidal neovascularization [7], to the best of our knowledge, the topical application of silicate particles in nano scale has not been investigated. This study was an attempt to investigate the in vitro penetration and diffusion of topical SiNps in the corneal stroma.

METHODS

Nanoparticles were prepared using two-phase nucleation method from tetraethoxysilane (TEOS) with arginine as a condensation catalyst. Fourier transform infrared spectroscopy (FTIR) was performed to indicate hydrolysis of TEOS to SiO2 in prepared nanoparticles. The mean particle size was determined by dynamic light scattering (DLS) and the morphology of the nanoparticles was investigated by scanning electron microscopy (SEM).

To study the penetration and distribution of SiNPs in corneal tissue, isolated corneal lenticules were treated with 5 µl of particle suspensions (1 mg/ml) for 5 and 30 minutes (on the epithelial side). Surface and cross-section of samples were then investigated using SEM. Cross-sections were prepared through cryosections with 10 µm thickness. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) was employed to determine the concentration of the SiNP suspension.

RESULTS

Presence of SiO2 peak (bands appearing at 1109 cm −1 and 473 cm −1) in nanpoparticle FTIR plot demonstrated the appropriate hydrolysis of TEOS in prepared NPs (Fig. 1). According to DLS results the mean size and polydispersity index (PDI) of prepared SiNPs were 34.3±5.6 and 0.10±0.11, respectively. SEM images showed semispherical shapes of particles (Fig. 2). DLS results revealed that the size of particles changed proportional to the concentration of incorporated reagents.

SEM images of corneal surface (Fig. 3) and cross-sections (Fig. 4) showed the penetration and distribution of SiNPs through the corneal tissue.

CONCLUSION

According to the results appropriate penetration of SiNPs were obtained following the administration of topical particle suspension. Prepared SiNPs showed acceptable permeability through the corneal epithelium and stroma. Consequently, SiNPs can be used as an effective modality in the intended for treatment of corneal neovascularization in future studies.

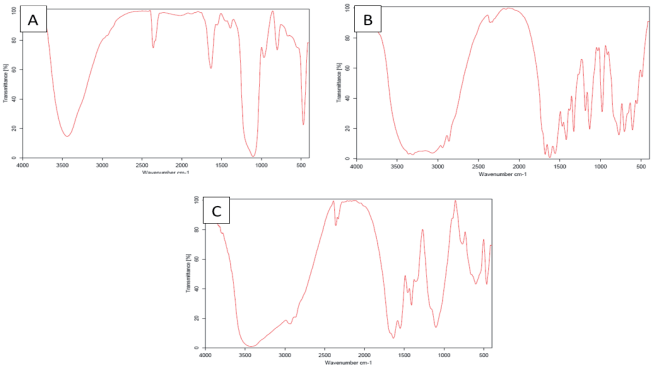

Fig. 1. *Fourier transform infrared spectroscopy (FTIR) plots of standard SiO2 nanoparticles (A), arginine (B), and prepared SiNPs containing arginine as catalyst (C)*

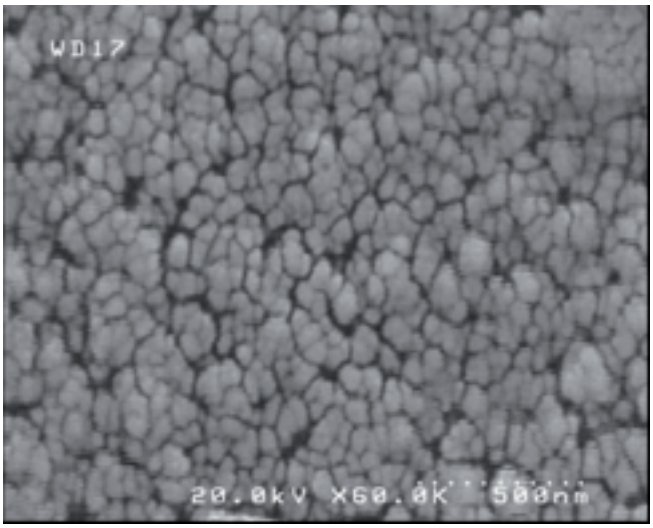

Fig. 2. *Scanning electron microscopy (SEM) images of prepared SiNPs*

A B C

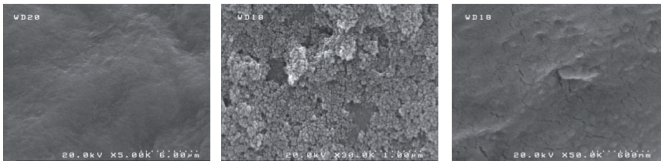

Fig. 3. *Scanning electron microscopy (SEM) images of untreated (A) and treated corneal lenticules with SiNPs after 5 (B) and 30 (C) minutes treatments.*

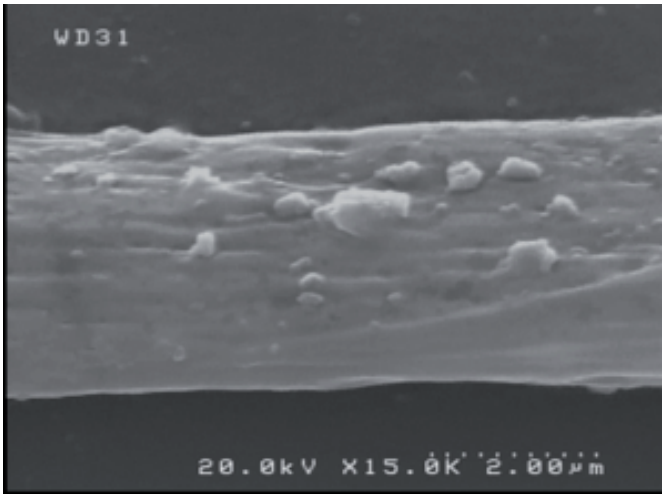

Fig. 4. *The cross-section scanning electron microscopy (SEM) images of treated cornea with SiNPs (after 30 min treatment). As can be observed the concentration of nanoparticles decreased from epithelium to endothelium.*

REFERENCES

- Chang JH, Garg NK, Lunde E, Han KY, Jain S, Azar DT. Corneal Neovascularization: An Anti-VEGF Therapy review, SurvOphthalmol. 2012 Sep;57(5):415-29.
- Bayens R, GurnyR. Chemical and physical parameters of tears relevant for the design of ocular drug delivery formulations. Pharm. ActaHelv. 1997;72:191-202.
- Schoenwald RD. Ocular drug delivery: pharmacokinetics considerations. Clin. Pharmacokinet. 1990;18(4):255-69.
- Kalishwaralal K, Sheikpranbabu S, Barathmanikanth S, Haribalaganesh R, Ramkumarpandian S, Gurunathan S. Gold nanoparticles inhibit vascular endothelial growth factor-induced angiogenesis and vascular permeability via Src-dependent pathway in retinal endothelial cells. Angiogenesis. 2011;14:29-45.
- Gurunathan S, Lee KJ, Kalishwaralal K, Sheikpranbabu S, Vaidyanathan R, Eom SH. Antiangiogenic properties of silver nanoparticles. Biomaterials. 2009;30:6341-50.
- Couleaud P, Morosini V, Frochot C, Richeter S, Raehm L, Durand JO. Silica-based nanoparticles for photodynamic therapy applications. Nanoscale. 2010;2:1083-95.
- Dong HJ, Kim JH, Young SY. Antiangiogenic effect of silicate nanoparticle on retinal neovascularization induced by vascular endothelial growth factor. Nanomedicine. 2012;8(5):784-91.

DISEASENET: A TOOL FOR IDENTIFICATION OF DRUG TARGETS

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BACKGROUND

Targeted approach of disease cure or its prevention is dependent upon information on the causative factors involved. Systems analysis of disease information provides insight to determine the causative factors that cause perturbed networks. The difficulty lies in obtaining useful biological information among diseases. With the accumulation of high throughput datasets, understanding the interaction and crosstalk between diseases is crucial. The underlying mechanisms by which these complications arise and manifest together remain poorly understood. Applying new methods for predicting critical nodes in disease gene network for identifying cellular perturbations and susceptibility to other diseases together with network analysis may serve as a tool for targeted therapeutic strategies.

RESULTS

To identify the critical players underlying diseases for targeted therapy, we performed network-based systems biology approach. We collected a list of candidates gene associated with human diseases from patient case studies. These list of genes were further expanded to include protein neighbours to depict high connectivity among similar diseases than random network. Further, we clustered disease genes in modules of pathways and observed that enriched pathways have dominant role in disease progression. We extended our work to identify the crosstalk among disease specific networks to uncover deregulation of multiple pathways (Fig.1). Finally we applied network approach to identify novel candidate genes, which may be used for targeted approach and as targeting sequences.

CONCLUSION

We present a computational approach based on disease specific network analysis tool to determine possible association among diseases. The approach enables to understand biological phenomena through analysis of interactions of cellular components. Our preliminary results provide the first comprehensive report on network and pathway characteristics of genes associated diseases. The given systems biology approach might prove as a promising tool for selection of critical nodes as target for drug discovery and ensuring targeted delivery of drugs for effective disease treatment or prevention.

CELLULAR TRAFFICKING OF NANOPARTICLES

DRITON VLLASALIU

The understanding and control of nanoparticle transport into and through cellular compartments is central to biomedical applications of nanotechnology. Our work shows that the transport pathway of 50 nm polystyrene nanoparticles surface decorated with vitamin B12 in intestinal epithelial cells is different compared to both soluble B12 ligand and unmodified nanoparticles. The data indicates that vitamin B12-conjugated nanoparticles might circumnavigate the lysosomal compartment, which is the destination of soluble vitamin B12 ligand. Cellular trafficking of soluble B12 is confirmed to occur via the clathrin-mediated pathway, whilst transport of B12-conjugated nanoparticles appears to predominantly take place by a route that is perturbed by caveolae-specific inhibitors. The work suggests that following surface conjugation of vitamin B12 nanoparticles display a dramatically increased cellular uptake, in addition to an alternative cell trafficking pathway compared to soluble B12. These results may have important implications for oral delivery of nanoparticulate diagnostics and therapeutics.

MULTIFUNCTIONAL MAGNETIC NANO-ASSEMBLIES FOR THE TARGETED DELIVERY OF CISPLATIN TO TUMORS: IN VITRO EVALUATION AND CELL STUDIES

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INTRODUCTION

Cisplatin is a potent anticacer agent. However, it exhibits serious side effects [1] due to lack of selectivity. Therefore, a more selective cisplatin delivery to tumors is pursued by incorporating cisplatin in targetable nanocarriers. In this work, hybrid magnetic nanoasssemblies loaded with ciplatin were prepared and evaluated in vitro. The nano-asssemblies were synthesized through grafting of poly(methacrylic acid)-graft-poly(ethyleneglycol methacrylate) (p(MAA-g-EGMA) on magnetite nanocrystallites. Poly(ethylene glycol) chains should confer to the nanocarriers bio-repellent properties. The formation of a distinct second inner polymeric corona with an abundance of carboxylate groups provides the binding sites for cisplatin molecules.

METHODS

Synthesis of Polymers

Different types of p(MAA-g-EGMA) copolymers with varying length and density of PEG chains were synthesized as described previously [2].

Preparation and characterization of nano-assemblies

Core-shell magnetic nano-assemblies were prepared by hydrolytic alkaline precipitation from a single iron molecular precursor in the presence of the above p(MAA-g-EGMA) copolymers [3]. The nanoassemlies were loaded with cisplatin by incubating them with cisplatin solutions of different cisplatin concentration. Non-entrapped cisplatin was removed by centrifugation and redispersion of the nano-assemblies in cisplatin free aqueous medium. The loading of the prepared magnetic nanoparticles with cisplatin was determined using atomic absorption spectroscopy and UV-Vis spectrophotometry with appropriate color developing reagents. The nanoassemblies were characterized with regard to their size and zeta potential at different salt concentrations using dynamic light scattering (DLS). The effect of pH on cisplatin loading was investigated. Drug release studies were performed in phosphate buffered saline (pH 7.4) at 37°C. The influence of applying an AC magnetic field on the release profile of cisplatin was also investigated using a homemade AC (400 kHz) magnetic field generator 5 mT.

Cell studies

The cytotoxicity of blank and cisplatin-loaded nano-assemblies against A549 human lung cancer cell line was assessed by flow cytometric measurement of cellular fluorescence after staining with propidium iodide (PI). The cellular uptake of the magnetic nano-assemblies was quantificated, as well. For this purpose, the magnetic nanoparticles were surface loaded with propidium iodide.

RESULTS

The chemical structure of the p(MAA-g-EGMA) copolymers synthesized is shown in Figure 1 and their structural properties are presented in Table 1.

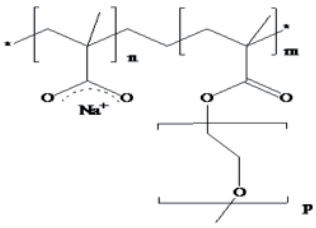

Figure 1. *Chemical structure of p(MAA-g-EGMA) copolymers.*

Table 1. *Structural characteristics of p(MAA-g-EGMA) copolymers used to prepare the nanoasemblies.*

Polymer	Length of chain p	Density of chains m:n	Mw (g/mol)	n	m	%n	Mol anionic sites
102-6	102	1:6	67000	76.8	12.8	12.4	$1.1\ 10^{-3}$
23-6	23	1:6	18900	64.3	10.7	36.7	$3.4\ 10^{-3}$
10-1	10	1:1	47000	86	71	19.74	$1.8\ 10^{-3}$
102-2	102	1:2	78000	32.4	16.2	4.48	$4.2\ 10^{-4}$

The hydrodynamic diameter of the nanoasssemblies was in the range of 100–150 nm depending on the length and density of the polyether chains. All nanoparticles types exhibited high colloidal stability and did not aggregate even in media of high ionic strength (2M NaCl). Relatively high cisplatin loading (8%) was obtained at pH 10 with the 23-6 nanocarriers having the highest carboxylate molar content (Figure 2 left panel). Sustained drug release from the nanoassemblies was observed, which could be triggered by AC magnetic field (Figure 2 right panel) indicating the magnetic responsiveness of the nanoassemblies. The blank magnetic nanoparticles did not show cytotoxicity. In the contrary, the cisplatin-loaded nano-assemblies exhibited comparable to free cisplatin cytotoxicity against A549 cancer cells , which was correlated to the degree of uptake of the different types of nano-assemblies by the cells and cisplatin release (Figure 3).

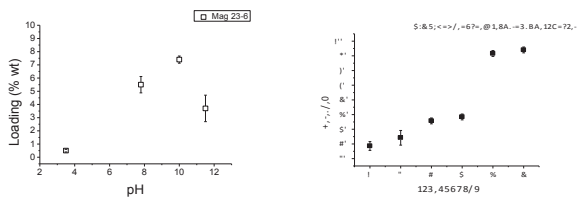

Figure 2. *Effect of pH on cisplatin loading in 23-6 nanocarriers (left panel) and magnetically triggered cisplatin release from 23-6 nanocarriers (after the first 4 hours, an AC magnetic field (4 kA/m, 400 kHz) was applied for the last 2 hours).*

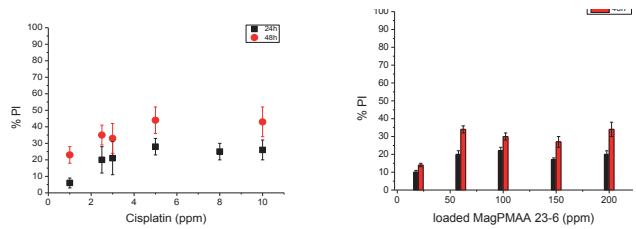

Figure 3. *Cytotoxicity (%PI) of cisplatin after 24 and 48 hours incubation (left panel) and cytotoxicity of cisplatin-loaded 23-6 nano-assemblies at the same concentrations on a cisplatin basis (right panel).*

CONCLUSIONS

The multifunctional p(MAA-g-EGMA) nanocarriers of cisplatin exhibit high colloidal stability, satisfactory drug-loading effficiency, magnetcally-triggered drug release and in vitro anticancer activity against A549 cancer cells comparable to free cisplatin. Thus, these nanocarriers appear to be promising candidates for magnetically-targeted cisplatin delivery to tumors and deserve further in vitro and in vivo evaluation.

REFERENCES

- C. A. Rabik and M. E. Dolan, Cancer Treatment Reviews 2007, 33, 1.
- F. Winnefeld , S. Becker , J. Pakush , T. Gotz , Cement Concrete Comp.2007 , 29 , 251.
- A. Bakandritsos , A. Papagiannopoulos , E. N. Anagnostou ,K. Avgoustakis, et al. Small 2012 8,15.

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CRIPECÒ DOCETAXEL – NANOMEDICINE WITH IMPROVED EFFICACY IN ONCOLOGY

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Cristal Delivery has developed a proprietary technology (CriPec®) that redesigns drug molecules into nanoparticulate compounds, thereby creating nanomedicines with improved efficacy and with acceptable safety and tolerability. As the Company's first product, CriPec® docetaxel has been designed and this product is currently in preclinical development in oncology indications. CriPec® docetaxel is differentiated from other nanomedicines; it entraps docetaxel covalently within stabilised nanoparticles, and entrapped docetaxel is subject to release by hydrolysis. By the nature of the distribution profile of the 70 nm sized nanoparticles, CriPec® docetaxel is designed to target drug delivery to the tumour, and to allow sustained exposure of the tumour to docetaxel. Consequently, CriPec® docetaxel is in development as a product with an improved efficacy/safety balance compared to conventionally administered docetaxel (Taxotere), potentially allowing higher dose levels of docetaxel.

CriPec® docetaxel is designed for the treatment of various solid tumours, with gastric adenocarcinoma as indication of current, initial focus. CriPec® docetaxel, dosed each 3 weeks (Q3W) as intravenous (i.v.) bolus, is aimed to have a superior therapeutic efficacy in cancer, compared to Taxotere, dosed Q3W as 1-hour infusion. At the dosing regimen with superior efficacy, CriPec® docetaxel is aimed to have a similar safety and tolerability profile as Taxotere standard treatment. By absence of polysorbate surfactants, CriPec® docetaxel is not expected to require premedication with corticosteroids or antihistamines.

CriPec® docetaxel releases only parent docetaxel over a period of more than a week, in a well controlled, predictable and reproducible manner. The nonclinical efficacy profile of CriPec® docetaxel is currently evaluated in vivo in xenograft subcutaneous tumour mouse models, including gastric and breast cancer models. In the NCI-N87 gastric cancer model, weekly i.v. doses of CriPec® docetaxel (75 mg/kg; 3 doses in total) resulted in a very much sustained suppression of tumour growth, compared to vehicle and compared to Taxotere dosed at its maximum tolerated dose (MTD) in the model, viz. 25 mg/kg. In the MDA-MB-231 breast cancer model, weekly i.v. CriPec® docetaxel (45 mg/kg; 3 doses in total) completely abolished tumour growth, compared to vehicle. Comparable efficacy was observed with Taxotere dosed at its MTD in the model, viz. 30 mg/kg, and CriPec docetaxel showed a clear advantage over Taxotere in terms of reduced effect on body weight. Findings are consistent with the approximately 50-fold higher tumour uptake of total docetaxel with CriPec® docetaxel compared to Taxotere, and with sustained presence of docetaxel for at least 96 hours after dosing.

Data to date support the potential of CriPec® docetaxel as novel, i.v. dosed, nanomedicinal product in oncology with essentially improved efficacy/safety balance. Clinical confirmation in a phase I/IIa study in patients with solid tumours is planned for 2014.

In parallel to the above, efforts are ongoing to develop CriPec® nanomedicines for various other oncological indications, and in indications other than cancer.

PEPTIDE-MODIFIED POROUS SILICON NANOPARTICLES VIA COPPER-FREE CLICK CHEMISTRY FOR TARGETING DRUG DELIVERY

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INTRODUCTION

The concept of targeted drug delivery is attractive because it recapitulates some of the advantages of typical application of drugs, such as high local concentrations and low systemic exposure.[1,2] A great number of biomarkers and cell-specific epitopes have been explored to show specific binding to certain antibodies, peptides or small molecules.[2] These differentially expressed biomarkers can be used as docking sites accumulating drug molecules and/or drug carriers at the tumor tissue, namely targeting drug delivery.

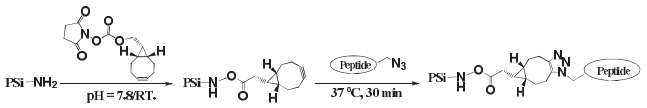
Porous silicon (PSi) nanoparticles have a number of unique properties that make it as a potential drug delivery vehicle, such as high loading capacity, controllable surface chemistry and structure, and controlled release properties.[3, 4, 5] Strain-promoted alkyne-azide click chemistry (SPAAC) as a high specific and efficient reaction has become a great deal of interest in developing new materials by introducing biofunctional molecules to biomaterials for different kind of applications.[6] RGD derivative peptides, such as RGDS and iRGD are specifically targeting to integrin avβ3/ avβ5, which are specially expressed by neovascular endothelial cells[7].

In this study, we aimed to efficiently conjugate peptides (RGDS and iRGD) to PSi nanoparticles by SPAAC for targeting therapy.

METHODS

Herein, the cyclootyne moiety was introduced to amine-functionalized PSi nanoparticles by reacting with (1R,8S,9s)-bicyclo[6.1.0] non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate (BCNOSu).

After that, the peptides RGDS and iRGD with azide-terminated group were incorporated into the PSi nanoparticles via SPAAC in aqueous solution (Scheme 1). The physicochemical properties and drug delivery efficiency were then investigated. The endothelial cell line EAhy.926 was used to investigate the targeting and drug delivery potential of the PSi nanoparticles. Cytotoxicity of nanoparticles was determined by an ATP-based CellTiter-GloTM luminescence assay. In vitro cell uptake was evaluated by transmission electron microscopy (TEM), confocal microscopy and flow cytometry. Antiangiogenic drug sorafenib was used as a model drug to study the peptide-modified PSi nanoparticles for drug delivery applications.



Scheme 1. *Peptides (RGDS and iRGD) conjugated to PSi nanoparticles via SPAAC.*

RESULTS AND DISCUSSION

Fourier-transform infrared (FTIR) spectra of the nanoparticles before and after modification were compared (Figure 1 (A)). FTIR

spectra of PSi-BCN, PSi-RGDS and PSiiRGD exhibited a characteristic peak corresponding to amide NC=O bending (1693 cm-1) after reaction of the amine-modified PSi nanoparticles with BCN-OSu, followed by peptided conjugation to the surface of the PSi nanoparticles. Element analysis was used to quantify the conjugation efficiency. The results showed that 15.2% and 3.4% of the surface functional group of the PSi nanoparticles was conjugated with RGDS and iRGD, respectively. The size of the nanoparticles was increased from 166.6 nm (bare PSi) to 179.1 nm (PSi-RGDS) and 193.7 nm (PSi-iRGD) after peptide-modification. The zeta-potential of the PSi nanoparticles changed from +35.1 mV (aminefunctionalized PSi) to +11.3 mV (PSi-RGDS) and +11.2 mV (PSi-iRGD) respectively.
The cell viability studies showed that the PSi nanoparticles have very low cytotoxicity (Figure 1 (B)). In vitro cell uptake of peptide-modified PSi (PSi-RGDS and PSi-iRGD) nanoparticles was evaluated by TEM (Figure 2), confocal microscopy and quantified by flow cytometry (Figure 3).

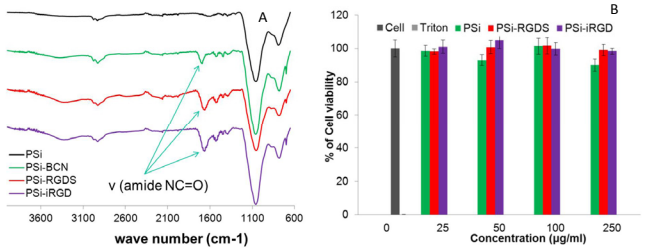


***Figure 1**. (A) FTIR spectra of PSi, PSi-RGDS and PSi-iRGD nanoparticles. Arrows indicate the peaks corresponding to the amide NC=O bond vibration. (B) Cell viability of PSi, PSi-RGDS and PSi-iRGD nanoparticles with EA.hy926 cell line after 6 h incubation at 37 oC determined by the ATP-based luminescence assay. Error bars represent s.d.*

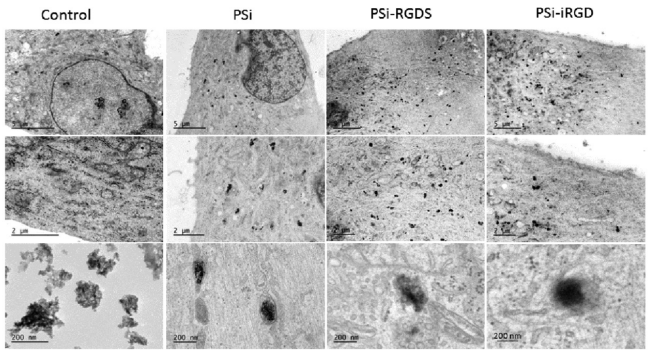


***Figure 2.** TEM pictures of PSi, PSi-RGDS, PSi-iRGD intracellular localization after 3 h incubation with EA.hy926 cells for each nanoparticle at 37 oC. Scale bars are 5 µm, 2µm and 200 nm.*

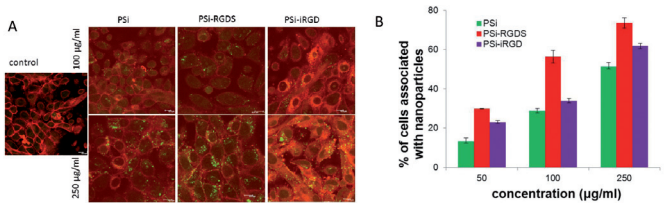


***Figure 3.** (A) Confocal microscopy images of Eahy.926 cell treated with PSi, PSi-RGDS and PSi-iRGD nanoparticles incubated for 3 h and showing PSi nanoparticles' internalization. PSi anoparticles were covalently labeled with FITC (green color), the cell membrane was stained with CellMask Organe (orange color). Scale bars are 10 µm. (B) Flow cytometry quantitative determination for the internalization of PSi, PSi-RGDS and PSi-iRGD nanoparticles, cells were treated with Trypan Blue (0.04% v/v) to quench their surface fluorescence and ten thousand events were evaluated for each measurement. Error bars represent s.d. (n = 2).*

The aforementioned results demonstrated that both peptide-modified PSi nanoparticles (PSi-RGDS and PSi-iRGD) were superior for endothelial cell uptake compared to bare PSi nanoparticles, despite of the lower positive charge of PSi-RGDS and PSi-iRGD nanoparticles. Considering that the nanoparticle's surface positive charge benefits non-specific nanoparticle cell uptake.[8] Our study demonstrated that peptide-modified PSi nanoparticles with less surface positive charge had better cell uptake, which showed that the surface peptide modification enhanced the PSi nanoparticles specific cell uptake.
Next, the antiangiogenic drug sorafenib was loaded to the three PSi nanoparticles. The loading degree of the three PSi nanoaprticles was 5.6% (PSi), 5.5% (PSi-RGDS) and 5.2% (PSi-iRGD),
which showed that the peptide-surface modification by RGDS and iRGD did not influence the drug loading to the PSi nanoparticles.
EA.hy926 cell lines were used to evaluate the in vitro antitumor activity of sorafenib-loaded PSi nanoparticles. The results showed a dose-dependent antiproliferative effect against tumor cells for the sorafenib-loaded PSi nanoparticles, whereas the bare PSi nanoparticles did not significantly inhibit the cell proliferation at the same range of concentrations tested (Figure 4). The antitumor activity of sorafenib-loaded PSi nanoparticles in vitro was similar as sorafenib dissolved in 20 mM DMSO.

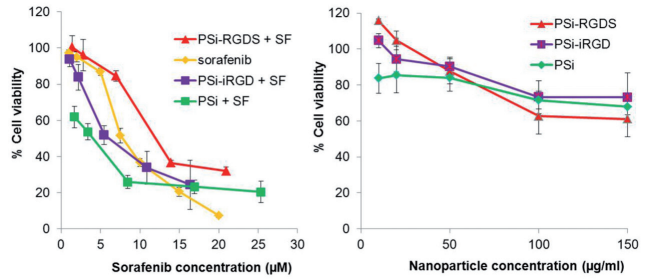


***Figure 4.** Growth inhibition of EAhy.926 cells treated with sorafenib (SF)-loaded PSi nanoparticles. Cells were exposed to pure sorafenib, bare PSi nanoparticles and SF-loaded PSi nanoparticles for 24 h at 37 oC. Pure SF was dissolved in 20 mM DMSO as stock solution. The cell viability of 20 mM DMSO is 82±7%. Error bars represent s.d.*

CONCLUSION

The peptides RGDS and iRGD were successfully conjugated to the amine-terminated PSi nanoparticles via SPAAC. Peptide-modified PSi nanoparticles (PSi-RGDS and PSi-iRGD) showed better cell uptake efficacy than bare PSi nanoparticles. After loading of the antiangeogenic drug sorafenib in PSi-RGDS and PSi-iRGD nanoparticles, similar in vitro antitumor activity was observed as for pure sorafenib dissolved in 1 % DMSO. This peptide-modified PSi drug delivery system can be further investigated for targeted cancer therapy.

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REFERENCES

[1] Ruoslahti E, Bhatia SN, Sailor MJ. J. Cell Biol., 2010, 188, 759–68.
[2] Ruoslahti E. Nat. Rev. Cancer. 2002, 2, 83–90.
[3] Santos HA, Bimbo LM, Lehto V-P, et al. Curr. Drug Discov. Tech. 2011, 8, 228–249.
[4] Bimbo LM, Mäkilä E, Raula J, et al. Biomaterials 2011, 32, 9089-9099.
[5] Liu D, Mäkilä E, Zhang H, et al., Adv. Funct. Mater., 2013, 23, 1893–1902.
[6] Lee DE, Na JH, Lee S, et al. Mol. Pharmaceutics 2013, DOI: 10.1021/mp300601r.
[7] Ruoslahti E. Adv. Mater. 2012, 24, 3747–3756.
[8] Kenzaoui et al. Int. J. Nanomedicine, 2012:7 1275–1286.

NANOPARTICLE-MEDIATED GENE DELIVERY SYSTEMS FOR SAFE CLINICAL APPLICATIONS

NADINE WILHELM

Mainly, cells for use in cell and gene therapy are modified by integrating viral vectors, which introduce therapeutic transgenes into the host genome and therefore carry potential safety risks for patients. A safer alternative to viral vectors are synthetic nanoparticle based systems for gene delivery. Therefore, biodegradable, ligand functionalized and cell specific nanoparticular delivery systems for enhanced gene transfer are developed, optimized and evaluated. These human serum albumin (HSA) based nanoparticles (NP) are loaded with DNA-vectors encoding either a suicide gene for the use in cell and cancer therapy or transcription-factors for the reprogramming of primary somatic cells to pluripotent stem cells (iPSC). After the transfection of difficult-to-transfect cells like adult stem cells and primary cells with the DNA-loaded NP, the transfection efficiency as well as the cellular uptake, intracellular distribution and degradation is evaluated in vitro. First nanoparticles generated were plasmid-loaded unmodified HSA-NP which showed a limited cell associated binding ability on the different tested cell types and were not taken up by the cells. Improvement of the nanoparticular system was required. To achieve a targeted binding and specific uptake of the DNA-loaded nanoparticles, the NP have to be modified with ligands for cell specific receptors. RGD-modified nanoparticles showed a binding to human mesenchymal stem cells (hMSC) by flow cytometry analysis as well as cellular uptake of the HSA-nanoparticles detected by Confocal Laser Scanning Microscopy (CLSM). Both applications, stem cell based cancer therapy as well as iPSC generation, have an enormous clinical impact and the use of non-viral nanoparticle based gene delivery systems, being a prerequisite for safer clinical application, are opening up broad future possibilities and markets.

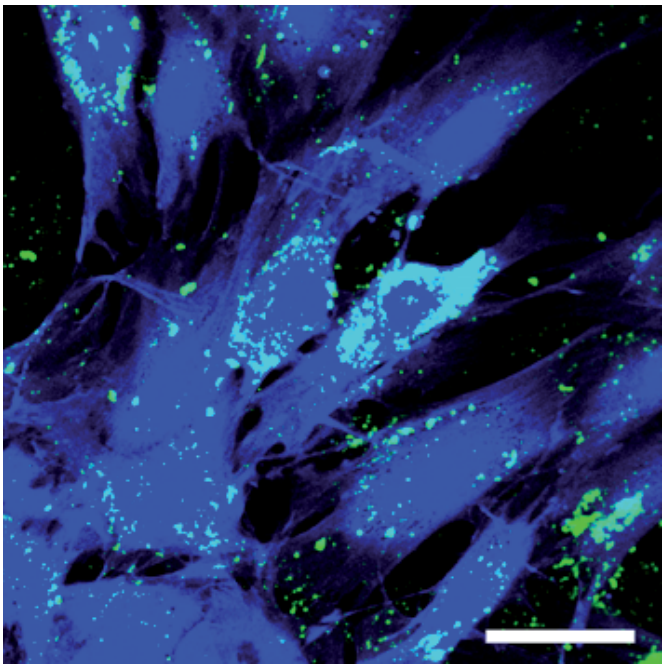

Figure 1. *Uptake of RGD-modified HSA-nanoparticles CLSM image of hMSC incubated for 24 hours with RGD-modified HSA-nanoparticles. The cytosol was stained with CellTracker™ blue (blue) and the HSA-nanoparticles were detected by their green autofluorescence (green). The scale bar is 50 µm.*

SYNTHESIS AND EVALUATION OF A γ-CYCLODEXTRIN DERIVATIVE FOR DOXORUBICIN DELIVERY

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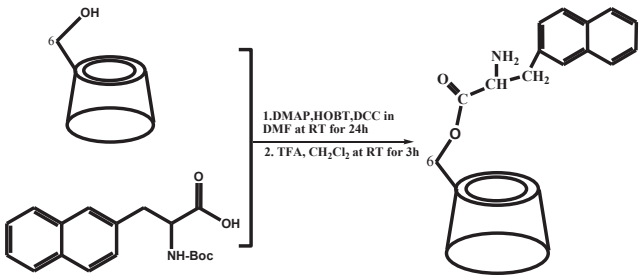
BACKGROUND AND PURPOSE

Cyclodextrins (CDs) have a variety of applications in pharmaceutical industry due to their complexation ability and other versatile characteristic.1 One of the most noteworthy facts is the application of CDs as drug carriers in controlled release.2, 3 In the present study, we aimed to design and synthesize a γ-CD based encapsulating vehicle for chemotherapeutic agent Doxorubicin (Dox) by chemical modification of the hydroxyl group of γ-CDs at the 6-position by a naphthalene containing molecule 2-((tert-butoxycarbonyl) amino)-3-(naphthalen-2-yl) propanoic acid. The rationale was that the π-π interactions between naphthalene and the aromatic ring of Dox would lead to enhanced drug binding hence improved encapsulation and delivery. The binding kinetics and the stability of the inclusion under varing temperatures and pHs were investigated.

METHODS AND MATERIALS

NMR spectra of Dox, the synthesized carrier and the drug-carrier complex were recorded on Bruker 400 MHz Advance III spectrometer with deuterated water (D2O) as a solvent. Fluorescence spectra were measured by fluorescence spectrophotometer (Agilent Technologies, USA Model Varian Cary Eclipse) at λex = 485.00nm, λem = 592.05nm.4, 5 The drug-carrier binding constant was determined by measuring fluorescence intensity of Dox using Tecan infinite M200 multi-function plate reader. The temperature-dependent drug release from the inclusion was assessed by dissolving the complex in PBS solution and by measuring the fluorescence intensity of Dox under differing temperatures. pH responsiveness of the drug-carrier compound was evaluated by dissolving the complex in different pH buffer solutions of 0.2M and 0.1M dibasic sodium phosphate, 0.1M monobasic sodium phosphate and 0.1M citrate acid monohydrate (Calbiochem). In vitro cell viability and drug uptake experiments in KB cells (human endothelial carcinoma, ATCC, USA) were performed using MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay and by measuring the fluorescence signal of intracellular Dox, respectively.

Synthesis of the γ -Cyclodextrin derivative:



RESULTS

Structure of carrier and carrier-Dox inclusion: The synthesized carrier contains a γ-CD backbone and a –CO–CH (NH2)–CH2–β–naphthalene side chain substituting the hydrogen atom of the hydroxyl group at the 6-position in the primary face of γ-CD. The side chain can be considered as a flexible cap equipped to γ-CD.6 The NMR data of 1H-NMR, COSY and NOESY (data not shown) and structural simulation by Arguslab 4.0.1 suggested that the encapsulation of Dox occurs inside of carrier's cavity (Fig.1a).

Binding affinity: The binding kinetics was analyzed at γ-CD/carrier ratios between 1000:1 and 0.13:1 at 37° C. The encapsulation efficiency was calculated according to the equation: Encapsulation%=100 × (I0-Ix)/I0. Where, I0 is the value of fluorescence intensity of Dox in the absence of carrier deducted by the background reading of blank control (PBS solution); Ix is the fluorescence inten-

sity of Dox in the presence of various concentrations (x) of carrier deducted by auto-fluorescence generated by the same concentrations of carriers alone in PBS. Encapsulation efficiency is increased up to 93% by the modified carrier compared the un-modified one, which was exponentially dependent on the carrier's concentration (up to 0.001M) or the ratio of carrier/Dox (up to 200), and reached a plateau thereafter (Fig.1b).

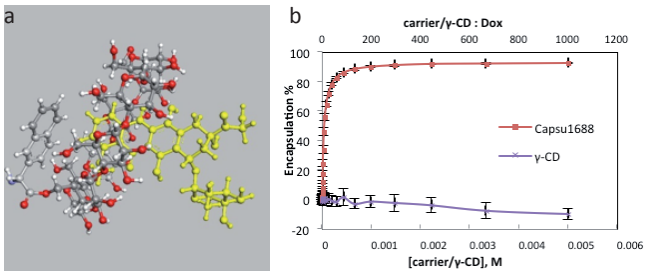


Fig. 1 *a) 3D modelling of drug-carrier inclusion; b) Comparison of binding affinity with Dox (5µM) of unmodified γ-CD and modified γ-CD carrier.*

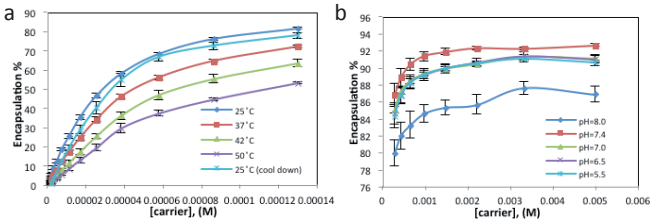


Fig.2 *a) Encapsulation efficiency of carrier for Dox (5µM) at different temperatures ; b) Encapsulation efficiency of carrier for Dox (5µM) in different pH buffer solutions.*

Thermal/pH stability of the drug-carrier complex: The encapsulation affinity of the carrier was reduced with increasing temperature, indicating that Dox was released from the complexation (Fig. 2a). Furthermore, a liner correlation can be obtained between 1/[carrier] and I0 / (I0-Ix), intercept and slope of the liner correlation were utilized for calculation binding constant of drug-carrier by K=Intercept/Slope. Where, K values were decreasing from 34433(25ºC) to 9960 (50ºC). These results suggested that the encapsulation of Dox by the novel carrier is thermal sensitive. The drug-carrier inclusion also demonstrated a pH-dependence as shown in Fig. 2b, with the highest encapsulation efficiency occurred at pH 7.4 and the lowest under the basic environment (pH 8.0). Small but significant amount of Dox was released in pH 7.0, 6.5 and 5.5 buffers compared with that of the neutral environment, indicating that the synthesized carrier has the pH-sensitive capacity of delivering Dox in weak acidic environment.

Cellular uptake of Dox from the inclusion: Inclusion of the drug by the carrier significantly reduced the toxicity of Dox to KB cells, over 75% of the cells was viable at the dose of Dox up to 100µM under which the free drug caused sharp reduction in cell viability (Fig.3a). Drug cellular uptake was decreased by 94% by the encapsulation as compared with free Dox where the uptake was assumed as 100% (Fig.3b), consistent with the binding affinity results.

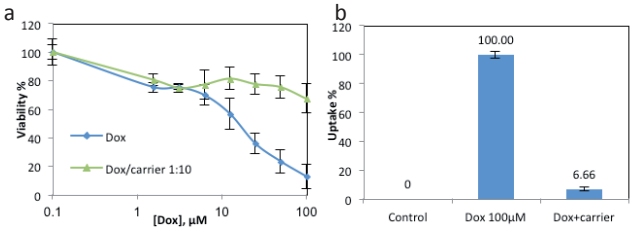


Fig.3 *a) KB cells viability test by Dox:carrier =1:10; b) Dox uptake investigation by free Dox and Dox-carrier inclusion*

CONCLUSION

Our study of γ-CD based novel carrier showed a much stronger encapsulation to Dox in comparison to unmodified γ-CD. Molecular modelling suggested a good fit of size, shape and binding of the carrier with Dox. Binding constant in the range of 104-105 was estimated for the drug-carrier complex by spectroscopic (fluorescence) analysis. Cell culture data also confirmed the synthesized compound as a potent encapsulating carrier for Dox to reduce its toxicity. This work will provide important information for the targeted delivery and controlled release of Dox in the future studies.

Acknowledgement: This work is supported by EU FP7 grant 'Nanoporation' (no. 230674). We acknowledge funding from the Northern Research Partnership (NRP).

REFERENCES

1. R. Arun, Scientia Pharmaceutica, 2008, 76, 567-598.
2. M. Tafazzoli and M. Ghiasi, Carbohydrate Polymers, 2009, 78, 10-15.
3. M. E. Brewster and T. Loftsson, Advanced Drug Delivery Reviews, 2007, 59, 645-666.
4. E. H. Z. T. Kerry K. Karukstis, Jennifer A. Whiles, and R. J. Rosenfeld, Biophysical Chemistry, 1998, 73, 249-263.
5. X. Dai, Z. Yue, M. E. Eccleston, J. Swartling, N. K. Slater and C. F. Kaminski, Nanomedicine : nanotechnology, biology, and medicine, 2008, 4, 49-56.
6. E. Engeldinger, D. Armspach and D. Matt, Chemical reviews, 2003, 103, 4147-4174.

DEVELOPMENT OF CHITOSAN/siRNA NANOPARTICLES FOLLOWING QBD PRINCIPLES; STRUCTURE/FUNCTION RELATIONSHIPS IN VITRO AND VIVO

KEWEI YANG[a], Pascale Holzerny[a,b], Baskim Ajdini[a], Wolf Heusermann[c], Katharina Bruno[a], Michael Schuleit[a], Lorenz Meinel[b] & Michael Keller[a]

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To date delivering siRNA to the biologically relevant target remains a key challenge. To this end, we investigated the use of fungal chitosans from agaricus bisporus as biocompatible cationic polymers to develop siRNA/chitosan nanoparticles. By applying QbD principles.- the focus of this concept is that quality should be built into a product with a concise understanding of the product and process - we endeavored to thoroughly understand starting material properties, formulation process and nanoparticle morphology and relate these parameters to in vitro and in vivo bioactivity.

Starting with a carefull characterization of four chitosans with different molecular weights (44–143kDa) using a gel permation chromatography (GPC) we then devised a formulation protocol that consisted of low N:P (4-8) ratios to generate bioactive nanoparticles. The effect of average molecular weight, polydispersity, hydrodynamic radius, intrinsic viscosity of the chitosans, thermodynamics of the chitosan/siRNA interactions, intracellular trafficking studies and morphological properties of the nanoparticles were investigated and related to in vitro bioactivity in human epithelial cell line H1299. This led to the identification of a superior formulation (44kDa chitosan/siRNA nanoparticle) which was investigated in vivo at 1mg/kg siRNA using oral delivery and a preliminary PK profile and PD readout established in mice.

CLINAM 6/13 SATELLITE SESSIONS
SWISS NATIONAL SCIENCE FOUNDATION
DEUTSCHE FORSCHUNGSGEMEINSCHAFT
SWISS COMPETENCE CENTRE TEDD

CLINAM Satellite Session 1

This is a Satellite Event within the CLINAM 6/13 Summit, organized by the Swiss National Science Foundation and the Deutsche Forschungsgemeinschaft. It is held on Tuesday, June 25 in Hall Osaka-Samarkand as Session 19 (10 -13 am) within the Summit Programme. All participants of the Summit are invited to join this Session that gives excellent overview on the activities of the programmes in Germany and Switzerland.

Biological Responses to Nanoscale Particles

The Priority Programme1313 of the Deutsche Forschungsgemeinschaft (DFG) aims at • the manufacturing and characterization of NPs • the transition of NPs into an interaction with the biological environment • the impact of NPs on fundamental biological functions.

The Swiss Research Programme 62 of the Swiss National Science Foundation aims at • developing new intelligent materials and combinations of such with new functions • establishing their application potential for various sectors of industry • serving as a model for future cooperation between SNSF and CTI.

The Swiss National Research Programme 64 of the Swiss National Science Foundation aims at • identifying opportunities arising from the use of nanomaterials for health care, the environment and natural resources. • revealing the potential risks that nanomaterials pose • developing tools that maximize the advantages of nanomaterials and minimize the risks for humans and the environment • enhancing expert knowledge and competencies for developing innovative nanomaterials and assessing risk in Switzerland.

10.10 ***German Priority Programme SPP 1313***

Chair **Prof. Dr. rer. nat. Dr. h.c. Reinhard Zellner,** Senior-Professor of Physical Chemistry, University of Duisburg- Essen and Coordinator of SPP1313, Essen (D)

Impact of the Nanoparticle - Protein Corona on Biomedical Applications
Prof. Dr. Roland Stauber, Molecular and Cellular Oncology/*ENT Department*/University Medical Center of the Johannes-Gutenberg University Mainz (D)

Quantitative Fluorescence Studies of Nanoparticles interacting with Proteins and Cells
Prof. Dr. G. Ulrich *Nienhaus,* Karlsruhe Institute of Technology (KIT) and Center for Functional Nanostructures (CFN), Karlsruhe (D)

The Fate of Nanoparticles in Vivo
PD Dr. Peter Nielsen, Head Department of Biochemistry and Molecular Cell Biology Universitätsklinikum Hamburg-Eppendorf, Hamburg (D)

11.05 ***Swiss National Research Program 62***

Chair **Prof. Dr. Louis Schlapbach,** President of NRP 62, Berne (CH)

Nanoparticle Transport Across the Human Placenta
Dr. Peter Wick, Group leader, Empa Swiss Federal Laboratories for Material Science and Technology, Laboratory for Materials-Biology Interactions, St. Gallen, Switzerland

Mechano-sensitive Nanocontainers for Innovative Targeted Drug Delivery
Dr. med. Till Saxer, Cardiology Department, University Hospitals of Geneva, Switzerland

Polymer Nanoparticles for Drug-delivery and Localized Synthesis of Antibiotics.
Dr. Nico Bruns, Chemistry Department of Chemistry, University Basel (CH)

12.05 ***Swiss National Research Program 64***

Chair **Prof. em. Dr. Peter Gehr**, University of Berne, President of NRP 64, Berne (CH)

On the Relationship between Cell Entry and Nanoparticle Toxicity
Prof. Dr. Francesco Stellacci, Institute of Materials, EPFL Lausanne (CH)

Biomedical Nanoparticles as Immune-modulators
PD Dr. med. Christophe von Garnier, Department of Clinical Research, University of Berne (CH)
Transport of Nanoparticles after Release from a Biodegradable Implant
Prof. Dr. med. vet. Meike Mevissen, Division Veterinary Pharmacology & Toxicology, Vetsuisse University of Berne, Berne (CH)

CLINAM Satellite Session 2

This is a Satellite Event within the CLINAM 6/13 Summit, organized by the Swiss Competence Centre TEDD in Zürich, Switzerland. It is held on Wednesday, June 26 in Hall Rio as Session 30 (09 -13 am) within the Summit Programme. All participants of the Summit are invited to join this Session that gives excellent overview on the research and findings of the Swiss Competence Centre TEDD and their related researchers.

CLINAM Satellite (Hall Rio)

Tissue Engineering for Drug Development and Substance Testing

Chair **Prof. Dr. Ursula Graf-Hausner**, Zürich University of Applied Sciences, Wädenswil (CH)

09.00 **3D Tissue Models for Drug Development and Substance Testing – an Overview**
Prof. Dr. Ursula Graf-Hausner, Zürich University of Applied Sciences, Wädenswil (CH)

Innovative Technologies for 3D Cell Culture - Biomimetic Materials
Dr. Martin Ehrbar, Zürich University, Zürich (CH)

Bioprinting – a Promising Approach for 3D Tissue and Organ Fabrication
Marc Thurner, CEO of regenHu Ltd., Villaz-St.-Pierre (CH)

3D Liver Microtissues to Test Nanoparticle Toxicity
Dr. Jens Kelm, CSO of InSphero AG, Zürich (CH)

Break (10.00 – 10.30)

Infectious Disease Model in Cell Cultures
Dr. Bruno Schnyder, University of Applied Sciences and Art, Western Switzerland, Sion (CH)

A Novel Approach to Print an Air-blood Tissue Barrier
Prof. Dr. Barbara Rothen-Rutishauser, Chair Bionanomaterials, and **Dr. Corinne Jud**, Adolphe Merkle Institute, Université de Fribourg, Marly (CH)

Co-culture of Human 3D Liver and Skin Equivalents in a Dynamic Two-tissue Microcirculation Chip
Dr. Uwe Marx, Program Head "Multi-Organ-Chips", Technical University, Berlin and CEO TissUse, Berlin (D)

3D Microtissues for Regenerative Medicine and their Application in Cardiovascular Surgery
Prof. Dr. Dr. Simon Philipp Hoerstrup, Head Cardiovascular Research and Swiss Center for Regenerative Medicine, Zürich University, Zürich (CH)

PORTRAITS OF THE EXHIBITORS

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BASEL INKUBATOR is a venture of the University of Basel, the University of Applied Sciences of Northwestern Switzerland FHNW, the Canton of Basel-Stadt, and "EVA - the Basel life sciences start-up agency". It opened its doors in January 2010 and was established to support high-tech spin-offs of the Universities in the region. Other applicants are welcome as long as there is space available. The location at the Stücki Business Park ensures an optimal access with public transport as well as by car.

Start-up projects are supported already in a very early phase. Typically, the first contact is already established before the decision to create a start-up is made. The BASEL INKUBATOR provides an affordable infrastructure (offices and laboratories) and coaching in various fields. A start-up company can stay up to three years within the BASEL INKUBATOR. The aim is that a company can successfully build up in this environment until either first sales are established or a major investment round is successfully closed which provides the financial resources to further develop.

Currently the BASEL INKUBATOR houses 5 life sciences companies, 4 ICT projects, 3 Med Tech company and one Nano-/Greentech company. There is apparently a positive trend to convert research result into an own business. The common infrastructure fosters a productive interaction where the start-up companies profit from each other's experiences.

The BASEL INKUBATOR is part of a interwoven network in the Basel area which is pushing the spirit of innovation in all domains. Additional needs of start-up companies like e.g. financing are covered within this network.

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Our technology consists in the conjugation of inorganic nanoparticles to selected active molecules. That way we create innovative materials with unique biological properties.

We apply our versatile and high technologic potential R&D platform in nanotechnology to develop products oriented to very diverse sectors, from oncology to dermatology.

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We have the facilities and know-how to fully characterize the active ingredient behavior in biological media, study how they interact with cells, what cell mechanisms are involved and how to ensure product security. The objective is to get the best candidate from the security and efficacy points of view.

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In 1956, IBM Research established its first West Coast laboratory in San Jose, helping to create what would eventually become Silicon Valley. In 1986, IBM Research - Almaden became home to a rapidly growing team of scientists and researchers. IBM Research - Almaden boasts a rich history of breakthroughs that include the distributed relational database; the ability to position individual atoms; the first data mining algorithms; the IBM Microdrive – the world's smallest disk drive; racetrack memory; and innovations in data storage technology. Today, the researchers there are focused on new breakthroughs in areas as diverse as nanomedicine, services science and storage at the atomic scale.

IBM Research – Australia was established in October 2010, and is located in the University of Melbourne precinct, on the fringe of the Melbourne Central Business District. The lab began operation in the first half of 2011 and encompasses the IBM Research Collaboratory in Life Sciences - Melbourne, which is located at the Victorian Life Sciences Computation Initiative (VLSCI). Projects at IBM Research – Australia include work in natural resource management, disaster management, bio-nanotechnology, life sciences and healthcare.

Izon Science

- Izon Science designs and manufactures precision instrumentation for multi-parameter measurement of nano- and micro- sized particles.

- Izon instruments use unique nanopore-based detection to enable the size, charge and concentration of 50nm 20 micron sized particles to be measured on a particle-by-particle basis.

- The level of detail, accuracy and repeatability in measurement enables Izon's systems to meet the high standards required for nanomedicines going to clinical trials and into routine medical use.

- Izon originated in New Zealand and now sells its products in 35 countries. It has its European headquarters in Oxford, UK and its US headquarters are in Cambridge, MA.

APPLICATIONS

The underlying measurement technique known as Tunable Resistive Pulse Sensing (TRPS) has been applied to enable high resolution analysis of a wide range of particle types in fields including:

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  E.g. Liposomes, Nanobubbles, Polymeric Drug Delivery

- **Virus Quantification / Vaccine Production**
  E.g. Viral Vaccines, Adenovirus, Lentivirus

- **Microvesicle Research & Haematology**
  E.g. Microparticles & Exosomes, While Blood Cells, Platelets

PRODUCTS

Two instruments based on TRPS technology are currently commerically available:

- **qNano** is a compact benchtop device for highly precise physical characterization (size, zeta-potential, concentration) of a wide range of particle types.

- **qViro-X** is a purpose-built virus analysis instrument ideal for assessment of viral titre and aggregation. It meets stringent decontamination requirements ideal for manufacturing and quality control environments.

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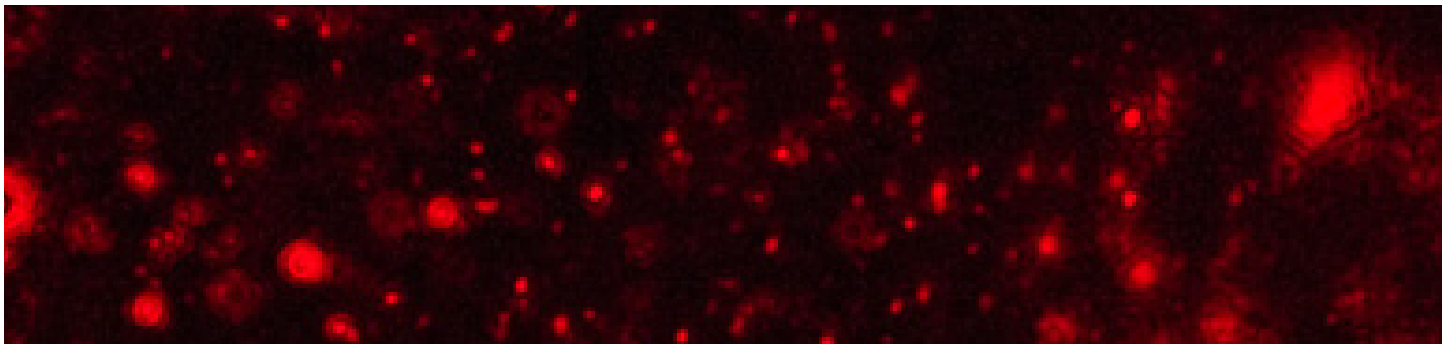

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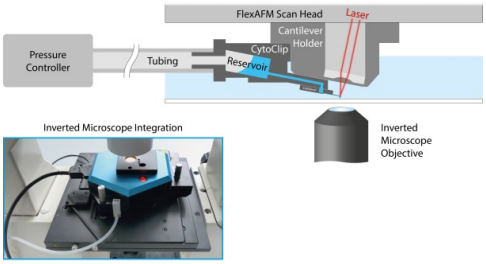

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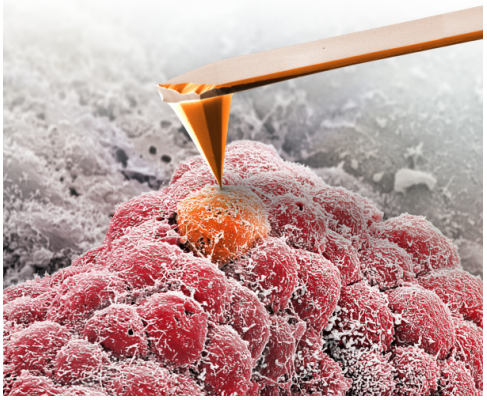
When a patient is suspected of cancer, time-consuming tissue analyses have thus far been necessary to obtain a reliable diagnosis. With ARTIDIS®, lengthy waiting times can now be reduced from days to hours. Nanomechanical measurements on suspect tissues provide a characteristic "fingerprint", which allows classification into healthy tissue, benign material, or malignant tumor [2].



References
1. Loparic M. *et al.* (2010), Biophysical Journal 98, 2731–2740.
2. Plodinec M. *et al.* (2012), Nature Nanotechnology 7, 757–765.

First devices are currently being installed in expert laboratories to acquire data on various tissue types and to develop a user-friendly diagnostic instrument.

Polymun Scientific - Biopharmaceuticals and Liposomes Contract Development and Manufacturing

Polymun Scientific GmbH is a family-owned company in Klosterneuburg, Austria, founded in 1992. Our core activities are contract development and manufacturing of biopharmaceuticals as well as liposomal formulations of APIs and vaccine antigens.
Polymun operates in accordance with current GMP guidelines and holds an Austrian production license thus meeting all EU requirements for drug manufacturing.
Polymun employs a team of more than 50 dynamic and highly qualified scientists, technologists and support staff. 45% hold an academic title, and about 45% have graduated from technical schools or colleges. With quality and reliability as a pre-requisite basis, innovation and creativity are strongly encouraged at Polymun. We are a flexible partner focused on the requirements of our clients.

Liposomal formulations

Polymun offers the development of liposomal formulations for all kinds of active pharmaceutical ingredients and vaccine antigens. We manufacture GMP-material, including all necessary documentation, and assist in planning of clinical trials.
License agreements for Polymun's patented liposome production technology are offered for the respective substance on an exclusive basis. Contracts can be arranged step by step - proof of concept, in-depth analysis, GMP-material production, product license - or all in one. Industrial applicability is the focus throughout each project.
Polymun's technology enables the industrial realization of pharmaceutical and cosmetic products for liposomal drug formats. The production technology is suitable for a broad range of substances formulated by passive entrapment, active loading or membrane incorporation.
The main characteristics of our technology are: Scalability, sterility, production of homogeneous, uniform vesicles, excellent batch to batch consistency and long term stability due to a mild procedure.

Contract development and manufacture of biopharmaceuticals

The core competence of Polymun is the development and GMP-compliant manufacture of bio-pharmaceuticals, using both mammalian and microbial cell technology. Polymun offers all steps from the gene to clinical grade material for innovator and generic products.
Contract manufacturing includes the preparation of IMPD and CMC documents. Based on our own experience we are able to support planning of phase I-III clinical trials.
As a small, independent and private company Polymun has no conflict of interests thus granting a high degree of flexibility and security for its clients including the possibility of technology transfer.

Capacity: Mammalian cells	- Stirred tank: 15l, 240l, 2 x 2,500l
	- Ultrasonic cell retention: up to 100l continuous perfusion culture
Yeast / bacteria	- Stirred tank: 50l, 750l
Downstream purification	- Process systems up to 600l/hr and 6 bar

Contact Details:

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Tel: +43 2243 25060 300 Fax: +43 2243 25060 399 office@polymun.com www.polymun.com

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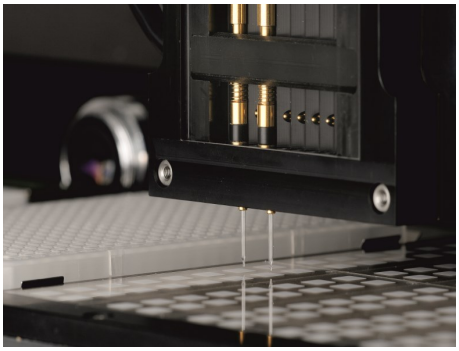

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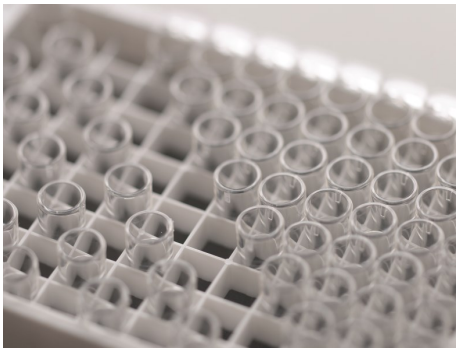

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SeroScience Ltd.

SeroScience Ltd. (www.seroscience.com) is a well-known Hungarian biotech company specializing in **immunotoxicology** and **liposomal formulation services**. SeroScience is a pioneer in studying pseudoallergic reactions (infusion reactions) to **nanomedicinal formulations**.

Immunotoxicology is a fast growing segment of drug safety testing and the most dominant toxicity of biologics (therapeutic antibodies, targeted liposomes, therapies based on nucleic acid) as well as imaging agents. Immunotoxicology is mandatory in drug development. **Nanomedicine** develops formulations in the nanoscale and is the most promising segment of present day drug development.

The Company's services contain both *in vitro* and *in vivo* testing. The pseudoallergy test panel is the **most complete testing portfolio in the market**.

Gewerbestrasse 10, 4450 Sissach, Switzerland, +41 (0)61 985 81 00, info@tecomedical.com, www.tecomedical.com

Biosafety testing

Medical devices, transplants, implants, pharmaceuticals and blood products require safety testing for their compatibility with human tissue and blood. The testing requirements are guided by regulatory agencies and notified bodies like FDA, EMA and ASTM.

Nanomedicine (e.g. liposomal drugs, polymer-protein conjugates, imaging agents, drug carrier nanosystems) and antibody therapeutics are in the frontline of modern pharmacotherapy but also carry unique toxicity issues. Stimulation of the immune system can cause hypersensitivity or infusion reaction, a major and potentially lethal haemoincompatibility. Infusion reactions can represent a real allergy (involving IgE) or a pseudoallergy (no IgE). The latter may arise at least in part, as a consequence of activation of the complement (C) system = C activation-related pseudoallergy (CARPA).

TECOmedical offers a range of biosafety assays and Test protocols for:

- Haemocompatability related to activation of the complement (C) system – Anaphylatoxins. Human and animal models.
- Complement C activation related to pseudoallergy (CARPA)
- Cytotoxicity

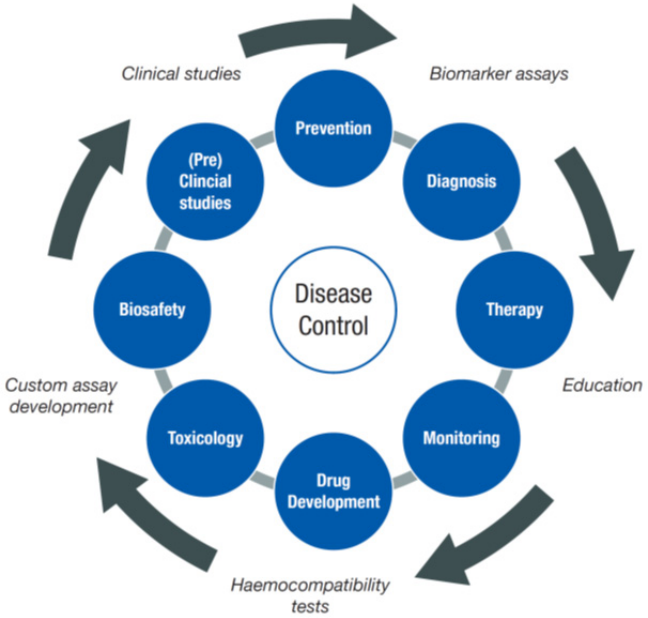
Toxicity testing:
drug-induced liver and kidney injury

Predicting which drugs will prove toxic to the liver and kidney is an important aspect during drug development. Current standards for liver and kidney injury have severe limitations and are not sensitive and specific enough. In order to reduce the risk of failure of new medicines, the European Innovative Medicines Initiative (IMI) and C-Path's Predictive Safety Testing Consortium (PSTC), a program run in partnership with the Food and Drug Administration (FDA) are validating new biomarkers for liver and kidney injury. The new biomarkers are validated for clinical application, regulatory decision making in clinical drug development and in a translational context.

TECOmedical offers a range of assays for monitoring drug-induced liver and kidney injury in:

- Drug development toxicity studies using kidney and liver cell culture models
- Preclinical toxicity studies in animals (rat, mouse)
- Clinical toxicity studies in humans

TECOmedical offers customized test development based on ELISA technology, e.g. immunogenicity and host cell protein assays. As well as scientific and regulatory compliance.



to-BBB technologies BV

Contacts

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Chief Executive Officer

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Telephone

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+31 84 8313409

Email

WillemVanWeperen@tobbb.com

Year Founded

2004

Financial Summary

Seed 2004-2006: ±€1M
Series-A 2007: ±€4M
Series-B 2011: ±€6M
Interim round 2013: ±€2.5M

Accum. grants/credits: ±€5M

Company Profile

to-BBB is a clinical stage blood-brain barrier company in the Netherlands with a proprietary technology (G-Technology®) to safely enhance brain drug delivery. Its lead product 2B3-101 is a brain-targeted formulation of Doxil/Caelyx, which has demonstrated higher brain levels but similar systemic exposure, resulting in therapeutic benefits in animal studies with 2B3-101. It is now achieving high therapeutic dose levels in a clinical phase I/IIa trial in patients with solid tumor brain metastases and glioma. Preliminary efficacy has been shown both intra- and extra-cranially. Upon determining maximum tolerated dose, the expansion phase of the trial will study preliminary efficacy in specific patient populations: 1) Breast Cancer brain metastases with or without trastuzumab (Herceptin). 2) Small Cell Lung Cancer brain metastases; 3) Melanoma brain metastases, and; 4) malignant glioma; The expansion phase will allow selection of most responsive tumor types for further development, while performing due diligence processes and negotiation discussions for licensing purposes.
to-BBB is developing several other products for brain diseases, either internally or in collaboration with pharma/biotech partners. Furthermore, it is evaluating oncology compounds that can benefit from the G-Technology by combining a slow release profile with enhanced brain delivery for metastatic disease including brain metastases or for primary brain tumors.

Management

Chief Executive Officer:
Willem van Weperen, MSc, MBA

Chief Scientific Officer/founder:
Pieter Gaillard, PhD

Chief Medical Officer:
Fredrik Lonnqvist, MD, PhD, Assoc. Prof.

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Basel, Switzerland, Monday, June 24 – Wednesday, June 26, 2013

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BioNanoNet Research Association **BioNanoNet)**, Graz (A)

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International Association for Pharmaceutical Technology **(APV),** Mainz (D)

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