COST TD1004 Action

Theranostics Imaging and Therapy: An Action to Develop Novel Nanosized Systems for Imaging-Guided Drug Delivery

Annual Meeting
September 1st- September 3rd, 2013

Meeting Venue:
Hotel Stratos Vassilikos, Athens, Greece

Meeting Programme

Sunday, September 1st, 2013

14:00 – 15:00 Registration

15:00 – 15:15 Opening of the COST Action TD1004 Annual Meeting, Silvio Aime (University of Torino, Chair of the COST Action TD1004)

15:15 – 15:30 Overall Lecture WG3, Robert Muller (University of Mons, Belgium): Preparation and selection of targeting vectors

Chairs: Sophie Laurent and Robert Muller

15:30 – 16:00 Lectures WG3

15:30 – 15:45 Development of high-relaxivity and luminescent silica nanoparticles as multimodal agents for medical imaging, E. Lipani, S. Laurent, M. Surin, L. Vander Elst, P. Leclère, R.N. Muller

15:45 – 16:00 Theranostic nanoparticles for lung tumors, F. Lux, A. Bianchi, S. Dufort, N. Tassali, J.-L. Coll, Y. Crémillieux, O. Tillement

16:00 – 16:30 Coffee Break

16:30 – 17:30 Lectures WG3 (continued)

16:30 – 16:45 Apoferritin loaded with Gd-HPDO3A and curcumin as a theranostic agent, Simonetta Geninatti Crich, Juan Carlos Cutrin, Diana Burghelea, Marta Cadenazzi, Walter Dastrù, Silvio Aime

16:45 – 17:00 Labeling of AGuIX Nanoparticles with Gallium-68: initial *in vitro* and *in vivo* results, C. Tsoukalas, T. Tsotakos, F. Lux, C. Truillet, S. Xanthopoulos, N. Kyza, M. Paravatou-Petsotas, O. Tillement, P. Bouziotis

17:00 – 17:15 In vivo evaluation of functionalized polymeric NPs on memory deficit of Alzheimer's disease mice model, D. Carrodari, B. Le Droumaguet, J. Nicolas, D. Brambilla, E. Nespoli, E. Salvati, L. De Kimpe, C. Zona, C. Airoldi, C. Balducci, M. Zotti, M. Canovi, M. Gobbi, O. Flores, F. Nicotra, W. Scheper, F. Re, M. Masserini, G. Forloni, M. Salmona, P. Couvreur, K. Andrieux

17:15 – 17:30 GN8 Fluorescent Analogues with Concomitant Therapeutic and Diagnostic Activities in Prion Diseases, M. Staderini, S. Aulic, V. González-Ruiz, G. Bianchini, L. Morelli, A. D'Onofrio, H. Ngoc Ai Tran, N. Cabezas, M.A. Martín, G. Legname, J.C. Menéndez, M.L. Bolognesi

18:00 Museum Visit

20:30 Dinner

Monday, September 2nd, 2013

09:00 – 09:45 Invited Lecture: Monitoring the effects of antiangiogenic therapies with DCE-US, Michalakis A. Averkiou

09:45 – 10:00 Overall Lecture WG4, Gerben Koning (Erasmus MC, Rotterdam, The Netherlands): Theranostic agents responsive to endogenous and external stimuli

Chairs: Chantal Pichon and Gerben Koning

10:00 – 11:10 Lectures WG4

10:00 – 10:25 Light-directed drug delivery by photochemical internalization, Anders Hogset

10:25 – 10:50 Enzymatic degradation induced drug release and imaging of sphingomyelin liposomes, Oula Penate Medina

10:50 – 11:10 Microbubble-mediated drug delivery, Twan Lammers

11:10 – 11:30 Coffee Break/ Poster Viewing

11:30 – 12:50 Lectures WG4 (continued)

11:30 – 11:45 New cationic liposomes bubbles for nucleic acids transfer, Anthony Delalande, Simona Manta, Michel Bessodes, Patrick Midoux, Nathalie Mignet, Chantal Pichon

11:45 – 12:00 Ultrasound-sensitive microbubbles for stroke therapy - toward understanding of the mechanisms of blood clot destruction with microbubbles, ultrasound and rtPA, E. Allémann, B. Petit, F. Yan , P. Bussat, E. Gaud, Yannick Bohren, F. Tranquart

12:00 – 12:20 Overcoming limitations in Nanoparticle Drug Delivery. Thermodox: An Update, David Needham

12:20 – 12:35 Characterization of supported lipid membranes – toward the development of nano-sized drug carriers for hyperthermia applications, Sofia Svedhem

12:35 – 12:50 Controlled Photothermal Drug Release using Smart Nanoplatforms, Mustafa Selman Yavuz, Mehmet Ulasan, Huseyin Sakalak, Emine Yavuz, Halit Cavusoglu, Burak Buyukbekar

12:50 – 13:40 Lunch

13:40 – 15:30 Management Committee Meeting

15:30 – 15:45 Overall Lecture WG2, Ruth Schmid (SINTEF, Trondheim, Norway): Nanocarriers for theranostic agents

Chairs: Maria J. Blanco-Prieto and Ruth Schmid

15:45 – 16:30 Lectures WG2

15:45 – 16:00 Application of mesoporous silica nanoparticles for improved solubility of poorly soluble drugs and targeted delivery vehicles, Adam Feiler, Nanologica Sweden

16:00 – 16:15 Folic acid conjugated polymeric nanocapsules for passive and active targeting of cancer, Khuloud Al-Jamal

16:15 – 16:30 Microbial versus human cells – implications for targeting, Nuno Azevedo

16:30 – 17:00 Coffee Break

17:00 – 18:00 Lectures WG2 (continued)

17:00 – 17:15 Edelfosine nanosystems overcome drug resistance in leukemic cell lines, Beatriz Lasa-Saracíbar, Ander Estella-Hermoso de Mendoza, Faustino Mollinedo, María D. Odero, María J. Blanco-Príeto

17:15 – 17:30 Biomimetic apatite nanocrystals: a new platform for nanomedicine, Michele Iafisco, Monica Sandri, Alessio Adamiano, Silvia Panseri, Josè Manuel Delgado-Lopez, Jaime Gomez-Morales, Maria Prata, Anna Tampieri

17:30 – 17:45 Selectively-modified porous silicon nanoparticles with the functionalities of imaging, targeting and triggered drug release, Wujun Xu, Jussi Rytkönen, Joakim Riikonen, Ale Närvänen and Vesa-Pekka Lehto

17:45 – 18:00 *In vivo* antitumor efficacy and biodistribution of polyaminoacid nanocapsules, Abellán-Pose, Borrajo-Alonso E, Delgado A, Evora C, Garcia-Fuentes M, Vidal A, Evora M, Csaba N, Alonso MJ

18:00 – 19:30 Cheese and Wine Reception with Poster Viewing

Tuesday, September 3rd, 2013

09:00 – 09:45 Invited Lecture: Production of High Purity and High Specific Activity Radioisotopes, Cathy Cutler, Nebiat Sisay, Matt Gott, Anthony Degraffenreid, James Kelsey, Silvia Jurisson

09:45 – 10:00 Overall Lecture WG1, Renata Mikolajczak (National Centre for Nuclear Research Radioisotope Centre POLATOM): Imaging reporters for theranostic agents

Chairs: Kristina Djanashvili and Frank Roesch

10:00 – 11:15 Lectures WG1

10:00 – 10:15 Radiopeptides in SPECT/MRI: Optimization of combined peptide receptor radionuclide therapy (PRRT) and temozolomide using SPECT/CT and MRI in mice, M. de Jong, S. M. Bison, J. C. Haeck, S. J. Koelewijn, H. C. Groen, S. Berndsen, M. Melis, M. R. Bernsen

10:15 – 10:30 Effects of metal-chelate and peptide chain-length on the biological profile of ^{99m}Tc/^{111}In-labeled Gastrin I analogs, A. Kaloudi, E. Lymperis, K. Markou, E. P. Krenning, M. de Jong, B. A. Nock, T. Maina

10:30 – 10:45 A Cu-64-labeled Sar-conjugated bombesin antagonist as new PET tracer for imaging of prostate cancer, Gourni E, Del Pozzo L, Kheirallah E, Donelly P, Reubi JC, Maecke HR

10:45 – 11:00 Multimodal imaging probes for the detection of amyloid plaques, André F. Martins, Jean-François Morfin, Adina Lazar, Charles Duyckaerts, Nicolas Arlicot, Denis Guilloteau, Carlos F.G.C. Geraldes, Éva Tóth

11:00 – 11:15 Systematic study for optimizing the Radiolabelling of AGuIX® Multimodal Nanoparticles, E. Ntsiba, C.Truillet, F.Lux, C. Alliot, F. Boschetti, S.Huclier, O. Tillement

11:15 – 11:45 Coffee Break/ Poster Viewing

11:45 – 13:45 Lectures WG1 (continued)

11:45 – 12:00 Nanozeolite-LTL with Gd^{3+} deposited in the large and Eu^{3+} in the small cavities as a bimodal MRI-Optical probe, Kristina Djanashvili, Florian Mayer, Wuyuan Zhang, Thomas Brichart, Olivier Tillement, Célia Bonnet, Éva Tóth, and Joop A. Peters

12:00 – 12:15 Substance P - nanozeolite labeled with ^{224}Ra and ^{225}Ra - new potential radiobioconjugate for internal alpha therapy E.Leszczuk, A. Piotrowska, P.Koźmiński A.Bilewicz, A.Morgenstern, F.Bruchertseifer

12:15 – 12:30 ^{64}Cu radiolabeled nanomaterials as bimodal contrast agent for optical imaging and Positron Emission Tomography (PET), A. M. Nonat, A. Roux, A. Yahia-Ammar, J. Brandel, V. Hubscher, C. Platas-Iglesias, L. Sabatier, L. J. Charbonnière

12:30 – 12:45 Efficient radiometal-labelling of drug delivery systems utilizing water/ethanol mixtures, F. Rösch, M. Malo-Cruz

12:45 – 13:00 Near-infrared Imaging in Living Cells with Lanthanides: Yb3+ nanoMOF, Alexandra Collet, Stephane Petoud

13:00 - 13:15 Equilibrium, kinetic and structural properties of lanthanide(III)- complexes formed with a sulphonamide derivative of DO3A, Anett Takács, Roberta Napolitano, Mihály Purgel, Attila Bényei, László Zékány, Ernő Brücher, Imre Tóth, Zsolt Baranyai and Silvio Aime

13:15 – 13:30 Development of Ca^{2+} Responsive Contrast Agents for fMRI, Sandip M. Vibhute, Martin E. Maier, Nikos K. Logothetis and Goran Angelovski

13:30–13:45 Nanobody-based Targeted Radiotherapy for Cancer Treatment, Matthias D'Huyvetter, Cécile Vincke, Catarina Xavier, Nick Devoogdt, An Aerts, Nathalie Impens, Sarah Baatout, Serge Muyldermans, Vicky Caveliers, Tony Lahoutte

13:45 – 14:30 Lunch

14:30 – 15:00 Lectures WG1 (continued)

14:30 – 14:45 High MRI performance mesoporous silica nanoparticles functionalized with Gd-chelates as potential theranostic systems, Lorenzo Tei, Mauro Botta, Fabio Carniato

14:45 – 15:00 Overall Lecture WG 5, George Loudos (Technological Educational Institute of Athens): Set-up of preclinical theranostic protocols

Chairs: Penelope Bouziotis and George Loudos

15:00 – 16:15 Lectures WG5

15:00 – 15:15 Superparamagentic iron oxide nanoparticles for local thermotherapy: potential and limitations, Olivier Jordan

15:15 – 15:30 Bimodal Imaging of Sentinel Lymph Node with $^{99m}Tc(CO)_3$-labeled Mannosylated Nanoparticles bearing a NIR-fluorophore, Maurício Morais, Catarina Xavier, Sophie Hernot, Tony Lahoutte, Vicky Caveliers, João D. G. Correia, Isabel Santos

15:30 – 15:45 Quantitative MRI approaches for tracking nano-sized systems, Klaas Nicolay, Rik P.M. Moonen, Gustav J. Strijkers

15:45 – 16:00 Non invasive temperature measurements during hyperthermia sessions with a near infrared camera, Spiridon Spirou, Lazaros Palamaris, Eirini Fragogeorgi, George Loudos

16:00 – 16.15 Preclinical protocols to monitor tumor growth, metastasis and nanotoxicity, Larissa Rizzo

16:15 – 17:00 Coffee break and closing of meeting

POSTERS

WG1

1. Quantum Dots bioconjugates for imaging and diagnostic, Akram Yahia-Ammar, Aline Nonat, Loïc Charbonnière

2. Development of a quantum-dot-labelled magnetic immunoassay method for circulating colorectal cancer cell detection, Gazouli M, Lyberopoulou A, Pericleous P, Nikiteas N, Anagnou NP, Efstathopoulos EP

3. Study on active targeting of Y-90 and Lu-177 radiolabelled ultra-small AGuIX nano particles functionalized by octreotate, M. Maurin, U. Karczmarczyk, P. Garnuszek, R Mikołajczak, Ch. Truillet, F. Lux, O. Tillement

4. Effects of Ln-DO3A-PiB Derivatives on the Amyloid Peptides: Self-Assembly and In Vitro Interactions, André F. Martins, David Dias, J-F Morfin, Douglas Laurents, Eva Toth and Carlos F.G.C. Geraldes

5. Kinetic studies of Zr DTPA and Zr DFO complex formation, Miroslav Pniok, Jan Kotek, Vojtěch Kubíček, Petr Hermann

6. Complexes of dibenzylamino-phosphinate DOTA analogue as albumin-binding MRI contrast agent, Peter Urbanovsky, Jan Kotek, Petr Hermann

7. The equilibrium and kinetic investigation of the complexes of H4OCTAPA, Imre Tóth, Ferenc Krisztián Kálmán, Andrea Végh, Carlos Platas-Iglesias, Martín Regueiro-Figueroa and Gyula Tircsó

8. Zinc responsive contrast agents for MRI, Célia Bonnet, Fabien Caillé, Agnès Pallier, Franck Suzenet, Eva Toth

9. Paramagnetic Solid Lipid Nanoparticles as a novel platform for the development of molecular MRI probes, Mauro Botta, Gabriele A. Rolla, Lorenzo Tei, Simona Ghiani, Claudia Cabella, Alessandro Maiocchi

10. MRI contrast enhancement of coated GdF3 nanoparticles, Mauro Botta, Fabio Carniato, Kalaivani Thangavel, Lorenzo Tei

11. Folate receptor targeted delivery of supramolecular drug-carriers monitored by PET, H. Schieferstein, A. Kelsch, J. Postema, B. Biesalski, H.G. Buchholz, N. Bausbacher, O. Thews, F. Roesch, R. Zentel, T.L. Ross

12. New methods for direct and mild 18F-labeling of macromolecules, J. Postema, T.L. Ross

13. Radiolabelling of different nanoparticles for dual-modality imaging, K. Stockhofe, D. Goempel, W. Tremel, T.L. Ross

14. Functionalized oligoproline as multivalent scaffold in tumor targeting, P. Wilhelm, H.R. Mäcke, H. Wennemers

15. Complexation of $[Gd(DTTA\_Me)(H_2O)_2]^-$ by F^- and its consequences to water exchange, Shima Karimi, Gail Hunter, Loïck Moriggi, Lothar Helm

16. TiO_2 nanoparticles as carries of $^{225}Ac/^{213}Bi$ and $^{212}Pb/^{212}Bi$ in vivo generators, E. Leszczuk A. Piotrowska, A. Bilewicz, A. Morgenstern, F. Bruchertseifer

17. Exendin-4 labeled with ^{99m}Tc, ^{111}In and ^{68}Ga - a comparative biodistribution evaluation, Dariusz Pawlak, Barbara Janota, Piotr Garnuszek, Renata Mikolajczak

18. Near-infrared Emitting Lanthanide Dendrimer Complexes for Biologic Imaging in Cells and in Small Animals, Stephane Petoud

19. A R2p/R1p ratiometric approach to visualize Matrix Metalloproteinase-2 activity by MRI, Valeria Catanzaro, Giuseppe Digilio, Valeria Menchise, Sergio Padovan, Martina Capozza, Linda Chaabane and Silvio Aime

20. Is the radiolabeling of DOTA-based phosphonic acids with ^{44}Sc efficient as NOTA-based phosphonic acids with ^{68}Ga ? R. Kerdjoudj, C. Alliot, P. Hermann, F. Rösch and S. Huclier-Markai

21. Synthesis of High Affinity Contrast Agents for Targeted MR Neuoroimaging, S. Gündüz, A. Power, Nikos K. Logothetis, and G. Angelovski

22. New macrocyclic imaging agents targeting chemokine receptor CXCR4, Sophie Poty, Pauline Désogère, Kati Nicholson, Shubhanchi Nigam, Christine Goze, Frédéric Boschetti, Steve Archibald, Helmut Maëcke, Franck Denat

23. Oligoprolines as Scaffolds for Tumor Targeting with Hybrid Bombesin Analogues, Carsten Kroll, Stefanie Dobitz, Rosalba Mansi, Friederike Braun, Helmut Maecke, Helma Wennemers

24. Theranostic nanoparticles for MRI-guided thermoablation of tumors, Vít Herynek, P. Jendelová, K. Turnovcová, Emil Pollert, Pavel Veverka, Daniel Jirák, Eva Syková, Milan Hájek

25. Labeling of HPMA-based, functionalized polymer-systems using metallic radionuclides, de la Fuente Ana, Eppard Elisabeth, Allmeroth Mareli, Zentel Rudolf, Roesch Frank

WG2

26. Ultrasound-enhanced accumulation in fat and efficient cellular uptake of hydrophobic drugs using a novel nanoparticle- microbubble platform, Catharina de Lange Davies, Sofie Snipstad, Sara Westrum, Siv Eggen, Kishia Søvik, Andreas Åslund, Per Stenstad, Yrr Mørch, Ruth Schmid

27. Targeted-receptor Bimodal Probe for Sentinel Lymph Node Detection, João D.G. Correia, M. Morais, M.P.C. Campello, C. Xavier, S. Hernot, T. Lahoutte, V. Caveliers, & I. Santos

28. Multimodal contrast agent for the imaging of pancreatic amyloids in type 2 diabetes, B, Stransky-Heilkron, M. Plissonneau, P. Mowat, M. Dumoulin, C. Louis, F. Lux, O. Tillement, C. Marquette, V. Forge, X. Montet & Eric Allémann

29. A tyrosine-based amphiphilic chelating molecule for magnetic resonance imaging. Synthesis steps and characterization, C. Xayaphoummine, A. Babic, L. Helm, A.S.Chauvin, E. Allémann

30. In vitro cell interaction and in vivo biodistribution of poly (dl-lactide-co-glycolide) nanospheres with encapsulated selenium nanoparticles for the treatment of liver diseases, Magdalena Stevanović, Jana Nunić, Jonghoon Choi, Miloš Filipović, Dragan Uskoković, Theodore Tsotakos, Eirini Fragogeorgi, Dimitris Psimadas, Lazaros Palamaris, George Loudos

31. Polymeric nanocapsule for triple-modal imaging as a theranostic system in cancer therapy, Jie Bai & Khuloud Al-Jamal

32. Small-core Gold Nanoparticles Stabilized with a Thiolated DOTA-based Ligand, F. Silva, M.P. Campello, L. Gano, A. Paulo & I. Santos

33. Polyglutamic acid-PEG block copolymer nanocapsules: biodistribution study following two administration routes, E. Borrajo, R. Abellán-Pose, A. Soto, N. Csaba, M.J. Alonso, A.Vidal, M. Garcia-Fuentes

34. A phase-shift concept for ultrasound mediated drug delivery, Andrew Healey, Per Christian Sontum, Svein Kvåle, Catharina de Lange Davies

WG3

35. Nano-thermometer with Thermo-sensitive Polymer Grafted USPIOs behaving as Positive Contrast Agents in low-field MRI, Hannecart, D. Stanicki, L. Vander Elst, R.N. Muller, S. Lecommandoux, J. Thévenot, C. Bonduelle, A. Trotier, P. Massot, S. Miraux, O. Sandre, S. Laurent

36. AGuIX® nanoparticles vectorization for an apoptosis targeting, M. Dentamaro, S. Laurent, F. Lux, L. Vander Elst, C. Truillet, M. Plisonneau, O. Tillement, R.N. Muller

37. Initial in vitro and in vivo assessment of Au@DTDTPA-RGD nanoparticles labeled with Ga-68, C. Tsoukalas, G. Laurent, T. Tsotakos, R. Bazzi, M. Paravatou-Petsotas, S. Xanthopoulos, S. Roux and P. Bouziotis

38. Carboxy-silane coated iron oxide nanoparticles: a convenient platform for bimodal imaging, D. Stanicki, S. Boutry, S. Laurent, L. Wacheul, E. Nicolas, L. Vander Elst, D.L.J. Lafontaine, R.N. Muller

39. Development and characterization of novel multimodal nanoplatforms of diamond for medical imaging, S. Montante, S. Laurent, L. Vander Elst, R.N. Muller

40. Targeting Immuno-liposomes using TCR-like antibodies directed against melanoma antigens, M. Saeed, E. Schooten, M. Bolkestein, T.L.M. ten Hagen, A.M.M. Eggermont, R.Debets, G.A. Koning

WG4

41. Identification of new bio-effects of ultrasound and microbubbles assisted drug delivery, Anthony Delalande, Lucie Pigeon, Chloé Leduc, Patrick Tauc, Eric Deprez, Patrick Midoux, Chantal Pichon

WG5

42. Superparamagnetically grafted multi-walled carbon nanotubes for dual-modality SPECT/MR biomedical imaging, Julie Tzu-Wen Wang

43. Comparative in vitro and in vivo evaluation of nanosized Liposome appropriately modified for being labelled with Tc-99m by two different radiolabelling approaches, Eirini Fragogeorgi

44. USPIO-labeled collagen scaffolds for non-invasive MR imaging in tissue engineering, M.E. Mertens, D. Moeckel, A. Hermann, A. Buehren, L. Olde-Damink, F. Gremse, J. Ehling, F. Kiessling, T. Lammers

45. ^{99m}Tc-Labeled aminosilane-coated iron oxide nanoparticles as dual modality imaging agents of tumor angiogenesis and in vivo hyperthermia evaluation, Irene Tsiapa, George Loudos, Alexandra Varvarigou, Penelope Bouziotis, Stavros Xanthopoulos, Eirini Fragogeorgi, Maria Paravatou-Petsotas, Eleni K. Efthimiadou, George C. Kordas, Dimitris Mihailidis, George C. Nikiforidis, George C. Kagadis

O1

Development of High-Relaxivity and Luminescent Silica Nanoparticles As Multimodal Agents for Medical Imaging

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[2] *CMMI - Center for Microscopy and Molecular Imaging, Rue Adrienne Bolland, 8, B-6041 Gosselies, Belgium*

The design and synthesis of a new bimodal contrast agent for magnetic resonance imaging and optical imaging are reported1. Tunable-sized silica nanoparticles were synthesized by a microemulsion-mediated pathway and used as carriers for paramagnetic and luminescent probes.

The near-infrared luminescent agent was a ruthenium complex that was directly entrapped in the silica shell to provide photo- luminescence enhancement and to make it highly photostable as it was protected from the surrounding environment.

The paramagnetic activity came from Gd-DTPA derivatives grafted on the silica surface. NMRD profiles showed a strong relaxivity enhancement (increase of 432% in the r1 value at 20 MHz) when the paramagnetic complex was grafted at the nanoparticle surface, because of a reduction of its mobility.

Polyethylene glycol was also grafted at the nanoparticle surface to enhance the nanoparticle residence time in the bloodstream.

A thorough characterization of the material confirmed its potential as a very effective bimodal contrast agent.

Further studies will consist in grafting an apoptosis-specific peptide on the nanoparticles surface in order to perform molecular imaging. Finally, the nanoparticles toxicity will be assessed through common cytotoxicity assays and through atomic force microscopy on cells to understand the interaction of nanoparticles with membranes and monitor changes in membrane roughness and cell elasticity.

Reference

1. E. Lipani, S. Laurent, M. Surin, L. Vander Elst, P. Leclère, R. N. Muller. "Highly Luminescent Silica Nanoparticles as Multimodal Agents for Molecular Imaging". Langmuir 2013, 29(10) : 3419--3427

O2

Theranostic nanoparticles for lung tumors

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A new type of ultrasmall gadolinium based nanoparticle has been recently developed by our team. These nanoparticle made of polysiloxane and surrounded by DOTA(Gd) chelates and Cy5.5 fluorophores covalently grafted to the inorganic matrix display a size around 3 nm [1]. They have been used for multimodal imaging [2] and radiosensitization [3] after intravenous injection.

Administration of the nanoparticles via the airways leads to an important increase of the MRI signal in the lungs. Due to their small sizes (inferior to 5 nm), the nanoparticles show a fast passage from the lungs to the bloodstream and then an accumulation in the kidneys before the final elimination through the urine. After intravenous injection in bioluminescent lung tumor bearing mice, an uptake of the nanoparticles is observed due to enhanced permeability and retention effect. After administration via the airways a more important increase of the signal in the tumor is detected by MRI. A good colocalization between the fluorescence signal of the nanoparticles and the bioluminescent signal of the tumor is observed by optical imaging. Finally, a radiotherapeutic protocol has been proposed thanks to the imaging data. After irradiation, an important increase of the median survival time is observed for the mice treated with irradiation and nanoparticles (120 days) in comparison with mice irradiated without nanoparticles (60 days).

Acknowledgements
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References

1. A. Mignot, C. Truillet, F. Lux, L. Sancey, C. Louis, F. Denat, F. Boschetti, L. Bocher, A. Gloter, O. Stephan, R. Antoine, P. Dugourd, D. Luneau, G. Novitchi, L. C. Figueiredo, P. C. De Morais, L. Bonneviot, B. Albela, F. Ribot, L. Van Lokeren, I. Dechamps-Olivier, F. Chuburu, G. Lemercier, C. Villiers, P. N. Marche, G. Le Duc, S. Roux, O. Tillement, P. Perriat, Chem. Eur. J., 2013, 19, 6122-6136.
2. F. Lux, A. Mignot, P. Mowat, C. Louis, S. Dufort, C. Bernhard, F. Denat, F. Boschetti, C. Brunet, R. Antoine, P. Dugourd, S. Laurent, L. Vander Elst, R. Muller, L. Sancey, V. Josserand, J.-L. Coll, V. Stupar, E. Barbier, C. Rémy, A. Broisat, C. Ghezzi, G. Le Duc, S. Roux, P. Perriat, O. Tillement, Angew. Chem. Int. Ed., 2011, 51, 12299-13303.
3. G. Le Duc, I. Miladi, C. Alric, P. Mowat, E. Bräuer-Krisch, A. Bouchet, E. Khalil, C. Billotey, M. Janier, F. Lux, P. Perriat, S. Roux, O. Tillement, « Towards an image-guided microbeam radiation therapy using gadolinium-based nanoparticles », ACS Nano, 2011, 5, 9566-9574.

O3

Apoferritin loaded with Gd-HPDO3A and curcumin as a theranostic agent.

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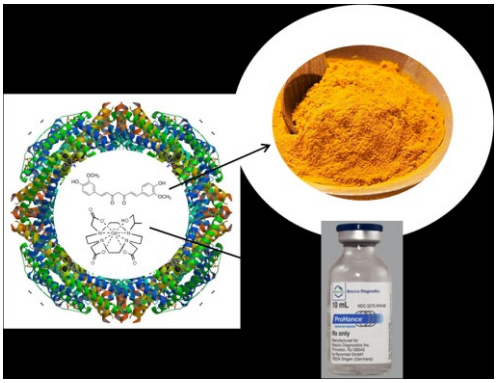
Ferritin is the primary iron storage protein. It is a 24-mer hollow 'nano cage' capable of sequestering iron in a nontoxic and bio-available form. Although functions of ferritin are traditionally associated with intracellular iron storage, additional roles have been recently discovered and investigated. For example, ferritin can act as a transferrin independent iron-delivery molecule to different target organs such us brain, liver and spleen by exploiting the scavenger receptor member 5 (SCARA5) for L-ferritin and TIM-2 and TfR1 for H-ferritin. Furthermore, perturbations in cellular ferritin are emerging as an important element in the pathogenesis of disease not only in the classic diseases of iron acquisition, transport, and storage, (hemochromatosis) but also in diseases characterized by inflammation, infection, injury, and repair; including neurodegenerative diseases (Parkinson and Alzheimer) vascular diseases (atherosclerosis) inflammatory states, breast, colon and liver cancers.

The aim of this study is to exploit the apoferritin nanocage to deliver simultaneously therapeutic and imaging agents (loaded into its internal cavity) to hepatocytes as this protein is efficiently taken up from blood by hepatocyte scavenger receptor class A type 5 via the ferritin transporting route. To this purpose the protein has been loaded with the MRI contrast agent GdHPDO3A and Curcumin, a polyphenolic substance endowed with multiple pharmacological actions namely: antioxidant, anti-inflammatory, antineoplastic. Curcumin and GdHPDO3A loaded apoferritin has been used with the aim to attenuate the thioacetamide-induced hepatitis together with the evaluation by MRI of drug delivery efficiency. Mice pre-treated by intraperitoneal administration showed significantly attenuated hepatic injury as assessed by measuring alanine aminotransferase (ALT) activity in plasma and by histology assessment. The encapsulation of curcumin inside the apoferritin cavity significantly increases its stability and bioavailability while maintaining its therapeutic anti-inflammatory properties.



References

1. Cutrin JC, Geninatti Crich S, Burghelea D, Dastrù W, Aime S. Mol Pharm. 2013 May 6;10(5):2079-85. doi: 10.1021/mp3006177. 2) Rivera-Espinoza, Y.; Muriel, P. Pharmacological actions of curcumin in liver diseases or damage. Liver Int. 2009, 29, 1457-1466.

O4

Labeling of AGuIX Nanoparticles with Gallium-68: initial *in vitro* and *in vivo* results

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Introduction

AGuIX nanoparticles are ultrasmall (smaller than 5 nm in size) nanoparticles, which are obtained in a top-down process starting from a core-shell structure (core=gadolinium oxide; shell=polysiloxane). They represent the first multifunctional silica-based nanoparticles that are sufficiently small to escape hepatic clearance and enable animal imaging by four complementary techniques (MRI, fluorescence imaging, SPECT, CT) [1]. The objectives of the present study were to radiolabel AGuIX nanoparticles using the generator-derived positron emitter Ga-68, and to assess their *in vitro* and *in vivo* properties.

Methods

For a typical preparation of ^{68}Ga-labeled AGuIX nanoparticles, the AGuIX nanoparticles were mixed with sodium acetate buffer, pH 5.6 and 200 μL of ^{68}Ga eluate were consequently added. The mixture was then incubated for 20 min at 40 °C. Radiochemical purity was determined by ITLC, using KCL 0.2M as the mobile phase. In vitro stability of ^{68}Ga-AGuIX was assessed in saline and serum, up to 3 h. In vitro cell binding experiments were performed on integrin $\alpha_v\beta_3$ receptor-positive U87MG cancer cells, in order to assess the targeting capability of ^{68}Ga-labeled c-RGD-functionalized AGuIX. A non-specific c-RAD-functionalized AGuIX nanoparticle was used for comparison. The *in vivo* behavior of the radiolabeled compound was initally evaluated in normal Swiss mice, as well as in athymic SCID mice.

Results

AGuIX nanoparticles were sucessfully labeled with Gallium-68 at high radiochemical purity (> 97%). They were stable at RT up to 3h, as well as in the presence of serum for up to 3 h, at 37 °C. The cell binding assay proved that ^{68}Ga-cRGD-AGuIX had specific recognition for U87MG glioma cells. Biodistribution studies in normal Swiss mice showed that AGuIX-68Ga cleared rapidly from the blood via the kidneys to the urine, resulting in extremely low background activity in all other analysed tissues (< 2% injected dose per gram at 120 min post-injection). A comparative pharmacokinetic study of ^{68}Ga-cRGD-AGuIX on U87MG tumor-bearing SCID mice showed that, when a high concentration of cRGD-AGuIX was labeled with ^{68}Ga, tumor uptake was much lower than with the low-concentration radiolabeled product (labeling of 500 nmol/cRGD compared to 7.5 nmol/cRGD, same labeling conditions). However, the tumor/muscle ratio of the high-concentration sample showed a slight increase from 60-120 min, while the low-concentration sample decreased noticeably.

Conclusions

The immobilization of paramagnetic gadolinium ions on AguIX nanoparticles permits their monitoring by MRI. We have shown that these nanoparticles can also be easily labeled with the positron-emitter Ga-68, via the remaining available NODA/DOTA chelator molecules found on their surface, thus leading to a potential dual-modality PET/MRI imaging agent. Passive tumor targeting was shown to be satisfactory, while in the case of active targeting, the concentration of the radiolabeled ^{68}Ga-cRGD-AGuIX is an issue to be taken under serious consideration, as it was shown that the less concentrated product had better tumor targeting properties, albeit a lower tumor/muscle ratio, comparable to the passively accumulated, non-targeted product ^{68}Ga-cRAD-AGuIX at 120 min p.i. Fine-tuning of the quantities of the injected ^{68}Ga-AGuIX nanoparticles needs to be done, in order to achieve the optimum tracer concentration for dual-modality PET/MRI imaging.

References

1. F. Lux et al, “Ultrasmall Rigid Particles as Multimodal Probes for Medical Applications”, Angewandte Chemie International Edition, 2011, 50, 1-6.

O5

In vivo evaluation of functionalized polymeric NPs on memory deficit of Alzheimer's disease mice model

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Purpose: The aim of this work was to synthetize nanoparticles (NPs) able to interact with the Aβ peptide in order to increase its elimination and to correct the memory defect observed in Alzheimer Disease.

Methods: Biotin-functionalized NPs were prepared by nanoprecipitation. After purification by ultracentrifugation, NPs were characterized by using DLS. Streptavidin-fluorescein isothiocyanate (SavFITC) was coupled with anti-Aβ monoclonal antibody (anti-Aβ mAb) and purified by gel filtration column using Superdex 200 gel. A semi-native electrophoresis was made and the gel was analyzed by florescence and coomassie blue. The conjugate's yield was quantified by spectrofluorimetry and Bradford's dosage. Biotin-NPs and SavFITC – anti-Aβ mAb conjugate were coupled in a nanoconstruct complex purified by ultracentrifugation and verified by spectrofluorimetry. Finally, anti-Aβ mAb - NPs have been evaluated in vivo on transgenic mice models (Tg2576 mice, 15 months old) receiving treatment (100 µL) by IV 3days by week during 3 weeks.

Results: Biotin- NPs showed a size around 100 nm, a good stability, a biotin amount of 9.6•1014 molecules by sample. The decorated NPs with anti-Aβ mAb have a similar size and are stable in water [1]. The object recognition memory test revealed complete correction of the memory defect on transgenic mice. The results are compared to the efficacy of nanoparticles decorated with curcumine derivatives.

Conclusion: Anti-Aβ mAb decorated NPs are promising for Alzheimer disease treatment. Their mechanism of action would be evidenced in further experiments.

Acknowledgements
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References

1. B. Le Droumaguet, et al. ACS Nano 6(7):5866-79 (2012).

O6

GN8 Fluorescent Analogues with Concomitant Therapeutic and Diagnostic Activities in Prion Diseases

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Prion diseases are a family of invariably fatal neurodegenerative disorders for which no effective therapy and diagnosis currently exist. The main hallmark of these maladies is the deposition of fibrillar aggregates of disease-associated prion protein (PrPSc) because of the misfolding of normal cellular prion protein (PrPC). These proteinaceous aggregates are considered to be responsible for the underlying neurodegeneration, and thus they represent a validated target either in the treatment or diagnosis of such disorders.

In recent work, we have shown that it is possible to design small molecules able to block capable of blocking PrPSc fibril formation and at the same time act as prion plaque fluorescent stains. Building on this approach and in an effort to locate different chemotypes, we focused on GN8, an antiprion compound that directly binds PrPC, preventing its conversion into the scrapie isoform. We modified the diphenylmethane core of GN8 by linking its two phenyl rings to generate a new class of carbazole and fluorene derivatives that, due to their fluorescent properties, offer the potential to act as therapeutic tools and chemical sensors for prion protein.

Two small libraries of fluorene and carbazole-based analogues of GN8 thus generated were studied on a cellular model of the disease, based on scrapie-infected mouse neuronal cells, and were revealed as capable to inhibit prion replication. We also studied their native fluorescence and their use to stain fibrillar plaques. These preliminary results suggest that our derivatives are able to interact with PrPSc preventing fibril formation and stain the scrapie aggregates, acting as potential theranostic agents.

Invited Lecture

Monitoring the effects of anitangiogenic therapies with DCE-US

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Imaging is a key factor in the accurate monitoring of response to cancer therapies targeting tumor vascularity to inhibit its growth and dissemination. Dynamic contrast enhanced ultrasound (DCE-US) is a relatively new quantitative method with the advantage of being non-invasive, widely available, portable, cost effective, highly sensitive and reproducible using microbubble contrast agents that are truly intravascular. Advances in nonlinear imaging techniques have enabled ultrasound imaging to visualize the macro- and micro-vasculature in real time. The image intensity of a region of interest (ROI) in the tumor is proportional to the microbuble concentration. Metrics of blood flow and blood volume may be extracted from indicator dilution models and thus offer a quantitative means of monitoring the effects of chemotherapy. The present talk will concentrate on the bolus injection method for contrast delivery and the analysis of the wash-in and wash-out of the microbubbles in the ROI. A review of the nonlinear imaging techniques for DCE-US will be presented first. Emphasis is placed on the utilization of this technique in the clinic and various issues and problems that are encountered will be addressed. Results from clinical trials with liver cancer patients undergoing vascular targeted therapies will be presented.

In the second part of the presentation, enhancement of drug delivery with *sonoporation* is discussed. Sonoporation is loosely defined as the “mechanical effects” of ultrasound and microbubbles on tumor cells and microenvironment and their ability to respond to chemotherapy (both cytotoxic and antiangiogenic). There are indications that sonoporation plays an important role in improving drug delivery, delaying drug resistance, and positively influencing the immune response. In the work discussed here a “modified” diagnostic ultrasound scanner is used and thus application of therapy while simultaneously imaging and monitoring the therapy outcomes is possible. The ultrasound conditions, bubble administration methods, and the related bubble physics are discussed. Initial results of a clinical trial with patients with metastatic liver disease are presented.

O7

Light-directed drug delivery by photochemical internalization

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Photochemical internalisation (PCI) is a technology for the release of endocytosed molecules into the cytosol. The technology is based on the light-activation of photosensitizers located in the membrane of endocytic vesicles, inducing damage to the membrane and release of drug molecules from the vesicles. Thereby endocytosed molecules can be released to reach their target of action either in the cytosol or in other intracellular compartments, such as the nucleus.

PCI has been shown to stimulate intracellular delivery of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including protein toxins and immunotoxins, oligonucleotides, genes, chemotherapeutic agents; and PCI also works very well with various types of nanocarriers. Since the PCI effect is induced by illumination PCI is a site-specific delivery technology where the effect will be confined to illuminated areas of body. The efficacy and specificity of PCI-mediated delivery can be further improved by the use of targeting moieties, such as the peptide ligands or antibodies to specific cell surface receptors.

PCI also has a very interesting application in vaccination and immunotherapy by enhancing antigen presentation by MHC class I in antigen presenting cells, thereby stimulating antigen-specific cytotoxic T-cell responses.

The efficacy and specificity of PCI have been demonstrated in various animal tumour models, and a phase I/II clinical study (with the cytotoxic agent bleomycin) has been completed in cancer patients with very promising results. Further clinical studies in head and neck cancer (with bleomycin) and bile duct cancer (with gemcitabine) have been started.

O8

Enzymatic degradation induced drug release and imaging of sphingomyelin liposomes

Oula Penate Medina

Lipid manipulating enzymes offers interesting target for liposome based delivery. Sphingomyelinase (SMase) is interesting target for targeted delivery and controlled release. SMase is one of the key enzymes in process of apoptosis. Here w show controlled release system that is based on the sphingomyelin (SM) containing liposomes that is converted to ceramide by SMase. Ceramides disrupt the liposome membrane and enable leakage of the contents of the liposomes. Ionizing radiation elevates activated SMase levels. This presents opportunities for novel approaches to image and selectively target therapies. Optical mouse experiments were done with nude mice bearing a PC3 prostate xenograft. Liposomes containing Cy 5,5, Alexa 680 or ICG,were administered into the mouse by tail vein injection. Mice were scanned by using Maestro fluorescence camera, FMT 2500 or Night Owl fluorescent camera. MRI Mouse experiments were done with nude mice bearing PC3 prostate xenograft. SM containing liposomes were loaded with multiple contrast agents and administered iv. Mouse with murine fibrosarcoma xenografts were imaged with rodent MRI following administration of gadolinium or Iron loaded SM liposomes. After initial imaging, mice were subjected to varying doses of external beam X-ray irradiation and the activation of the liposomes by Acid SMase was read by further MR imaging.

Results

Liposomes were able to rapidly release their contents when acid SMase was introduced to the liposomes or when radiated cells or apoptotic cells were introduced to the liposomes. There were a radiation dose dependent increase in the liposome leakage in vitro and vivo. There were dramatic difference in the fluorescence intensity with the irradiated tumors versus non-irradiated tumors. In MRI animal experiments there was a radiation dose dependent increase of MRI contrast in tumors. SM liposomes can act as an imaging tool and site activated delivery vehicle in vitro and in vivo. The liposomes can release the payload either by SMase activity due apoptosis, inflammation or necrosis or it can be induced with moderate amount of radiation to the endothelial cells.

O9

Microbubble-mediated drug delivery

Twan Lammers

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Ultrasound (US) imaging is clinically well-established for routine screening examinations of breast, abdomen, neck and other soft tissues, as well as for therapy monitoring. Microbubbles as vascular contrast agents improve the detection and characterization of cancerous lesions, inflammatory processes and cardiovascular pathologies. Taking advantage of the excellent sensitivity and specificity of ultrasound for microbubble (MB) detection, molecular imaging can be realized by binding antibodies, peptides, and other targeting moieties to the shell of MB. Molecular MB directed against various targets such as vascular endothelial growth factor receptor-2, vascular cell adhesion molecule 1, intercellular adhesion molecule 1, selectins and integrins have been developed and have been shown to be able to selectively bind to tumor blood vessels and atherosclerotic plaques. Currently, the first MB formulation targeted to angiogenic vessels in prostate cancers is being evaluated clinically. However, MB can be used for more than diagnosis: disintegrating MB emit acoustic forces that are strong enough to induce thrombolysis, and they can also be used for facilitating drug and gene delivery across biologic barriers. In the present lecture, I will summarize several studies recently performed in our laboratories in which MB and US are used for drug delivery purposes. These will include experiments aiming to use VEGFR2-targeted polymeric MB loaded with the fluorescent model drugs rhodamine and coumarin, for image-guided, targeted and triggered drug delivery to tumors, as well as ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle-loaded MB for mediating and at the same time monitoring permeation of the blood-brain barrier (BBB).

Acknowledgements

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References

1. Fokong S, Theek B, Wu Z, Koczera P, Appold L, Jorge S, Resch-Genger U, van Zandvoort M, Storm G, Kiessling F, Lammers T. Image-guided, targeted and triggered drug delivery to tumors using polymer-based microbubbles. J Control Release 163: 75-81 (2012)
2. Kiessling F, Fokong S, Koczera P, Lederle W, Lammers T. Ultrasound microbubbles for molecular diagnosis, therapy and theranostics. J Nucl Med 53: 345-358 (2012)
3. Lammers T, Koczera P, Fokong S, Gremse F, Vogt M, Storm G, Van Zandvoort M, Kiessling F. Theranostic USPIO-loaded microbubbles for MR-controlled blood-brain barrier permeation (submitted)

O10

New cationic liposomes bubbles for nucleic acids transfer

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Ultrasound and microbubbles-mediated gene transfer is a non-invasive, targetable and controlled DNA delivery technique. Under ultrasound microbubbles are known to interact with cells and to permeabilize the plasma membrane leading to sonoporation [1]. The main limitation of this technique is the low transfection efficiency of the commercially available microbubbles. Our project concerns the design of cationic microbubbles with the aim of binding DNA on the microbubble shell and having the capacity to fuse with cellular membranes. Several microbubble formulations were produced using three kinds of cationic lipids: Lipid 1(triple cationic cationic lipid), Lipid 2 (mono charged cationic lipid) and Lipid 3 (fusogenic lipid). Microbubble stability was analysed by optical observations, counting, sizing and flow cytometry. Their acoustic activity and interaction in the presence of cells has also been measured by attenuation measurements and high-speed imaging microscopy. Microbubbles produced by mechanical shaking showed required properties: a proper stability up to 3 hours, a size distribution centered at 2 µm and a resonance frequency around 1.5 MHz. Microbubbles produced presented a zeta potential of +12 mV and a good DNA binding capacity. In vitro sonoporation of HeLa cells using the produced microbubbles resulted in almost 30% of GFP transfected cells. In vivo, gene transfer was achieved on a previous animal model developed on the Achilles tendon [2]. A stable luciferase expression lasting over two weeks was obtained after local injection of microbubbles bearing DNA and sonoporation. Further experiments on in vivo ultrasound microbubble imaging and DNA delivery after systemic injection are in progress.

Acknowledgements

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References

1. Delalande, A., et al., Sonoporation at a low mechanical index. Bubble science, Engineering and Technology, 2011. 3(1): p. 3-11.
2. Delalande, A., et al., Ultrasound and microbubble-assisted gene delivery in Achilles tendons: Long lasting gene expression and restoration of fibromodulin KO phenotype. J Control Release, 2011. 156(2): p. 223-30.

O11

Ultrasound-sensitive microbubbles for stroke therapy – toward a better understanding of the mechanisms of blood clot destruction

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Ischemic stroke results from a cerebral vessel occlusion by a blood clot and vascular debris. Treatments are intended to restore the cerebral blood flow as soon as possible to avoid major brain damage and permanent disability. Currently, recombinant tissue plasminogen activator (rtPA) is the only thrombolytic drug approved for acute ischemic stroke treatment in Europe and by the FDA.

Sonothrombolysis (STL) is a recent and promising approach for the treatment of ischemic stroke. Indeed, in vitro and in vivo studies have shown that the efficiency of thrombolytic drugs can be increased when combined with ultrasound (US) and microbubbles (MB) [1, 2]. However the exact mechanisms involved in the STL process remain misunderstood. In the present study the ability of US, combined with MB, to degrade the fibrin network of the clot was evaluated on an in vitro model.

In vitro human blood clots with 125I-labeled fibrin were produced. Thrombolysis efficacy was assessed in vitro by measuring clot diameter changes over time [3] and fibrin degradation by measuring radioactivity release. The results show an increase of fibrin degradation with rtPA+US+MB compared with rtPA alone. This increase is in accordance with the larger diameter loss observed optically. On the contrary, US+MB without rtPA led to a significant diameter loss without any fibrin degradation. In addition histological observations show an intact fibrin network in the sonicated area for this last condition. This is certainly suboptimal since it could promote subsequent re-clotting

This study led to a better understanding of the mechanisms involved in the STL process. It clearly demonstrated that US and MB are able to potentiate the rtPA efficacy, which is promising for the improvement of stroke treatment and reduction of adverse effects.

References

1. B. Petit, F. Yan, F. Tanquart, E. Allémann, J. Drug Del. Sci. Tech., 22 (5), 381-392 (2012).
2. S. Meairs, W. Culp, Cerebrovasc Dis, 27 Suppl 2, 55-65 (2009).
3. B. Petit, E. Gaud, D. Colevret, M. Arditi, F.Yan, F. Tanquart, E. Allémann, Ultrasound Med Biol, 38 (7), 1222-1233 (2012).

O12

Overcoming limitations in Nanoparticle Drug Delivery. Thermodox: An Update

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This presentation will briefly describe the engineering design of the LTSL. And will present new data that shows how LTSLs in conjunction with local Hyperthermia can release drug within seconds of entering the warmed tumor vasculature. Moreover the released, and now free, drug diffuses into the tumor interstitium with greater penetration distance and to much higher concentrations than those achievable by either free drug administration or via the putative EPR effect required for the more traditional long circulating liposome formulations. The high interstitial doxorubicin concentrations achievable with LTSLs + HT drive drug into the cells and reach their nucleus target. Real-time confocal imaging of doxorubicin delivery to murine tumor-bearing window chambers and histologic analysis of flank tumors illustrates that intravascular drug release provides a mechanism to increase both the time that tumor cells are exposed to maximum drug levels and the penetration distance achievable by drug diffusion. These improvements in drug bioavailability establish a new paradigm in drug delivery: rapidly triggered drug release in the tumor blood stream providing for greater concentrations of drug and maximal penetration depths into tumor tissue, saturating neoplastic cells as well as endothelia, pericytes and stroma with the anti-cancer drug. Additionally, an update of progress in human clinical trials will be given including Phase III for liver cancer, Phase II Recurrent Chest Wall cancer after mastectomy (RCW), and new considerations for Pain reduction in Bone Metastases, MR guided HIFU (w/Phillips) Pancreatic Cancer, MR guided HIFU, Colorectal Liver Mets, Metastatic Liver Cancer, HIFU (w/Univ. of Oxford, England)

O13

Characterization of supported lipid membranes – toward the development of nano-sized drug carriers for hyperthermia applications

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The understanding of the fundamental physicochemical properties of lipid membranes, in particular the distinct transition between the gel phase and the fluid phase in response to temperature, is an important key to successful designs of hyperthermia-responsive nanodrugs for medical applications. Towards this end, we have studied the formation and the function of lipid membrane coatings on planar solid supports, focusing on lipid compositions yielding a gel to liquid phase transition temperature in the range 40 - 45 °C. More specifically, a method of preparing asymmetric lipid membranes on solid surfaces by combining two leaflets in different phase states is demonstrated [1]. As a proof-of-concept, phase transition induced flip-flop between the lipid leaflets is employed to control what lipid head groups are presented at the membrane surface. The process was monitored by the surface-sensitive analytical techniques quartz crystal microbalance with dissipation (QCM-D) and dual polarization interferometry (DPI). The asymmetric structure was stable at a temperature below the effective Tm of the lower leaflet, while lipid flip-flop was induced upon increasing of the temperature above the effective Tm. Transmembrane lipid exchange was demonstrated by detecting, through streptavidin binding, biotinylated lipids appearing at the surface of the top leaflet after 'temperature-activation' of an asymmetric structure where these lipids were first located in the lower leaflet. These results may serve as an inspiration for the design of future, lipid-based drug carriers.

[1] Jing, Y; Kunze, A., and Svedhem, S; submitted

O14

Controlled Photothermal Drug Release using Smart Nanoplatforms

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Although the delivery techniques of biologically active species have been improved over the last few decades, site-specific, time- and dosage-controlled release of these species remain obstacle. Gold nanoparticles (AuNPs) possess strong visible and near-infrared (NIR) light absorption (several orders of magnitude more intense than organic dyes) due to their localized surface plasmon resonance (LSPR) characteristics. The absorbed light (visible or NIR) can be transferred to heat energy via using metal nanostructures. Gold nanocages represent a novel class of nanostructures characterized by hollow interiors and ultrathin, porous walls. These nanocages can have relatively strong absorption (for the photothermal effect) in the NIR region (800-900 nm) where the soft tissue is highly transparent. When the surface of Au nanocage is covered with a smart polymer to form a hybrid system, the pre-loaded effectors can be released with high spatial and temporal resolutions by means of a pulsed NIR laser. In vitro polymeric dye, an anti-cancer drug- (doxorubicin (Dox)) and enzyme (lysozyme) releases from smart golden nanocapsules were successfully demonstrated.

Thermoresponsive polymeric colloids attract great attention in several biotechnological applications owing to their ability to manipulate drug release characteristics in a controlled manner. Majority of these applications utilized N-isopropylacrylamide (NIPAAm)-based particles for controlled drug release. Despite its advantages, such as easy chemical modification and well-documented literature, a potentially important bottleneck for NIPAAm in biological applications is its tendency for nonspecific protein adsorption. Recently, we reported a simple way to prepare novel thermoresponsive colloids composed of oligo(ethylene glycol) side chains via precipitation polymerization technique. In addition to displaying highly reversible thermal response, these particles also have considerably low nonspecific protein adsorption when compared with NIPAAm counterparts. These crosslinked poly(ethylene glycol) ethyl ether methacrylate particles (PEGMA NPs) were characterized using dynamic light scattering and transmission electron microscopy. The effects of co-monomer, crosslinker and initiator on particle characteristics were investigated. Particle toxicity studies were carried out using 3T3 fibroblast cell lines in MTT cytotoxicity assay. Recently, we have also synthesized biocompatible PEGMA NPs with labile bond containing crosslinkers by surfactant free emulsion polymerization (SFEP) method. We showed that these crosslinked PEGMA NPs can be used as a controlled dual drug carrier system.

Acknowledgements

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References

1. M. S. Yavuz, Y. Cheng, J. Chen, C. M. Cobley, Q. Zheng, J. Xie, C. Kim, K. Song, A. G. Schwartz, L. V. Wang, Y. Xia, Nature Materials 2009, 8, 935.
2. M. S. Yavuz, F. Buyukserin, Z. Zengin, S. T. Camli. Journal of Polymer Science Part A: Polymer Chemistry 2011, 49, 4800-4808.
3. F. Buyukserin, S. T. Camli, M. S. Yavuz, G. G. Budak, Journal of Colloid and Interface Science 2011, 355, 76-80.
4. H. Erdogan, H. Sakalak, M. S. Yavuz, G. Demirel, Langmuir 2013, 29, 6975–6982.

O15

Application of mesoporous silica nanoparticles for improved solubility of poorly soluble drugs and targeted delivery vehicles

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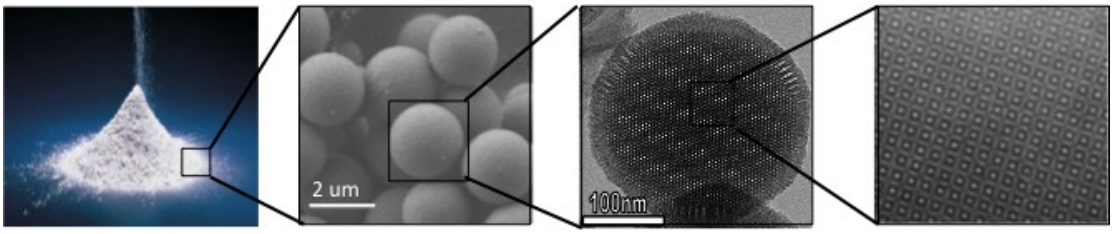
Nanologica is a material development company based in Stockholm with proprietary nanoporous silica platform. Nanologica's mesoporous silica particles are used for drug delivery formulations providing enhanced solubility for poorly soluble drugs and controlled release properties. Encapsulation of drug molecules within the nanopores gives protection against degradation and inhibits drug-drug interactions as has been demonstrated in mice with enhanced bioavailibitly of the HIV/AIDs drug Atazanavir.

Mesoporous silica particles have excellent potential for use as combined diagnostic and therapeutic agents since they can be loaded with a large variety of therapeutic agents and the particles be readily chemically modified with optical and chemical markers for specific targeting and their extremely high internal surface area makes them attractive contrast agents.



Mesoporous silica in powder form seen with the naked eye and magnified to nanometer scale showing the internal porous structure in detail

O16

PEGylated Polymeric Nanocapsules for Targeted Delivery of Hydrophobic Drugs to Folate-expressing Cancer Cells In-vitro and In-vivo

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In this work we describe the formulation and characterization of chemically modified polymeric nanocapsules encapsulating hydrophobic drugs for the passive and active targeting to tumors. Folic acid was conjugated to poly (lactide-co-glycolide) (PLGA) polymer to facilitate active targeting to cancer cells. Two different methods for the conjugation of PLGA to folic acid were employed utilising polyethylene glycol (PEG) as a spacer. Characterisation of the conjugates was performed using FTIR and 1H-NMR studies. The PEG and Folic acid content was independent on the conjugation methodology employed. PEGylation has shown to reduce the size of the nanocapsule, moreover, Zeta potential was shown to be polymer-type dependent. Comparative study on the cytotoxicity and cellular uptake of the different formulations by HeLa cells, in the presence and absence of excess folic acid, were carried out using MTT assay and Confocal Laser Scanning Microscopy, respectively. Both results confirmed the selective uptake and cytotoxicity of the folic acid targeted nanocapsules (containing the drug) to the folate enriched cancer cells in a folate-dependent manner. Finally the passive tumor accumulation and the active targeting of the nanocapsules to folate-expressing cells were confirmed upon intravenous administration in HeLa tumor-bearing mice. The developed nanocapsules thus constitute new opportunities for applying such systems for targeted delivery of a range of hydrophobic anti-cancer drugs in-vivo.

O17

Microbial versus human cells – implications for targeting

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Microbial cells outnumber animal cells within the human body, and under specific circumstances are able to cause serious infections that lead to serious illness and even death (Turnbaugh et al., 2007). A theranostics strategy directed to microbial cells has however some specificities when compared to the counterpart strategy aimed at animal cells. For a start, microbial cells are mostly located in the gastrointestinal tract, or, when an infection occurs, in the blood or in specific organs. Secondly, as they lack a endocytosis mechanism and possess a cell envelope that typically includes a cell wall, the use of molecules that act at the cytoplasm level presents significant challenges.

A possible action to target intracellular compounds in microbial cells is then to use small molecules that have an acceptable diffusion rate through the microbial cell wall. Using small oligonucleotide variations comprising locked nucleic acids (LNA) and 2'-O-methyl RNAs (2'OMe) with two types of backbone linkages (phosphate or phosphorothioate), we were able to show that passive diffusion and specific duplex formation inside Helicobacter pylori occurs at detectable levels at 37 □C and in the presence of very few adjuvant compounds (Fontenete et al., submitted). The diffusion and hybridization also occurred for a broad range of pH, opening prospects for future applications in the stomach. Strategies to improve H. pylori cellular uptake will be tested in the future.

Acknowledgements

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References

1. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007). The human microbiome project. Nature; 449:804-10.
2. Fontenete S, Guimarães N, Leite M, Figueiredo C, Wengel J, Azevedo NF. Hybridization-based detection of Helicobacter pylori at human body temperature using advanced Locked Nucleic Acid (LNA) probes. Submitted.

O18

Edelfosine Lipid Nanosystems Overcome Drug Resistance in Leukemic Cell Lines

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Cancer is one of the leading causes of death worldwide [1] and its treatment still remains challenging. Leukemic represents 7.9% of total cancer cases. Taking this into consideration, considerable research activity is focusing on the benefits of applying nanomedicine to cancer therapy. In this sense, lipid nanoparticles (LN) have been proved to improve drug efficacy and decrease toxicity [2]. The aim of this study was to evaluate the antileukemic efficacy of edelfosine encapsulated in LN in different leukemic cell lines. Previous in vivo studies have demonstrated that LN improve edelfosine's oral bioavailability and decrease its gastrointestinal toxicity [3]. It has also been shown that some leukemia cells are resistant to the entrance of the drug into the cell and that they therefore present resistance to its action [4]. We postulated that LN may vary the drug internalization mechanism in the cell. Nanoencapsulated drug might accumulate in the cell at a higher rate and, therefore, nanomedicine might overcome the resistance to the free drug. To achieve this purpose, leukemia cells with different sensitivity to the treatment were treated with free and nanoencapsulated drug. The drug effect against sensitive cell lines was not affected when edelfosine was encapsulated; the encapsulated drug showed a comparable effect to the free drug at the end of the treatment. Proliferation studies revealed that LN were able to inhibit proliferation in resistant cells whereas the free drug was ineffective. Besides, only edelfosine LN were able to induce apoptosis in resistant cells. Both treatments induced G2/M arrest. Moreover, extrinsic and intrinsic apoptosis pathways were triggered by the free drug but not by the encapsulated edelfosine. These results lead us to think that LN containing edelfosine act through caspase-independent pathways. In conclusion, edelfosine-LN was able to improve the efficacy of the drug in a resistant leukemia cell line whereas the free-drug was practically devoid of efficacy.

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References

1. WHO: Cancer, 2012. Available at: http://www.who.int/mediacentre/factsheets/fs297/en/ [Last accessed 24 March 2012]
2. Estella-Hermoso de Mendoza A, Campanero MA, Mollinedo F, et al. Lipid nanomedicines for anticancer drug therapy. J Biomed Nanotechnol 2009;5:323-43
3. Estella-Hermoso de Mendoza A, Preat V, Mollinedo F, et al. In vitro and in vivo efficacy of edelfosine-loaded lipid nanoparticles against glioma. J. Control. Release. 2011;3:421-6
4. Tidwell T, Guzman G, Vogler WR. The effects of alkyl-lysophospholipids on leukemic cell lines. I. differential action on two human leukemic cell lines, HL60 and K562, Blood. 1981;57:794-7.

O19

Biomimetic apatite nanocrystals: a new platform for nanomedicine

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Nanocrystalline apatite, due to their structural and chemical similarity with the mineral component of mammalian bones and teeth, are ideal materials for healthcare applications. Traditionally, apatite based materials are used for bone repair due to their well-known properties such as osteoinductivity and bioactivity. The recent progresses in the structural characterization at the nanoscale level and in the colloidal stabilization of apatite nanocrystals have opened new perspectives in their use in nanomedicine. They possess several advantages in comparison with the most commonly nanomaterials used for the same aim, such as: (i) favourable biodegradability and biocompatibility properties in general; (ii) solubility and scarcer toxicity than silica, quantum dots and carbon nanotubes; (iii) higher biocompatibility and pH-dependent dissolution. Apatites dissolve into their ionic constituents (Ca2+ and PO43-), which are already present in relatively high concentration (1-5 mM) in the cells and the bloodstream. This dissolution allows actively to prevent undesirable nanoparticle accumulation in cells and tissues, a setback often encountered with inorganic and metallic nanoparticle systems. Nanocrystalline apatites are well known for their capability to bind a wide variety of molecules and therapeutic agents due their high surface area and presence of available surface ionic sites (Ca2+ and PO43-) [1]. In this way, active targeting moieties as well as the therapeutic agent can also be incorporated into the nano-devices to specifically enhance their internalization by the target cells. In this presentation we will display some recent findings in this field of our research activity. In particular the preparation and characterization of nanocrystalline apatites and the possibility to functionalize them with drugs (doxorubicin) and with active targeting moieties (monoclonal antibodies directed against the ectodomain of the TKR for Hepatocyte Growth Factor (HGF-R), which is over-expressed on different types of carcinomas) for the selective cancer cells internalization, will be displayed [2,3]. In addition, the preparation and characterization of superparamagnetic (Fe2+/Fe3+)-doped apatite nanoparticles will be also highlighted [4,5]. The magnetic functionalities could allow to control inside the body the nanocarriers by an external magnetic field and to use them for bioimaging applications. Furthermore, the magnetic feature of the nanoparticles could allow to tailor the release of the therapeutic agent by switching (on-off) the external magnetic field.

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References

1. Iafisco M., Margiotta N. Journal of Inorganic Biochemistry (2012), 117, 237-247.
2. Iafisco M., et al. Small (2013), in press.
3. Rodriguez-Ruiz I., et al. Langmuir (2013) 29(26), 8213−8221.
4. Tampieri A., et al. Acta Biomaterialia (2012), 8(2), 843-851.
5. Iafisco M. et al. Chemistry of Materials (2013), 25(13), 2610–2617.

O20

Selectively modified porous silicon nanoparticles with the functionalities of imaging, targeting and triggered drug release

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The application of nanomaterials in biomedicine offers excellent prospects for the development of novel strategies for drug delivery.[1, 2] Herein an effective drug delivery platform of mesoporous silicon (PSi) was engineered with multiple functionalities of nanoscaled imaging, targeting, and pH-triggered drug release. To achieve the above purposes, firstly a novel method based on "solvent stopper" was developed to selectively modify the PSi prepared by electrochemical etching. The functional components of undecylenic acid and folic acid (FA) were successfully grafted on the internal and external surface of PSi, respectively. Subsequently fluorescein isothiocyanate (FITC) were conjugated on surface of selectively modified PSi for imaging through the linkage of aminosilane.[3] The PSi nanoparticles modified externally with FA showed significant higher intracellular cellular uptake when compared to the control sample without FA modification. Moreover, the engineered PSi nanoparticles presented the effective intracellular drug delivery with pH-triggered functionality. The nanoporous platform with multiple functionalities developed in the present study was proven to be an effective drug delivery system for cancer therapeutic applications.

Scheme 1. Selective modifications of PSi for a multi-functionalities drug delivery plateform

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References

1. Bimbo LM, Sarpanta M, Santos HIA, Airaksinen AJ, Makila E, Laaksonen T, Peltonen L, Lehto VP, Hirnoven J, Salonen J. ACS Nano. 2010;4:3023-32.
2. Barreto JA, O'Malley W, Kubeil M, Graham B, Stephan H, Spiccia L. Advanced Materials. 2011;23:H18-H40.
3. Xu W, Riikonen J, Nissinen T, Suvanto M, Li B, Deng F, Lehto, VP. J Phys Chem C. 2012; 116: 22307-22314.

O21

In Vivo Antitumor Efficacy and Biodistribution of Polyaminoacid Nanocapsules

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Recently we have developed polymer-coated lymphotrophic nanocapsules with potential application for preventing metastatic cancer cell invasion. These nanocapsules consist of an oily core, coated with polyaminoacids such as polyglutamic acid (PGA) or pegylated polyglutamic acid (PGA-PEG), among others (1). Nanocapsules with different sizes (100nm and 200nm) have been efficiently loaded with the anticancer drug docetaxel and their stability in biological media and release profiles have also been evaluated (2).

Biodistribution was performed using optical imaging tools (IVIS) with fluorescently labelled nanocapsules of two different sizes (100nm and 200nm) and two different administration routes (i.v. and s.c.). These studies confirmed the affinity of the carriers to accumulate in lymphatic tissues, being this effect more pronounced for the smaller nanocarriers.

Based on these findings, more recently we have also performed quantitative biodistribution studies with radiolabelled PGA and PGA-PEG nanocapsules of 100 nm. Radiolabelling was carried out by the incorporation of a chelating agent (phospholipid-modified DTPA) and subsequent addition of 111In to the nanocapsules core. Ex vivo counting revealed significant accumulation of the carriers in lymph nodes 24 hours post i.v. administration. In particular, PGA-PEG nanocapsules exhibited high affinity to lymphatics. Administration of nanocapsules by s.c. route showed a slow but effective drainage of nanocapsules. Further studies are being currently conducted to evaluate the lymphatic uptake of the carriers by this latter route.

Antitumor efficacy of PGA-PEG (100 nm) nanocapsules has been evaluated in an endogenous metastatic lung cancer (implanted A549-luc cells). Different doses of docetaxel (5mg/kg and 10mg/kg, encapsulated or as Taxotere®) have been administered to the animals in two separate experimental setups, i.e. before and after the development of lymph node metastasis. Overall, results show that docetaxel encapsulated into PGA-PEG nanocapsules inhibit primary tumor growth and metastatic spreading more efficiently than the same dose of Taxotere. In addition, survival rates and complementary MTD studies also corroborate the better performance of our nanocapsules as compared to the current commercial docetaxel formulation.

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References

1. M.J. Alonso, M. Garcia-Fuentes, T. Gonzalo, G. Lollo, F. Oyarzún, G. Rivera Rodriguez, D. Torres Lopez, Nanocapsules with a polymer shell, PCT/ES2012/00000, WO2012095543 A1.
2. T. Gonzalo, G. Lollo, M. Garcia-Fuentes, D. Torres, J. Correa, R. Riguera, E. Fernandez-Megia, P. Calvo, P. Aviles, M.J. Guillen, M.J. Alonso, A new potential nano-oncological therapy based on polyamino acid nanocapsules, J. Control. Release 169, 10-16, 2013.

Invited Lecture

Production of High Purity and High Specific Activity Radioisotopes

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High purity and high specific activity radionuclides are critical for use in environmental studies, evaluation of biological processes in plants and animals and in medicine for treatment and diagnosis. A need exists for isotopes that can be used for associated diagnosis and therapy coined as “Theranostic Medicine.” This can either be done through the use of a single isotope that can be utilized in both imaging and therapy such as Lu-177 and many of the radiolanthanides, or the use of “matched diagnostic/therapeutic” pairs of radionuclides such as Tc-99m and Re-186 or Au-199 and Au-198. MURR is actively developing novel production and purification methods of radioisotopes with potential use in a variety of applications. Current efforts have focused on developing high specific activity radioisotopes which can be attached to biomolecules or nanoparticles that are taken up selectively by diseased tissues, thus delivering toxic radioactivity to diseased tissue while minimizing or sparing damage to healthy or normal cells. The development of these production methods and radioisotopes will be discussed in the context of their physical and chemical properties as related to their potential utility in medical research.

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O22

Radiopeptides in SPECT/MRI: Optimization of combined peptide receptor radionuclide therapy (PRRT) and temozolomide using SPECT/CT and MRI in mice.

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Aim: Successful treatment of patients with somatostatin receptor overexpressing neuroendocrine tumours (NET) with Lutetium-177-labelled octreotate, (PRRT) or temozolomide (TMZ) as single treatments has been described. Their combination might result in additive response, so we studied tumour characteristics and therapeutic responses after different administration schemes in mice to obtain the optimal strategy to combine PRRT and TMZ. Materials and Methods: Initially we performed imaging studies of nu/nu mice, (n=5-8) bearing somatostatin receptor-expressing human H69 small cell lung carcinoma xenografts, after single administration of ^{177}Lu-octreotate (30MBq/µg) or TMZ therapy (50mg/kg/day (d) 5x/week for 2 weeks). Weekly tumour perfusion was measured by DCE-MRI and tumour ^{111}In-uptake 24h after administration of 30MBq ^{111}In-octreotide was quantified using SPECT/CT. Based on the imaging results, seven groups were included in a combination therapy study in H69 tumour-bearing mice (n=8-9): 1: control (saline), 2: TMZ, 3: PRRT, 4: PRRT + TMZ both d1, 5: PRRT d1, TMZ from d15, 6: TMZ from d1, PRRT d15, 7: PRRT d1 and d15. Study endpoint was tumour volume >1800-2000 mm3. Results: Single treatment with ^{177}Lu-octreotate or TMZ therapy resulted in reduction of tumour size, which led to changes in MRI characteristics such as intrinsic T2, T2* and perfusion values. Moreover, TMZ treatment not only showed tumour size reduction 9d after start of treatment and an increase in MRI perfusion parameters but uptake of ^{111}In-octreotide peaked at d15 followed by a decrease afterwards. In the combination therapy study no complete cure was found in control, single TMZ and single and double PRRT groups, while in the TMZ/PRRT combination groups resp. 44%, 38% and 55% of mice (groups 4, 5 and 6) showed cure without recurrence of tumour growth during follow-up. This was also reflected in an extended median survival time (MST), resp. 101, 107 and 120d. MST for controls only was 35d; single TMZ resulted in a MST of 83d, while PRRT showed a MST of either 56d after single and 74d after double administration. Conclusions: All three TMZ and PRRT combination groups showed additional anti-tumour effect compared to the single treatment groups, including fractioned PRRT. MRI tumour perfusion and SPECT/CT uptake studies proved that administration of ^{177}Lu-octreotate 15d after start of TMZ treatment is the optimal combination as confirmed in the best anti-tumour effects in the model studied. Therefore TMZ administration prior to PRRT might be the best option for clinical practice to increase tumour responses in NET patients as well.

O23

Effects of metal-chelate and peptide chain-length on the biological profile of $^{99m}Tc/^{111}In$-labeled Gastrin I analogs

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Introduction: Radiolabeled gastrin analogs have been proposed for diagnostic imaging and radionuclide therapy of CCK2R-positive human tumors, such as medullary thyroid carcinoma [1-5]. Modifications in amino acid chain-length and type of metal-chelate coupled at the N-terminus may influence the biological profile of resulting radioligands. With the aim to study such effects, we have coupled either N4 or DOTA at the N-terminus of full length and truncated gastrin I (GI, pGlu-Gly-Pro-Trp-Leu-(Glu)5-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH2)-related sequences to allow for ^{99m}Tc or ^{111}In labeling, respectively. As a result, the following analogs were developed: SG1 ([(DOTA)Gln1,Nle15]GI), MG11 ([(DOTA)DGlu10]GI(10-17), SG6 ([(N4)Gln1]GI) and DG4 ([(N4)DGlu10]GI(10-17)).

Materials and Methods: Binding affinities for the CCK2R were determined by [^{125}I-Tyr12,Leu15]GI displacement assays in A431-CCK2R(+) cell membranes (22°C, 1 h incubation). Labeling of SG1/MG11 with ^{111}In and SG6/DG4 with ^{99m}Tc was conducted following established methods [2-4]; products were analyzed by RP-HPLC. Radioligand internalization was studied by 1 h incubation at 37oC in confluent monolayers of A431-CCK2R(+) cells. Radiopeptides were injected in the tail vein of healthy Swiss albino mice and blood was collected 5 min postinjection (pi) and analyzed by RP-HPLC. Subcutaneous tumors grew in the flanks of SCID mice after inoculation of AR4-2J cells, endogenously expressing the CCK2R. After injection of a radiopeptide bolus (100 μL, 2-4 μCi, 10 pmol peptide) in the tail vein, animals were sacrificed at 4 h pi and biodistribution was conducted; excess DG2 (100 μg) was used for in vivo CCK2R-blockade [2].

Results: CCK2R binding affinity was high for truncated analogs (IC50: MG11, 1.1 nM; DG4, 0.9 nM) declining for SG1 (3 nM) and SG6 (9.3 nM). Labeling resulted in high yield and high purity radioligand formation, as verified by RP-HPLC analysis. All analogs internalized in A431-CCK2R(+) cells with comparable efficiency via a CCK2R-mediated process, except for [^{99m}Tc]DG4 which showed weaker internalization. Analysis of mouse blood collected 5 min pi showed that full length radiopeptides were more stable than truncated ones ([^{111}In]SG1= 28% intact, [^{99m}Tc]SG6= 34% intact, [^{111}In]MG11= 4% intact, [^{99m}Tc]DG4= 10% intact). The AR4-2J tumor uptake in SCID mice was superior for [^{111}In]SG1 (5.7%ID/g) and [^{99m}Tc]SG6 (3.3%ID/g) and lower for [^{111}In]MG11 (1.2%ID/g) and [^{99m}Tc]DG4 (1.8%ID/g) at 4 h pi. However, renal uptake was unfavourably high for [^{111}In]SG1 (104.4%ID/g) and lower for [^{99m}Tc]SG6 (26.8%ID/g). In line with previous reports [3-5], the short radiopeptides displayed minimal kidney accumulation (1.8%ID/g).

Conclusions: These results demonstrate that metal-chelate and peptide chain-length greatly affect key-biological parameters of gastrin-based radioligands. [^{99m}Tc]SG6 is more stable in mouse blood stream and displays high tumor uptake combined with low renal accumulation. As a result, [^{99m}Tc]SG6 shows the most promising overall pharmacokinetic profile among this series of compounds.

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References

1. J.C. Reubi et al. Curr. Top. Med. Chem. 2007, 7(12), 1239-1242.
2. B.A. Nock et al. Eur. J. Nucl. Med. Mol. Imaging 2005, 46, 1727-1736.
3. A.C. Fröberg, et al. Eur. J. Nucl. Med. Mol. Imaging 2009, 36(8), 1265-1272.
4. W. A. Breeman, et al. Nucl. Med. Biol. 2008, 35, 839-849.
5. P. Laverman, et al. Eur. J. Nucl. Med. Mol. Imaging 2011, 38,1410-1416.

O24

A Cu-64-labeled Sar-conjugated bombesin antagonist as new PET tracer for imaging of prostate cancer

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The increasing evidence of peptide receptor overexpression in various tumors has generated interest in the development of radiolabeled peptides for radiodiagnosis and/or targeted radiotherapy. One example of this approach is the gastrin-releasing peptide receptor (GRPr), which is currently being targeted with radiolabeled bombesin derivatives. Taking into account our previous findings which support that the presence of a positive charge at the N-terminal of radiolabeled bombesin-based agonists, may improve the binding affinity of the derived ligand, this study aims at developing a statine bombesin-based antagonist suitable to be labeled with ^{64}Cu for PET imaging of GRPr-positive tumors.

Methods: The bombesin antagonist D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH2, was conjugated to the MeSar chelator via a PEG4 spacer (15-amino-4,7,10,13-tetraoxapentadecanoic acid) and radiolabeled with ^{64}Cu. The GRPr affinities of natCu-MeSar-AR and MeSar-AR were determined using ^{125}I-Tyr4-BN as radioligand and native BN as control peptide. In vitro evaluation including the determination of the lipophilicity and internalization - dissociation studies were performed using the human prostate cancer cell line PC3 which overexpresses the GRPr. The in vivo evaluation consisted of biodistribution and PET animal studies in PC3 tumor bearing mice.

Results: MeSar-AR was labeled with ^{64}Cu with a high specific activity of 67.8 MBq/nmol without any further purification. ^{64}Cu-MeSar-AR exhibits a high affinity to GRPr (IC50 = 1.4 ± 0.1 nmol/L). Internalization, dissociation and calcium flux measurements validate the antagonist behavior of the radiopeptide. Biodistribution studies as well as PET imaging revealed the high and specific tumor and GRPr positive tissue uptake of ^{64}Cu-MeSar-AR. Tumor was the tissue with the highest uptake of 19.59 ± 4.71 % IA/g at 1 h p.i.. Biodistribution studies also demonstrate the relatively fast washout from the blood circulation and positive GRPr organs such as the pancreas resulting in increasing tumor-to-background ratios at later time points. Biodistribution studies and PET images highlight the predominant renal excretion pathway.

Conclusion: This study demonstrates the high affinity of ^{64}Cu-MeSar-AR to GRPr. The overexpression of those receptors, the long life-time of ^{64}Cu, the stable encapsulation of ^{64}Cu by MeSar and the suitable biodistribution profile of ^{64}Cu-MeSar-AR leads to PET images with high contrast.

References

1. Abiraj K, Mansi R, Tamma ML, et al. Bombesin antagonist-based radioligands for translational nuclear imaging of gastrin-releasing peptide receptor-positive tumors. J Nucl Med. 2011;52:1-9.
2. Cai, H., J. Fissekis, and P.S. Conti, Synthesis of a novel bifunctional chelator AmBaSar based on sarcophagine for peptide conjugation and ^{64}Cu radiolabelling. Dalton Trans. 2009;27:5395-400.

O25

Multimodal imaging probes for the detection of amyloid plaques

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Alzheimer's disease (AD) is the most frequent form of intellectual deterioration in elderly individuals, characterized by the brain deposition of amyloid plaques and neurofibrillary tangles. Early detection of the β-amyloid (Aβ) deposits in vivo is very difficult. Recently ^{11}C-radiolabeled small-molecules have been developed, capable of entering the brain and specifically targeting amyloid plaques for imaging with PET, such as several Thioflavin T derivatives [1,2]. In particular, the uncharged analogue 6-OH-BTA-1 (Pittsburgh compound B- PiB) is highly efficient both in crossing the BBB and in selective binding to AD amyloid aggregates. The use of Aβ marker linked to a MRI CA would constitute an attractive noninvasive in vivo imaging approach.

In an attempt to label Aβ plaques using small metal complexes for the diagnostics of Alzheimer disease, we have synthesized a series of new PiB-derivatives of DO3A and studied its dissociation constants (KD) with Aβ1-40 peptides by SPR and 1H STD NMR. In vivo studies with mice confirmed moderate BBB passage of the [^{111}In]DO3A-PiB derivative denoting the potential utility of these derivatives [3].

1. C. Mathis, et al., Bioorg. Med. Chem. Lett., 2002 , 12 , 295-298.
2. E. Nesterov, et al., Angew. Chem. Int. Ed. Engl., 2005 , 26 , 5452-5456.
3. A. Martins et al., ACS Med Chem Lett, 2013, 4, 436.

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O26

Systematic study for optimizing the Radiolabelling of AGuIX ® Multimodal Nanoparticles.

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Over the past two decades, nanoparticles have been developed in the field of theranostic applications [1]. A new type of ultrasmall nanoprobes has recently been developed [2]. The AGuIX® particles have been characterized for their size (3-5 nm), mass (8.5kDa) and imaging properties [3]. They are composed of a polysiloxane inorganic matrix and surrounded by chelates which can entrap gadolinium for MRI and also radioisotopes for scintigraphy.

The aim of the present work consists in studying and optimizing the radiolabelling of these particles with $^{44m/44}$Sc, ^{64}Cu and ^{67}Ga in various physico-chemical conditions such as pH, temperature, metal-to-nanoparticle ratio, time. These 3 radiotracers are of interest for PET imaging. In addition to this, the influence of the ligand ratio at the surface of the polysiloxane matrix and the nature of the ligand on the radiolabelling yield will be also studied.

Results have shown that Gadolinium-based nanoparticles can be radiolabelled with ^{44}Sc, ^{64}Cu, and ^{67}Ga with high yields. The stability of these nanoparticles in serum has been studied and challenging studies towards bone substitutes have been performed too.

This paper will present a complete description of the physico-chemical study and the first biological tests.

References

1. C.Rivière, S.Roux, O. Tillement, C. Billotey, P. Perriat, [2006] Ann. Chim. Sci. Mater., 31, 351-357.
2. F. Lux et al., Angew. Chem. Int. Ed., 2011, 123, 12507-12511.
3. G. Le Duc et al, [2011] ACS Nano 5, 9566.
4. F. Lux et al, [2011] Angew. Chem. Int. Ed 123, 12507.

O27

Nanozeolite-LTL with Gd3+ deposited in the large and Eu3+ in the small cavities as a bimodal MRI-Optical probe

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The immense structural diversity of more than 200 known zeolites is the basis for the wide range of applications of these either natural (>40) or synthetic materials that vary from molecular filtration and catalysis to biological uses. The anionic aluminosilicate framework of every zeolite is arranged in a unique manner, where the loosely bound counter ions in many cases can easily be exchanged by any other cations that fit into the cavities. In this study, we explore the unique structural properties of nanozeolte-LTL, which is composed of both, large channels that are well accessible for water and are less accessible small cavities [1].

Eu3+-ion was chosen as an optical reporter because of its sharp emission bands in the visible region (570-720 nm). The removal of the highly quenching water molecules coordinated to the Eu centre is responsible for a dramatic increase of the luminescence intensities (from 120 to 718 p.d.u.) and lifetimes upon locking of this lanthanide into the small cavities as result of thermal treatment (600 °C). On the other hand Gd3+ was envisaged as an MRI reporter. Since the r1 relaxivity is directly proportional to the number of water molecules coordinated to the Gd-centre, loading of Gd3+ into the big pores of the LTL-zeolite pre-loaded with Eu3+ into the small cages, offers an elegant approach for the exploitation of the particular physical properties of each lanthanide in one probe.

NMRD measurements revealed that the exchange of water between the interior of the zeolite and bulk water was much faster than for previously studied mesoporous materials,[2] most probably due to the unlimited diffusion conditioned by the linear channels. As result of the fastest water exchange that was found in Gd-loaded mesoporous up to now, the 1H relaxivities as high as 44 mM-1s-1 (25° C, 60 MHz) with a high relaxivity density (13.7 s–1Lg–1) could be achieved.

Based on this findings, high performance dual imaging probes can be developed and their application can be extended to nuclear imaging/therapy by loading with radioactive lanthanides.

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References

1. Newell, P. A.; Rees, L. V. C. Zeolites, 1983, 3, 22.
2. Peters, J. A.; Djanashvili, K. Eur. J. Inorg. Chem., 2012, 1961.

O28

Substance P - nanozeolite labeled with ^{224}Ra and ^{225}Ra - new potential radiobioconjugate for internal alpha therapy

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The ^{223}Ra, ^{224}Ra and ^{225}Ra radioisotopes exhibit very attractive nuclear properties for application in radionuclide therapy. Unfortunately the lack of appropriate bifunctional ligand for radium was the reason why these radionuclides did not find application in receptor targeted therapy. In the present work the potential usefulness of the NaA nanozeolite as a carrier for radium radionuclides has been studied. ^{224}Ra and ^{225}Ra, the α-particle emitting radionuclides, have been absorbed in the nanometer-sized NaA zeolite through simple ion-exchange. ^{224,225}Ra-nanozeolites have shown very good stability in solutions containing: physiological salt, EDTA, amino acid and human serum. To make NaA nanozeolite particles dispersed in water their surface has been modified with silane coupling agent containing poly(ethylene glycol) (PEG) molecules. In the next step short peptide substance P were covalently attach to the PEG-nanozeolite surface. The stability, cell affinity and cytotoxicity studies of the obtained radiobioconjugate are in progress.

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O29

64Cu radiolabeled nanomaterials as bimodal contrast agent for optical imaging and Positron Emission Tomography (PET)

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By combining the strengths of two complementary imaging techniques, bimodal contrast agents are powerful tools for diagnostic, gathering a large panel of informations at the anatomic, physiologic and molecular levels.

Fluorescent nanocrystals made of semiconducting material, also called Quantum Dots, are ideal agents for long-term or real-time optical imaging. They have been found to outperform traditional organic fluorescent dyes in many ways (size-tunable optical properties, high quantum yields, high extinction coefficients, resistance to photobleaching). We have developed a straightforward microwave method for the synthesis of highly luminescent water soluble CdTexS1-x nanocrystals (Φ= 53% at 600 nm), which can be easily functionalized at their surface with proteins and antibodies for targeting.

The same coupling strategy will be used to incorporate 64Cu-radiotracers for PET imaging (β+, t1/2 = 12.7 h) at the surface of the Quantum Dots. Our approach focuses on the synthesis of new ligands based on the bispidine (bispidine = 3,7-diazabicyclo[3.3.1]nonane) backbone substituted by nitrogen- and oxygen- containing groups.[2] Physicochemical studies on ligand L have demonstrated the formation of Cu(II) complexes with high kinetic and thermodynamic stabilities, a fast kinetic of complexation and a particularly good selectivity for Cu(II).

References

1. T. J. Wadas, E. H. Wong, G. R. Weisman, C. J. Anderson, Chem. Rev., 2010, 110(5), 2858-2902.
2. T. Legdali, A. Roux, C. Platas-Iglesias, F. Camerel, A. M. Nonat, L. J. Charbonnière, J. Org. Chem., 2012, 77, 11167-11176.

O30

Efficient radiometal-labelling of drug delivery systems utilizing water/ethanol mixtures

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OBJECTIVES

Radiopharmaceuticals are typically synthesized in pure aqueous solutions. Labeling yields are defined by temperature, period of labeling, amount of labeling precursor, pH etc. In many cases, labeling requires relatively high temperatures. Those conditions may not be applicable to various drug delivery systems. Consequently, the effect of improving the efficacy of radiolabelling was studied by means of adding non-aqueous solvents to the aqueous solutions.

METHODS

^{68}Ga eluates obtained from an EZAG / Obninsk generator were used with and without cation-exchange based post-processing. The fraction of non-aqueous solvents added was varied in the range from 10 to 40 volume-%. Solvents such as methanol, ethanol, isopropanol, octanol, acetonitrile, DMF, DMSO, PEG-derivatives etc. were added to aqueous solutions containing DOTA- and NOTA-conjugated precursors. Labeling parameters according to standard protocols were modified in terms of lower temperature, shorter reaction time and lower amount of precursor. Labeling yields were analyzed by TLC and HPLC.

RESULTS

For many non-aqueous solvents, labeling yields improved significantly at lower temperatures, shorter reaction times and less amounts of precursor. For example, while aqueous systems at 70°C, 30 µg DOTANOC and 10 min reaction time give 40% radiochemical yield for ^{68}Ga-DOTA-NOC, these yields are 55%, 75% and 90% for 10, 30 and 40 vol-% of ethanol, isopropanol and acetonitrile, respectively. When amounts of precursors are reduced from 30 to 20 or 5 µg, labeling yields are higher for 30 vol-% isopropanol or ethanol mixtures by factors of about 2, 3 and 5, respectively, which represents significantly increased specific activities.

CONCLUSIONS

There is experimental evidence, that mixtures of aqueous + non-aqueous solvents significantly improve ^{68}Ga labeling efficacies in terms of temperature, time and concentration. For specific parameters, yields improve by a factor of 2 or more. Meanwhile, this effect was also demonstrated for other trivalent metal such as ^{44}Sc and β-emitting ^{177}Lu. It may result in higher specific yields of the labeled products and may allow labeling reactions at significantly lower temperature which is relevant for sensitive biological molecules such as e.g. proteins. The most probable explanation is the perturbation of the hydration sphere of the central cation, which allows easier access to complex formation with the ligands present.

O31

Near-infrared Imaging in Living Cells with Lanthanides: Yb3+ nanoMOFs

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We have created novel near-infrared-emitting nanoscale metal-organic frameworks (nano-MOFs) incorporating a high density of Yb^{3+} lanthanide cations and sensitizers derived from phenylene. We establish here that these nano-MOFs can be incorporated into living cells for near-infrared (NIR) imaging.
The use of near-infrared (NIR) photons is beneficial for improved detection sensitivity in biological systems because 1) biological systems have lower autofluorescence in the NIR, resulting in higher signal-to-noise ratios and detection sensitivity; and 2) the lower scattering of NIR photons allows for the acquisition of higher resolution images. Furthermore, the luminescence of NIR emitting lanthanide cations is very attractive due to its emission wavelength and the photostability of the luminescent reporters. In order to take advantage of such luminescence, lanthanide cations need to be sensitized. In order to maximize the number of photons per unit volume, we have chosen to create a nanomaterial incorporating a high density of lanthanide cations and lanthanide sensitizers.

We present here a new nanoscale metal-organic framework (nanoMOF) based on a (PVDC) sensitizer-ligands and Yb^{3+} NIR-emitting lanthanide cations. This material has been structurally characterized, its stability in various media has been assessed, and its luminescent properties have been studied. We demonstrate that it is stable in certain specific biological media, does not photobleach, and has an IC_{50} of 100 µg/mL, which is sufficient to allow live cell imaging. Confocal microscopy and ICP measurements reveal that nano-Yb-PVDC-3 can be internalized by cells with a cytoplasmic localization. Despite its relatively low quantum yield, nano-Yb-PVDC-3 emits a sufficient number of photons per unit volume to serve as a NIR-emitting reporter for imaging living HeLa and NIH 3T3 cells. NIR microscopy allows for highly efficient discrimination between the nanoMOF emission signal and the cellular autofluorescence arising from biological material. This work represents one of the first demonstrations of the possibility of using NIR lanthanide emission for biological imaging applications in living cells with single photon excitation.

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O32

Equilibrium, kinetic and structural properties of lanthanide(III)- complexes formed with a sulphonamide derivative of DO3A

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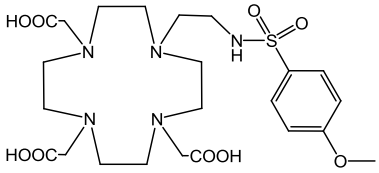
The GdDO3A-arylsulphonamide (DO3A-SA) complex is reported1 to be a promising pH-sensitive MRI agent, but detailed characterization of the complex has not been carried out so far. The study on structure, thermodynamic stability and kinetics of formation and dissociation reactions of the lanthanide(III)- and Mg^{2+}-, Ca^{2+}-, Mn^{2+}-, Zn^{2+}- Cu^{2+}-complexes formed with DO3A and DO3A-arylsulphonamide (DO3A-SA) will be presented in our talk.



H_4DO3A-SA

The stability constants of the DO3A-SA and DO3A complexes formed with divalent metal ions are similar, whereas the logKLnL values of Ln(DO3A-SA) complexes are 2 orders of magnitude higher than those of DO3A complexes. In the Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} -complexes of DO3A-SA, the protonation constant (logKMHL) of the sulphonamide nitrogen is very similar to that of the free ligand, whereas the logKLnHL values of the Ln(DO3A-SA) complexes are lower by about 4 logK units, indicating strong interaction between the Ln3+-ions and the sulphonamide N-atom.

The protonated Ln(HDO3A-SA) complexes are formed via the formation of tri-protonated (*Ln(H3DO3A-SA) intermediates followed by to the final complex. This mechanism and reaction rates are similar as it has been found for the Ln(DOTA) complexes for the OH- -ion assisted pathway. The transmetallation reaction of Gd(HDO3A-SA) with Cu^{2+} is very slow ($t_{1/2}$=5.6×103 hour at pH=7.4) and mainly occurs through proton assisted dissociation of the complex near physiological conditions. Interestingly, this exchange reaction is significantly slower in the presence of *β*-cyclodextrin ($t_{1/2}$=9.9×103 hour at pH=7.4), due to non-covalent interactions between the Gd(HDO3A-SA) complex and *β*-cyclodextrin. This behavior of Ln(DO3A-SA) complexes may be advantageous for biological applications.

1H and 13C-NMR spectra of the La-, Eu-, Y- and Lu(DO3A-SA) complexes have been assigned using 2D correlation spectroscopy (COSY, EXSY, HSQC). Two sets of signals are observed for Eu-, Y- and Lu(DO3A-SA) showing two coordination isomers in solution, i.e. square antiprismatic (SAP) and twisted square antiprismatic (TSAP) geometries with ratios of 86 – 14, 93 – 7 and 94 – 6 %, respectively. Line shape analysis of the ^{13}C-NMR spectra of La-, Y- and Lu(DO3A-SA) gives higher rates and lower activation entropy values compared to Eu(DOTA) for the arm rotation, which indicates that the Ln(DO3A-SA) complexes are less rigid due to the larger flexibility of the ethylene group in the sulphonamide pendant arm.

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References

1. M. P. Lowe, D. Parker, O. Reany, S. Aime, M. Botta, G. Castellano, E. Gianolio, R. Pagliarni, J. Am. Chem. Soc., 2001, 123, 7601.

O33

Development of Ca2+ Responsive Contrast Agents for fMRI

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Magnetic resonance imaging (MRI) using contrast agents has been widely employed in diagnostic imaging and biomedical research. For this purpose, Gd3+ based complexes are commonly utilized. Recently, responsive (smart) contrast agents (SCAs) are being developed in order to aid better understanding of biological processes [1] They are able to report physiological or pathophysiological changes by altering the MR signal they produce.

However the routine in vivo use of SCA is hampered due to challenges such as lack of tools to localize or quantify the agents, low MR signal, non-specific delivery etc. To overcome these challenges, one of the meaningful strategies is to conjugate SCA to various functional molecules such as dendrimers, nanoparticles, delivery vectors or fluorescent tags. The essential requirement when coupling SCAs to functional molecules is retaining their crucial physico-chemical properties in terms of MRI activity.

Hence, the overall objective of our approach was to develop synthetic strategies in which modified DO3A chelator is appended with different linkers for further functionalization. Diverse synthetic strategies were successfully developed using liquid, as well as solid phase chemistry [2]. The SCA were modified and they still robustly response to Ca2+. The newly developed strategies open pathways to improve in vivo applicability of DO3A-based SCAs and to serve as better in vivo reporters in future fMRI experiments.

References

1. Chang, C. J.; Que, E. L., Responsive magnetic resonance imaging contrast agents as chemical sensors for metals in biology and medicine. Chemical Society Reviews, 2010, 39, 51-60.
2. Vibhute, S. M.; Engelmann, J.; Verbic, T.; Maier, M. E.; Logothetis, N. K.; Angelovski, G., Synthesis and characterization of pH-sensitive, biotinylated MRI contrast agents and their conjugates with avidin. Organic & Biomolecular Chemistry , 2013, 11, 1294-1305.

O34

Nanobody-based Targeted Radiotherapy for Cancer Treatment

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Introduction: There is an unmet need for neo-adjuvant strategies in the treatment of residual and micrometastatic disease in cancer therapy. Nanobodies are the smallest natural antigen-binding fragments, occuring from heavy chain-only Camel antibodies. These small proteins have a size of 15 kDa and are characterized by improved solubility and stability, efficient and specific tumor targeting, and rapid blood clearance. Their potential as diagnostic markers have been reported intensively, targeting a viariety of extracellular tumor cell targets like CEA (Vaneycken et al., 2010), EGFR (Gainkam et al., 2008), HER2 (Vaneycken et al., 2011 ; Xavier et al., 2013), and PSMA (Evazalipour et al., 2013). Radiolabeled with a radioisotope, biodistribution is characterized by high uptake values in target tissue and low background activity except for the kidneys. Indeed, in the referred reports high kidney uptake (up to 150 % IA/g) is observed. For therapeutic purposes, we recenty described the development of a 177-Lutetium radiolabeled anti-HER2 nanobody (D'Huyvetter et al., 2012). In the current abstract we describe the selection of an optimized antiHER2-nanobody, with a focus on reducing the kidney retention. The optimized anti-HER2 nanobody was used in a first experimental targeted radionuclide therapy study in HER2 overexpressing tumor xenografts.

Materials & Methods: The anti-HER2 nanobody was produced with 3 different c-terminal amino-acid sequences (-MycHistag, -Histag, untagged). Nanobody production and radiolabeling was performed as described elsewhere (Xavier et al., 2013; D'Huyvetter et al., 2011). The impact of the amino acid-tags on biodistribution was evaluated in tumor xenografts at 1 h p.i. The most favorable nanobody construct was further evaluated for dosimetric calculations. Next, an experimental targeted radionuclide therapy study was conducted. Tumor xenografts with tumor volumes of 20-30 mm3 were injected with 7 doses (once a week) of either PBS (Group 1), 177Lu-labeled control BCII10 nanobody (Group 2), or with 177Lu-labeled anti-HER2 2Rs15d nanobody (Group 3), all co-injected with 150 mg/kg Gelofusin. Tumor growth was monitored using caliper and bioluminescence. After 125 days, results were combined in a event-free survival curve. Animals were excluded from the event-free survival curve when 20 % weight loss, necrotic tumor tissue, or a tumor volume that exceeded 250 mm3 was observed.

Results: Important differences in kidney uptake were observed in tumor xenografts with 195.8 ± 23.7 %; 127.7 ± 2.9 %; 25.8 ± 1.3 % and 10.4 ± 1.7 % IA/g for the MycHis-tagged, His-tagged, untagged, and untagged nanobody + 150 mg/kg Gelofusin, respectively. Uptake values in tumor and additional organs and tissues did not differ significantly.
In the experimental targeted radionuclide therapy study, xenografts in both the PBS-treated (n=8) and the 177Lu-BCII10-treated groups (n=8) reached a tumor volume of 250 mm3 between day 33 and 75 after inoculation. All animals from the control groups were euthanized at day 85, due to the development of large tumors (> 1 cm3). No statistical significant difference was observed in event-free survival between both control groups. Among the mice treated with untagged 177Lu-2Rs15d (n=8), no tumor volume above 250 mm3 was observed until day 125. Even more so, 5 out of the 8 mice were completely free of tumor burden, confirmed with bioluminescence. The other 3 mice gave rise to small, but not palpable tumors. Thus, event-free survival was significantly different for the untagged 177Lu-2Rs15d treated group compared to the PBS-treated ($P < 0.0001$) and the 177Lu-BCII10-treated ($P < 0.0001$) groups.

Conclusion: Kidney uptake could be reduced from a value of 195 % IA/g to only 25% IA/g, at 1h p.i., by removing the c-terminal amino-acid sequence. A co-injection with Gelofusin could further lower kidney uptake values to 10 % IA/g at 1h p.i. The 177Lu-radiolabeled untagged anti-HER2 2Rs15d nanobody was able to inhibit tumor growth in HER2 over expressing tumor xenografts, as a significant difference in event-free survival was observed between treated group and the control groups.

Vaneycken I et al., 2010. J Nucl Med;51(7):1099-106 ; Gainkam LO et al., 2008. J Nucl Med;49(5):788-95 ; Vaneycken I et al., 2011. FASEB J;25(7):2433-46 ; Xavier C et al., 2013. J Nucl Med [Epub ahead of print] ; D'Huyvetter M et al., 2012. Contrast Media Mol Imaging;7(2):254-64.

O35

High MRI performance mesoporous silica nanoparticles functionalized with Gd-chelates as potential theranostic systems

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Introduction. The characteristic properties of mesoporous silica nanoparticles (MSNs), such as uniform mesopores, thermal stability, ease of chemical functionalization, and high biocompatibility, have attracted great interest for a number of biomedical applications.1 In particular, mesoporous materials with tunable pores size are relevant for an efficient delivery of drugs for either a controlled release or to overcome a possible low solubility, so that their potential in anticancer therapy has been recently investigated [1]. These materials are also ideal platforms for the development of magnetic resonance imaging (MRI) probes of high efficiency and high sensitivity owing to their ability to carry a high payload of paramagnetic units.2-4 The use of different types of MSNs as Gd-complexes carriers was explored in detail in order to enhance the relaxivity per Gd and per particle [2-4].

Methods. Initially, different Gd(III)-chelates were immobilized on nanosized (20-50 nm) MCM-41 nanoparticles in order to investigate the chemical role of the porous support on the magnetic properties of the systems. This study allowed to determine the most efficient Gd(III)-chelate and to demonstrate that the best results were obtained with the attachment of the chelates only on the external surface of the nanoparticle.3 Thus, MSN with both surfactant and aminopropyl triethoxysilane were synthesised to allow the presence of NH2 groups on the external surface of the particles. The attachment of GdDOTAGA followed by extraction of the surfactant left the Gd-complexes on the exterior of the MSNs and the pores empty [4].

Results. A chemical interaction of the Gd(III) complexes with the MCM-41 surface was found to be responsible of unexpectedly low relaxivity values. In order to account for this result, the protonated amino groups present on the silica surface were transformed in neutral amide groups resulting in a large increase of the longitudinal molar relaxivity (per Gd) of the material passing from 20.3 to 37.8 mM-1s-1 (r1p per particle = 29500 mM-1s-1). Optimized nanoparticles GdDOTAGA-MSNs are slightly larger (80 nm by TEM and hydrodynamic diameter of 200 nm by DLS measurements), with a lower amount of GdDOTAGA attached (ca. 10%) and a relaxivity per Gd ca. 250% higher than the previous NPs based on MCM-41. Transformation of the amino groups into amides enhances further the relaxivity reaching a value of almost 80 mM-1s-1 per Gd (67×103 mM-1 s-1 per particle).

Conclusions. The selective anchoring of Gd(III) complexes on the outer surface gives rise to porous systems optimized for MRI applications, but the presence of empty internal channels and free functionalities would allow their use in dual imaging and theranostic applications.

References

1. J. L. Vivero-Escoto, R. C. Huxford-Phillips, W. Lin, Chem. Soc. Rev. 2012, 41, 2673.
2. F. Carniato, L. Tei, et al., Chem. Commun., 2009, 1246.
3. F. Carniato, L. Tei, et al., Chem Eur J, 2010, 16, 10727.
4. F. Carniato, L. Tei, et al., Chem Eur J, 2013, 19, 1421.

O36

Superparamagnetic iron oxide nanoparticles for local thermotherapy: potential and limitations

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Hyperthermia, or more specifically thermotherapy, is an established adjuvant in oncology when combined with chemo- or radiotherapy. To achieve a local, mild heat tumor treatment, superparamagnetic iron oxide nanoparticles (spions) may be used, under exposure to an external alternating magnetic field.

Direct injection of spion suspension - so called magnetic fluid hyperthermia - has shown promises in clinical trials. Alternatively, local high spion concentration can be administered as in-situ forming depots at tumor sites, an approach developed in our labs. The technique should allow for repeated treatment of deep-seated tumors, in contrast to other existing modes of heat application. It might be particularly attractive for bone and spine tumors. However, a tight control of target tissue temperature still needs to be established. Moreover, the limited magnetic field strength applicable to humans should also be taken into account in view of a safe and efficient clinical application.

O37

Bimodal Imaging of Sentinel Lymph Node with 99mTc(CO)3-labeled Mannosylated Nanoparticles bearing an NIR-fluorophore

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Sentinel lymph node detection (SLND) is very important not only for cancer management, but also for the establishment of the most adequate therapy.1, 2 SLND is performed preoperatively by intradermal injection of a radiolabeled colloid, whereas its intraoperative localization depends on the acoustic signal coming from the hand-held gamma probe and visual confirmation of the radioactive node that is stained with a blue dye.2, 3 Despite being widely used in the clinical setting for SLND, 99mTc-based colloids (e.g. 99mTc-human serum albumin colloids) and blue dyes present a set of properties that are far from ideal, which hamper the surgeon's ability to identify and excise the sentinel lymph node in minimal invasive manner.2, 3

The mannose receptor (MR) expressed on lymphatic macrophages is an attractive target to design receptor specific diagnostic agents for SLND.4 The encouraging results obtained with 99mTc(CO)3-mannosylated dextran,5, 6 namely high SLN extraction, prompted us to design a bimodal probe that might improve the current clinical approach. In this communication we report on the synthesis, characterization and biological evaluation of a fluorescent 99mTc(CO)3-labeled mannosylated dextran that would enable the preoperative visualization of SLN by Single Photon Emission Computed Tomography (SPECT) as well as the intraoperative real-time guidance for surgical excision by optical imaging in the near infrared (NIR) field. This probe has been prepared by functionalizing the dextran-backbone with mannose units, specific for SLN detection, pyrazolyl-diamine chelating units for stabilization of the fac-[99mTc(CO)3]+ moiety and NIR fluorophore units for optical imaging.

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References

1. Leong, S. P.; Nakakura, E. K.; Pollock, R.; Choti, M. A.; Morton, D. L.; Henner, W. D.; Lal, A.; Pillai, R.; Clark, O. H.; Cady, B. Unique patterns of metastases in common and rare types of malignancy. J Surg Oncol 2011, 103, 607-14.
2. Sharma, R.; Wendt, J. A.; Rasmussen, J. C.; Adams, K. E.; Marshall, M. V.; Sevick-Muraca, E. M. New horizons for imaging lymphatic function. Lymphatic Continuum Revisited 2008, 1131, 13-36.
3. Ravizzini, G.; Turkbey, B.; Barrett, T.; Kobayashi, H.; Choyke, P. L. Nanoparticles in sentinel lymph node mapping. Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology 2009, 1, 610-623.
4. Marttila-Ichihara, F.; Turia, R.; Miiluniemi, M.; Karikoski, M.; Maksimow, M.; Niemela, J.; Martinez-Pomares, L.; Salmi, M.; Jalkanen, S. Macrophage mannose receptor on lymphatics controls cell trafficking. Blood 2008, 112, 64-72.
5. Morais, M.; Subramanian, S.; Pandey, U.; Samuel, G.; Venkatesh, M.; Martins, M.; Pereira, S.; Correia, J. D.; Santos, I. Mannosylated dextran derivatives labeled with fac-[M(CO)]+ (M = (99m)Tc, Re) for specific targeting of sentinel lymph node. Mol Pharm 2011, 8, 609-620.
6. Pirmettis, I.; Arano, Y.; Tsotakos, T.; Okada, K.; Yamaguchi, A.; Uehara, T.; Morais, M.; Correia, J. D. G.; Santos, I.; Martins, M.; Pereira, S.; Triantis, C.; Kyprianidou, P.; Pelecanou, M.; Papadopoulos, M. New Tc-99m(CO)(3) Mannosylated Dextran Bearing S-Derivatized Cysteine Chelator for Sentinel Lymph Node Detection. Molecular Pharmaceutics 2012, 9, 1681-1692.

O38

Quantitative MRI approaches for tracking nano-sized systems

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This presentation will focus on our quantitative MRI approaches for in vivo measurements of the spatial distribution of nanoparticles for diagnostic and therapeutic applications. Nanoparticles are attractive candidates for such applications, as they can carry high payloads of both diagnostic and therapeutic agents. MRI has a lot to offer to theragnostics imaging as it provides high-spatial resolution images with excellent soft-tissue contrast. Most of our research in this area is devoted to the development of quantitative MRI techniques, which allow longitudinal monitoring of both tissue status as well as the local concentration and the integrity of contrast agent and/or drug-loaded nanoparticles. Along similar lines we aim to develop MRI techniques, which enable the estimation of the local release of contents from stimulus-sensitive drug-loaded nanoparticles. Examples of studies of nanoparticle behaviour in mouse models of cancer [1-3] and cardiovascular diseases [4,5] will be shown, and in terms of nano-sized systems the emphasis will be on liposomes [6,7], iron oxide particles [8] and perfluorocarbon emulsions [9].

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References

1. Kluza E, van der Schaft DW, Hautvast PA, Mulder WJ, Mayo KH, Griffioen AW, Strijkers GJ, Nicolay K. Synergistic targeting of alphavbeta3 integrin and galectin-1 with heteromultivalent paramagnetic liposomes for combined MR imaging and treatment of angiogenesis. Nano Lett 2010;10(1):52-58.
2. Kluza E, Jacobs I, Hectors SJ, Mayo KH, Griffioen AW, Strijkers GJ, Nicolay K. Dual-targeting of alphavbeta3 and galectin-1 improves the specificity of paramagnetic/fluorescent liposomes to tumor endothelium in vivo. J Control Release 2012;158(2):207-214.
3. Kluza E, Strijkers GJ, Nicolay K. Multifunctional magnetic resonance imaging probes. Recent Results Cancer Res 2013;187:151-190.
4. Paulis LE, Geelen T, Kuhlmann MT, Coolen BF, Schafers M, Nicolay K, Strijkers GJ. Distribution of lipid-based nanoparticles to infarcted myocardium with potential application for MRI-monitored drug delivery. J Control Release 2012;162(2):276-285.
5. Geelen T, Paulis LE, Coolen BF, Nicolay K, Strijkers GJ. Passive targeting of lipid-based nanoparticles to mouse cardiac ischemia-reperfusion injury. Contrast Media Mol Imaging 2013;8(2):117-126.
6. Langereis S, Geelen T, Grull H, Strijkers GJ, Nicolay K. Paramagnetic liposomes for molecular MRI and MRI-guided drug delivery. NMR Biomed 2013;26(7):728-744.
7. de Smet M, Hijnen NM, Langereis S, Elevelt A, Heijman E, Dubois L, Lambin P, Grull H. Magnetic resonance guided high-intensity focused ultrasound mediated hyperthermia improves the intratumoral distribution of temperature-sensitive liposomal doxorubicin. Invest Radiol 2013;48(6):395-405.
8. Starmans LW, Burdinski D, Haex NP, Moonen RP, Strijkers GJ, Nicolay K, Grull H. Iron oxide nanoparticle-micelles (ION-micelles) for sensitive (molecular) magnetic particle imaging and magnetic resonance imaging. PLoS One 2013;8(2):e57335.
9. Kok MB, de Vries A, Abdurrachim D, Prompers JJ, Grull H, Nicolay K, Strijkers GJ. Quantitative (1)H MRI, (19)F MRI, and (19)F MRS of cell-internalized perfluorocarbon paramagnetic nanoparticles. Contrast Media Mol Imaging 2011;6(1):19-27.

O39

Non invasive temperature measurements during hyperthermia sessions with a near infrared camera

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Magnetic nanoparticles have attracted enormous attention during the past years, since they possess a number of attractive properties in Theragnostics. They can be drug loaded and used as nanocarriers for targeted drug delivery, can be guided via a magnetic field and can be radiolabelled, thus imaged with SPECT or PET. In addition, they can be heated by the application of an external magnetic field and either allow controlled drug release or even selective destruction of cancer cells via hyperthermia. Although a number of studies have shown rather encouraging results for magnetic induced hyperthermia, the methods for studying temperature changes in vivo have not been standardized yet. A number of different methods have been assessed, but they fail to provide accurate in vivo information of temperature increase during a hyperthermia session. In this work we have carried out comparative measurements between optical fibers and near infrared camera in nanoparticle solutions, which provide contradictory results. Different magnetic fields and coils, as well as various types of magnetic nanoparticles have been tested using both techniques. Although the placement of the near infrared camera, seems to affect the results, it is clear that this method can be more accurate for in vivo measurements if properly calibrated. In vivo measurements on mice have been carried out during hyperthermia, as well as on organs that were removed post biodistribution. Our results demonstrate that it is necessary to carefully interpret temperature measurement both in vitro, as well as in vivo. However, new solutions –already tested in other domains- could be properly adjusted and exploited in the field of Theragnostics.

O40

Preclinical protocols to monitor tumor growth, metastasis and nanotoxicity

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The use of endogenously expressed near-infrared fluorescent (NIRF) proteins within cancer cells holds great potential for facilitating preclinical research, enabling the monitoring of cancer both at early-stages and in longitudinal studies where metastatic colonization of distant organs occurs. Our efforts focus on the development of optical imaging techniques and NIRF protein-expressing breast cancer models to non-invasively and longitudinally monitor tumor growth, metastasis and therapy responses in nude mice. In our research group, the fluorescence intensity of iRFP-transfected MDA-MB-231 and 4T1 breast cancer cells was evaluated both in vitro and in vivo. The fluorescent signal intensity was shown to correspond to the amount of cells (in vitro) and to tumor growth (in vivo), with fluorescence properly correlating tumor volume. Organs were scanned for fluorescence ex vivo and immunohistochemistry validated our in vivo and ex vivo findings. Metastatic lesions were found in lungs, bones, lymph nodes, ovary, back and shoulder region, with fluorescent iRFP-foci overlapped with proliferative (HE detected) areas, providing initial evidence for efficient distant organ colonization by NIRF-transfected metastatic breast cancer cells. In addition to the solid and metastatic fluorescent models, we also developed protocols to assess the toxicity of theranostic nanoparticles employing the zebrafish embryo assay as an intermediate in vivo screening tool. By making use of the zebrafish assay, the toxicity effects of nanoparticles can be analyzed at different developmental stages, providing more relevant information on particle toxicity than do cells in culture. Taking everything into account, the developed protocols are considered to hold significant potential for facilitating preclinical research, enabling both studies on tumor growth and metastasis, as well as non-invasive treatment monitoring and nanotoxicity evaluation.

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References

1. Rizzo, Golombek, Mertens, Pan, Laaf, Broda, Jayapaul, Möckel, Subr, Hennink, Storm, Simon, Jahnen-Dechent, Kiessling, Lammers. In vivo nanotoxicity testing using the zebrafish embryo assay. J. Mater. Chem. B, 1, 3918-3925 (2013).
2. Rizzo, Theek, Storm, Kiessling, Lammers. Recent progress in nanomedicine: therapeutic, diagnostic and theranostic applications. Current Opinion in Biotechnology (2013, in press).

Antibody-conjugated quantum dots for the diagnostic of prostate cancer by time-resolved fluoro-immunAssays

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Research on fluorescent semiconductor nanocrystals (also known as quantum dots or QDs) has evolved over the past two decades from electronic materials science to biological applications. The unique optical properties of QDs make them appealing as *in vivo* [1,2] and *in vitro* [2] fluorophores in a variety of biological investigations.

In this work we present the two steps synthesis of bioconjugated-QDs. At first, fluorescent Glutathione (GSH)-capped $CdTe_xS_y$ nanoparticules are obtained in aqueous phase through a facile one-pot microwave irradiation (MWI) strategy. QDs with maximum emission wavelengths at 595 nm and 609 nm with PLQY of 50% and 44% respectively are easily prepared through this new MWI strategy and characterized. In the second part, these water-soluble QDs are linked via classic peptide coupling to proteins like the Bovine serum albumin (BSA), the Streptavidin and some antibodies.

Currently our work focuses on the detection of total prostate specific antigen (TPSA) biomarker of prostate cancer. We successfully labeled the QDs with anti-TPSA (PSR 222) (Fig1) and we are developing sandwich fluoro-immunassays based on Fluorescence Resonance Energy Transfer (FRET) between these bioconjugated-QDs (acceptor) and luminescent lanthanide complexes (donor)[3] (Fig 2) for the detection and quantification of the antigen.

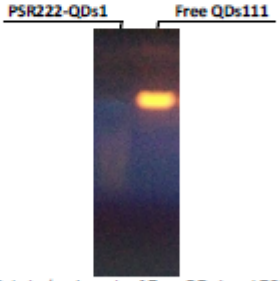

Fig 1. Gel electrophoresis of Free QDs1 and PSR222-QDs1 (HEPES 25mM pH 7.4, Agarose 1%, 30V, 1h30).

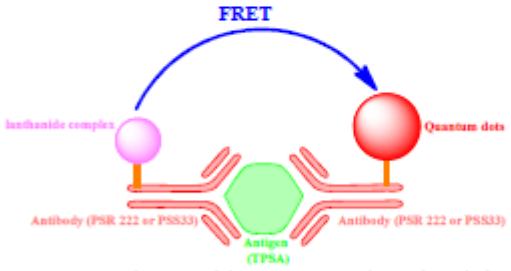

Fig 2. Schematic representation of sandwich system for the detection of total prostate specific antigen (PSA) by FRET.

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[1] Y. Lu, Y. Su, Y Zhou, J. Wang, F. Peng, Y. Zhong, Q. Huang, C. Fan, Y H, *Biomaterial*. 2013, 34, 4302-4308.
[2] C. Zhang, X. Ji, Y. Zhang, G. Zhou, X. Ke, H. Wang, P. Tinnefeld, Z. He, *Anal.Chem*. 2013, 85, 5843−5849.
[3] K. Nchimi-Nono, K. D. Wegner, S. Lindén, A. Lecointre, L. Ehret-Sabatier, S. Shakir, N. Hildebrandt. L.J. Charbonnière, *Org. Biomol. Chem*. 2013, 48.

P2

Development of a Simple and Sensitive Quantum Dot Labelled Magnetic Immunoassay Method for Circulating Colorectal Cancer Cell Detection

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Background/Aim: The detection of circulating tumor cells (CTCs) is of great importance in the clinical management of patients with solid cancers like colorectal cancer (CRC), since they have long been considered as a reflection of tumor aggressiveness. However, owing to the rarity of CTCs in peripheral blood, their detection requires methods combined with high sensitivity and specificity, which sets tremendous challenges for the implementation of these assays into clinical routine. Therefore, the development of a simple, sensitive and specific method for the detection of CTCs is crucial.

Methodology: Here, we present an assay incorporating cadmium selenide quantum dots (QDs) for the detection of CRC CTCs surface antigens. The principle of the assay is the immunomagnetic separation of CTCs from body fluids, in conjunction with QDs, using specific antibody biomarkers: epithelial cell adhesion molecule (EpCAM) antibody, and monoclonal cytokeratin 19 (CK19) antibody. The detection signal was acquired from the fluorescence signal of QDs. For the evaluation of the performance, the method under study was used to isolate DLD-1 human CRC cell line and CTCs from CRC patients' peripheral blood.

Results: The minimum detection limit of the assay was defined to 10 DLD-1 CTCs cells/ml, after the measurement of the fluorescence with a spectrofluorometer. FACS analysis and Real Time RT-PCR have also been used to evaluate the performance of the described method.

Conclusions/Future perspectives: We developed a simple, sensitive, efficient and of lower cost (than the existing ones) method for the detection of CRC CTCs in human samples. The method described here can be easily adjusted for any other protein target of either the CTC or the host. Future research will be focused on improving the detection limit and blood sample treatment using microfluidic systems. In addition, multi-colored QDs can also be conjugated with different antibodies, to enhance the detection specificity.

P3

Study on active targeting by Y-90 and Lu-177 radiolabelled ultra-small AGuIX nano particles functionalized by octreotate.

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AGuIX particles are the nano particles consisting of polysiloxane backbone with DOTA chelator groups attached via amide groups. Until now particles have been characterized for their size (3-4nm), mass (8.5kDa), imaging properties in MRI (after inclusion of Gd atoms), fluorescence reflectance imaging and SPECT of ^{111}In labeled AGuIX. Previously we labeled AGuIX with ^{90}Y and ^{177}Lu and found out that AGuIX with 5 DOTA show favorable biodistribution pattern, suitable for planning its further clinical application. The aim of the present work was the investigation of ^{90}Y labeling of AGuIX particles functionalized with a somatostatin analogue – Octreotate (TATE) and the in vitro evaluation of their binding to somatostatin receptors.

The radiolabelling conditions were investigated for non-functionalized and TATE functionalized Gd-substituted AGuIX particles containing approximately one or five non-substituted DOTA chelators. The optimal radiolabelling conditions were: 1:400 molar ratio of Y to the particles (calculated based on the their Gd substitution), ascorbic acid/NaOH pH 4.5, and 15 min incubation at 900C. The labeled nanoparticles were purified on AmiconUltra 3kDa cut-off filters. The AGuIX and functionalized TATE-AGuIX were labeled with 90Y with yields >75% and 45%, respectively. The radiochemical purities after filtration were >96 and 91%, respectively, maintained for over 24h.

Binding to the AR42J cells cell line expressing somatostatin receptors was investigated for ^{90}Y labeled AGuIX, TATE-AGuIX and DOTA-TATE as reference. The specific binding of functionalized particles was determined in presence of excess of cold nanoparticles or DOTA-TATE. Our study revealed over 10 times higher binding of the TATE functionalized AGuIX (ca. 4%) to AR42J cells than the parent AGuIX particles (ca.0.4%) while for ^{90Y}-DOTA-TATE it was around 9%. However, the binding of ^{90}Y-TATE-AGuIX to the cells when blocked by excess of DOTA-TATE was at the same level as the binding to the cells with no blocking. This suggests the unspecific mechanism of ^{90}Y-TATE-AGuIX interaction with AR42J cells.

Conclusions: The TATE-functionalized nanoparticles could be effectively labeled with ^{90}Y. The biological activity of ^{90}Y-TATE-AGuIX differs from that of ^{90}Y-labeled AGuIX. In future experiments, the change of approach to functionalization of particles is needed in order to achieve the specific binding similar to that of the radiolabeled DOTA-TATE peptide. A higher binding to cell membranes will enable further investigation of the theranostic effect of labeled particles and radio sensitization effect of the Gd substituted particles.

P4

Effects of Ln-DO3A-PiB Derivatives on the Amyloid Peptides: Self-Assembly and In Vitro Interactions

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As a contribution towards the visualization of β-amyloid plaques by in vivo imaging techniques for early detection of Alzheimer's disease (AD), we have recently reported a promising candidate to be used as a metal-based multimodal imaging probe for the detection of such plaques (L1). These are stable, non-charged M^{3+} complexes of a DO3A-monoamide derivative of Pittsburgh compound B (PiB), a well-established marker of Aβ amyloid plaque [1,2]. (L1) coordinated with Gd^{3+} showed a good relaxivity (r1), reasonable binding properties to $A\beta_{1\text{-}40}$ and also to human serum albumin (HSA), excellent specificity to ex vivo amyloid deposits and moderate in vivo brain uptake of its radioactive $^{111}In^{3+}$ labeled probe in normal Swiss mice [3]. Here we report on the synthesis and characterization, as well as the in vitro interaction with the amyloid peptide $A\beta_{1\text{-}40}$, of Gd3+ complexes formed by another DO3A-monoamide PiB derivative (L2), that differs from the original one in the nature and size of the C6 spacer linking the macrocyclic reporter probe and the PiB targeting moiety.

In this work we describe the interaction of these Ln3+ complexes with the $A\beta_{1\text{-}40}$ peptide in the aggregated or monomeric form using a series of different biophysical techniques. These include studying their affinity and mode of binding to immobilized or aggregated $A\beta_{1\text{-}40}$ by Surface Plasmon Resonance (SPR) and Saturation Transfer Difference (STD) NMR, leading to moderate in vitro affinities (KD values in the 67-170 μM range. The group epitope mapping (GEM) for the corresponding La^{3+} complexes, obtained by STD NMR, shows that the complexes interact with immobilized $A\beta_{1\text{-}40}$ mainly through the benzothiazole ring and the attached methoxy group. Their mode of interaction with the ^{15}N-labeled $A\beta_{1\text{-}40}$ peptide monomer was studied at the atomic level using 1H−15N Heteronuclear Single Quantum Coherence (HSQC) NMR. The assessment of their effect on the secondary structure and aggregation process of $A\beta_{1\text{-}40}$ was studied by Circular Dichroism (CD), ThT Fluorescence, Dynamic Light Scattering (DLS) and Transmission Emission Microscopy (TEM). This study suggests that the Ln^{3+}-$L_{1,2}$ complexes interact weakly with the $A\beta_{1\text{-}40}$ peptide monomer, but much more strongly with $A\beta_{1\text{-}40}$ aggregates. They do not affect its self-association in the same way. They promote the early formation of α-helical or β-sheet ordered structures, depending on their nature and concentration. As a consequence, they show inhibition or promotion of the formation of amyloid fibrils [4−6]. These studies give important clues to improve the targeted specificity and affinity of this type of multimodal imaging probes.

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References

1. Mathis, C. A.; Bacskai, B. J.; Kajdasz, S.T. et. al. W.E. Bioorg. Med. Chem. Lett. 2002, 12, 295-298.
2. Vandenberghe, R.; Van Laere, K.; Ivanoiu, A. et. al. Ann. Neurol. 2010, 68, 319-29.
3. Martins, A.F., Morfin J.-F., Kubíčková A, et al.. ACS Med. Chem. Lett. 2013, 5, 436–440.
4. Lendel, C.; Bolognesi, B.; Wahlstrom, A. et al. Biochemistry 2010, 49,1358-1360.
5. Jarvet, J.; Danielsson, J.; Damberg, P.; et. al. J. Biomol. NMR 2007, 39, 63–72.
6. Abelein, A.; Bolognesi, B.; Dobson C.M. et.al. Biochemistry 2012, 51, 126–137.

P5

Kinetic studies of Zr DTPA and Zr DFO complex formation

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Zirconium-89 is one of the most promising metallo-radionuclide for positron emission tomography (PET). Due to favourable decay characteristics it has been used for the design and synthesis of new radioimmunoconjugates for Immuno-PET.1 On the other hand, aqueous coordination chemistry of the Zr4+ ion has not been excessively studied. The lack of fundamental research is evident for radioconjugates of Zr-DFO complex (DFO = Deferoxamine B). Although Zr-DFO complex has been used in the pre-clinic practice, neither stability constant for Zr DFO complex nor solid-state or NMR structural studies have been yet published. The kinetic data on Zr4+ complexes are also rather rare. While in vitro stability studies of Zr DTPA and Zr DFO have been reported, to the best of our knowledge, no formation kinetic study has been published.

This work reports on new analytical methods enabling investigations of the kinetics for Zr DTPA and Zr DFO complexes formation. These methods are based on transchelatation from the weak zirconium(IV) fluoride or oxalate complexes. In order to monitor formation kinetic by a fluoride selective electrode, the fluoride ions have been chosen as precomplexing agent. Formation kinetics of Zr DTPA and Zr DFO complexes via ligand exchange reaction from zirconium(IV) oxalate complexes have been studied using UV-Vis spectrophotometry. The oxalate ions have been chosen since it is the most often used precomplexing agent in the radiolabelling procedures with 89Zr.

Our kinetic studies show that the Zr-DFO complex formation from both weak complexes is considerably faster than the Zr-DTPA complex formation. Moreover, the presented kinetic data give mechanistic insight into the formation of Zr-DTPA and Zr-DFO complexes. Understanding of solution chemistry of Zr4+ complexes is a basic for design of more efficient ligands for zirconium-89 complexation. So such analytical methods are necessary to evaluate of new potential ligands.

References

1. Holland, J. P.; Williamson, M. J.; Lewis, J. S. J. Nucl. Med. 2010, 9, 1–20.

"Complexes of dibenzylamino-phosphinate DOTA analogue as albumin-binding MRI contrast agent"

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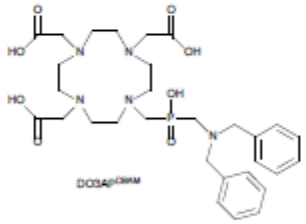
To increase the contrast in MR images, contrast agents employing Gd^{3+} ion are commonly used. However, a toxicity of free gadolinium cation is significantly high and, therefore, this paramagnetic ion has to be wrapped by ligand molecule in thermodynamically stable and kinetically inert complex. To achieve relaxation enhancement, gadolinium compounds directly bonding at least one water molecule should be used. Such requirements have been met by usage of octadentate polyaminocarboxylate compounds, especially derivatives/analogues of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and diethylenetriamine pentaacetic acid (DTPA). From point of view of kinetic inertness, the derivatives of macrocyclic DOTA are more preferred. Further improvement of relaxivity is brought by slowing down of local movement (i.e., by increase in rotational correlation time). It is commonly reached by covalent bonding to macromolecules or by non-covalent interactions with (bio)macromolecules.

The novel ligand based on $DO3AP^R$ skeleton with dibenzylaminomethyl pendant side-group was synthesized. The simple four-step synthesis involving Mannich-type reactions and hydrolysis of protecting groups had overall 60 % yield. pK_a's of Eu(III), Tb(III) and Yb(III) complexes as well as of free ligand itself were determined by NMR spectra acquired in range from pH 1.0 to 12.5 and 1.5 to 10.0 (ligand and complexes, respectively). The abundance of square antiprismatic and twisted-square antiprismatic isomers of complexes was determined from ^{1}H-NMR and ^{31}P-NMR spectra of the complexes and from temperature-dependent high resolution UV-Vis spectra of Eu(III) complex.

The relaxivity of Gd(III) complex was measured *in vitro* at 37 °C and at 0.5 T. The complex has pH-dependent relaxivity with pK_a = 5.8 attributable to protonation of pendant amino-group (r_1 = 6.1 mM^{-1} s^{-1} in protonated form, r_1 = 5.1 mM^{-1} s^{-1} in deprotonated form). In the presence of 4 % bovine serum albumin, the relaxivity of non-protonated complex is increased by a factor of 2.5 (r_1 = 15.6 mM^{-1} s^{-1}). Contrary, the relaxivity of complex with charged (protonated) amino-group is close to that of unbound complex (r_1 = 8.1 mM^{-1} s^{-1}). It points to much higher affinity of the deprotonated complex to albumin molecule comparing to the protonated one.



DO3AP^{CBRM}

Acknowledgements
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P7

The equilibrium and kinetic investigation of the complexes of H4OCTAPA

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In this work the effect of the replacement of two acetate functional groups of EDTA ligand by picolinate ones on the complexation properties was studied. In addition, the results give proof of the fact that this kind of modification of the EDTA ligand decreases the protonation constants of the new ligand OCTAPA. In spite of this, the OCTAPA's overall basicity is two orders of magnitude higher and 5 protonation constants can be determined because this new ligand is an octadentate chelator.

In the knowledge of the protonation constants the stability constants of some metal complexes were determined. In the case of lanthanide and transition metal ions (Cu2+ and Zn2+) the stability constants of their complexes formed with the OCTAPA are several orders of magnitude higher, while the stability constants of Mg2+ and Ca2+ complexes are significantly smaller than those of the corresponding EDTA ones.

In order to gain information on kinetic inertness of the [Gd(OCTAPA)]– complex, metal exchange reactions were investigated. Interestingly, the proton assisted reaction pathway was negligible, contrary to the significant role of the Cu2+ catalyzed pathway.

According to this results one can assume, that our complex can be an acceptable candidate for some kind of in vivo applications, based on the fact that the concentration of the endogenous metals (e.g. Cu2+, Zn2+) in body fluids is really low.

Acknowledgement: The authors thank the TÁMOP-4.2.2.A-11/1/KONV-2012-0043 project implemented through the New Hungary Development Plan, co-financed by the European Social Fund and the European Regional Development Fund as well as the Hungarian Scientific Research Foundation (Grants OTKA PD-83253 and OTKA K-84291) for financial support of this work.

References:

1. E.W. Price et al., JACS, 2012, 134, 8670-8683.

P8

Zinc responsive contrast agents for MRI

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Magnetic Resonance Imaging has been devoted for a long time to obtaining anatomical and functional images. Recently emerging applications in molecular imaging seek information at the molecular level, looking at the biochemical or physiological abnormalities underlying the disease. This allows for a better understanding and earlier diagnosis. Unlike anatomic imaging, molecular imaging always requires an imaging probe that is selectively responsive to the parameter to detect. Gd^{3+}-based contrast agents are particularly well-adapted for this purpose and most often the changes on the efficacy (relaxivity) are based on changes of the hydration number and/or rotational dynamics of the complexes; these two parameters being the easiest to be tailored by the chemist[1].

Endogenous cations are known to play vital role in many fundamental biological processes. Their concentration is tightly regulated by the cell, and misregulation of these ions is connected to different pathologies. Zinc is the second most abundant transition metal ion in humans, and it plays a central role in controlling gene transcription and metalloenzyme function. Exposure to high zinc concentration can lead to neuronal death. It is also an important signalling ion in the brain, which is implicated in neurodegenerative diseases like Alzheimer's Disease.

We have recently developed zinc responsive contrast agents based on a pyridine unit already used for Gd^{3+} complexation [2], to which a zinc complexing unit has been added through a linker (cf Figure). Potentiometric studies have shown that the presence of the amide is necessary for the stability of Gd^{3+} complexes in the presence of zinc. GdL2 and GdL3 show a relaxivity response to zinc, and analyses of the 17O NMR, and the NMRD profiles prove that changes of the rotational correlation time of the complexes are responsible for this behaviour. Finally the selectivity of the zinc complexing unit has been studied by relaxometry.

References

1. C.S. Bonnet, L. Tei, M. Botta, E. Toth, Responsive Probes in "The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging", 2013, John Wiley & Sons, Chichester, 343-385.
2. C.S. Bonnet, F. Buron et al., Chem. Eur. J. 2012, 18, 1419-1431.

P9

Paramagnetic Solid Lipid Nanoparticles as a novel platform for the development of molecular MRI probes

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Introduction. Among the various MRI nanoprobes so far reported (e.g. dendrimers, lipidic nanoparticles, apoferritin, viral capsids), Solid Lipid Nanoparticles (SLNs) having amphiphilic Gd(III) complexes uploaded on the surface (pSLNs) can be particularly effective in reducing both T1 and T2. Their long circulation time and the size < 100 nm may allow the accumulation of the imaging reporter in the targeted site. A DOTA-like GdIII complex functionalized with two hydrophobic chains on adjacent pendant arms (GdDOTA(GAC12)2), found to greatly enhance the relaxivity (r1) of liposomes and micelles, was uploaded in these pSLNs.2 Then, the improvement in r1 of these nanoprobes with respect to pSLNs loaded with non-optimized GdIII amphiphilic complexes (GdDOTAMA(C18)2)3 and the applicability as molecular imaging probes was demonstrated by an in vitro study.

Methods. pSLNs were prepared through a microemulsion composed by the Gd(III) complex, lipids, stealth agent, emulsifiers and co-emulsifiers components. Size and zeta potential were measured by DLS. The NMRD profiles were measured in water at pH 7.4 and in Seronorm™. A multifunctional pSLNs dispersion (FA-pSLNs) was also prepared by adding DSPE-PEG(2000)-Folate as targeting agent and a rhodamine phospholipid to the pSLNs. Their specific targeting properties were assessed by in vitro experiments on human ovarian carcinoma IGROV-1 cells.

Results. The size of pSLNs loaded with GdDOTAMA(C18)2 and with GdDOTA(GAC12)2 were 52 and 76 nm with ζ-potential of -23 and -26 mV, respectively. The relaxometric study (Fig. 1c) showed a 81% enhancement of r1p at 20 MHz and 310 K, in Seronorm™ (r1p = 41.1 mM-1 s-1) for the pSLNs loaded with GdDOTA(GAC12)2 with respect to pSLNs with GdDOTAMA(C18)2 (r1p = 22.7 mM-1 s-1). The specific targeting properties of FA-pSLNs were successfully demonstrated at 0.10 and 0.25 mM concentrations of incubated complex and 4h incubation time. Fluorescence microscopy allowed also to observe a marked fluorescence at the cell border, near the plasma membrane, due to nanoparticles clusters accumulation, whereas a more diffuse signal was observed into the cytoplasm.

Conclusions. The results reported indicate that these pSLNs can be powerful optical/MRI dual diagnostic tools for molecular imaging applications.4

References

1. M. Botta, L. Tei, Eur. J. Inorg. Chem., 2012, 1945.
2. P.L. Anelli, L. Lattuada, et al., MAGMA, 2001, 12, 114.
3. F. Kielar, L. Tei et al., J. Am. Chem. Soc., 2010, 132, 7836.
4. G. A. Rolla, M. Botta, L. Tei et al., Chem. Eur. J., 2013, 19,11189

P10

MRI contrast enhancement of coated GdF3 nanoparticles

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Introduction: Gd-based MRI nanoprobes allow delivering to the site of interest a large number of metal ions, thus increasing the sensitivity of the technique.1 Moreover, the paramagnetic nanoparticles (NPs) can incorporate different functionalities, such as a vector for specific targeting, dyes or drugs for multimodality imaging or therapeutic delivery. Among the large number of inorganic NPs investigated, GdF3 (and NaGdF4) attract growing interest due to their chemical versatility and high r1p values.2 In addition, they show the ability to exchange both cations and anions on the surface which enables multimodal imaging approaches. GdF3 NPs with size < 5 nm and coated with citrate, EDTA, EDTA-PEG and polyacrylate (PAA) ligands were prepared and their relaxometric behaviour was investigated in order to unravel the mechanisms underlying the magnetic interaction with water.

Methods: GdF3 NPs were prepared in aqueous solution by reacting NaF and GdCl3 in the presence of the different ligands. The solutions were stirred at 348 K (3h) and mixed with ethanol to promote precipitation of the NPs. For the characterization we measured X-ray diffraction patterns, HRTEM images, IR spectra, TG and DLS data. The magnetic field dependency of r1 (NMRD profiles) was measured in water and in Seronorm™. 17O NMR R2 data were measured as a function of temperature at 11.4 T. A MRI phantom study was also carried out at 7 T on a Bruker scanner.

Results: GdF3 functionalized with citrate, polyacrylate and EDTA-PEG show in water a high hydrophilicity, whereas EDTA-based GdF3 tends to aggregate in aqueous suspension as a consequence of the reduced charge density. In fact, the NMRD profile of citrate-based GdF3 NPs presents a shape typical of slowly tumbling systems, with a peak centred at 80-120 MHz (r1p ~ 5.8 mM-1s-1). Similar behaviour is found for GdF3 NPs functionalized with polyacrylate and EDTA-PEG. Instead, EDTA-based GdF3 shows a completely different profile, characterized by a decrease of r1p value with increasing frequency. The combined 1H and 17O relaxometric data demonstrate the presence of Gd-bound water molecules and a significant contribution from water molecules H-bonded to the organic coating in addition to highlighting the predominant role of the metal ions exposed on the surface.

Conclusions: The nature of the organic coating on GdF3 NPs surface can significantly affect the r1p values of these nanomaterials and then their MRI contrast enhancement. We think this information will help to guide the synthetic design in order to optimize the characteristic of the NPs as MRI probes.

References

1. M. Botta, L. Tei, Eur. J. Inorg. Chem., 2012, 1945.
2. N.J.J. Johnson et al., Chem. Mater., 2011, 23, 3714.

P11

Folate receptor targeted delivery of supramolecular drug-carriers monitored by PET

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Objectives: The field of targeted drug delivery by nanoparticles and polymers, loaded with diagnostic tools or therapeutics, has become more and more an important field of research. In this line, pHPMA-based systems are well-known for being non-cytotoxic, non-immunogenic and providing a determined excretion pathway [1]. pHPMA-based copolymers have already been coupled to native folic acid (FA) to enhance tumor uptake [2]. On the other hand, radiolabeled folic acid derivatives have already shown high affinity to folate receptor-positive tumors and have been used in PET imaging, but most PET-radiofolates revealed an unfavorable fast blood clearance and high hepatobiliary excretion [3]. The first approach with respect to in vivo imaging of radioactive labeled pHPMA-based polymers was performed by M.M. Herth et al. [4]. Therefore, we aim to combine the favorable in vivo properties of pHPMA-polymers and the high affinity of folic acid to the folate receptor for polymer-based tumor imaging using PET.

Methods: pHPMA-based polymers carrying alkyne functions were coupled to an regiospecific γ-azido-PEGylated folic acid derivative via copper catalyzed click reactions. pHPMA was synthesized in different molecular weights with an alkyne content of 12-15%. These alkyne-pHPMA systems have been coupled to FA using the Cu(II)SO4 system with sodium ascorbate. The FA incorporation was between 8-10% (~65% efficacy). The radiolabeling was facilitated through the phenolic hydroxyl functions of incorporated tyramine groups (~3%) using [18F]FETos in DMSO at 125 °C and purified using size exclusion chromatography (PD-10 desalting column). Preliminary µPET studies were performed using Walker carcinoma bearing rats by employing a new 18F-labeled 10 kDA FA-polymer. Additionally, ex vivo biodistribution studies were performed at different time points (2 and 4 h p.i.).

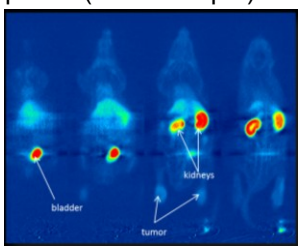

Figure 1. Injection of 28 MBq of a 10 kDa FA-polymer via tail vein; of a Walker carcinoma carrying rat (224 g); images of the whole body scan in different depths 120-135 min p.i.

Results In this work, the first focus was on the synthesis of a γ-azido-PEGylated folic acid derivative, which was utilized for coupling to pHPMA via CuAAC. To ensure regioselectivity at the γ-position, a build-up synthesis for the FA-derivative was applied. In addition, a pHPMA copolymer was synthesized by RAFT polymerization, which was carrying alkyne functionalities for targeting vector conjugation and tyramine units for 18F-labeling. The degree of tyramine units was kept constantly at ~3%. Due to the incorporation of FA, the tumor uptake could be enhanced of about 4-times. Furthermore, blocking experiments showed a highly selective block in the tumor and kidneys with 80-90% specificity.

Conclusions: The coupling reaction of the azido-PEG FA and the alkyne carrying polymers provides relatively high yields, although a complete conversion of all alkynes could not been reached. The preliminary in vivo studies showed expected results with an enhanced tumor uptake of the polymer and an increased kidney uptake due to the FA and the low molecular weight of the polymer. In further studies, the impact of a heavy pHPMA polymer (~60 kDa) on circulation time by overcoming the renal threshold and additionally the influence of spacer variations will be addressed.

Acknowledgements: This work is supported by the research cluster SAMT of the Johannes Gutenberg-University Mainz.

References:

1. Duncan R, et al. Nature Reviews of Drug Discovery (2003), 2, 347–360. [2] Canal F, et al. Journal of Controlled Release (2010), 146, 388–399. [3] Ross TL, et al. J. Nucl. Med. (2010), 51, 1756 – 1762. [4] Herth MM, Barz M, et al. Biomacromolecules (2009), 10, 1697 – 1703.

New methods for direct and mild ^{18}F-labeling of macromolecules

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Objectives: Current methods for ^{18}F-labeling of macromolecular compounds often involve the use of prosthetic groups or harsh reaction conditions such as high temperatures. This research is aimed at developing activated aromatic systems for direct nucleophilic labeling with [^{18}F]-fluoride under mild conditions. The new labeling methods will be tested on novel molecular and supramolecular carriers as well as new HPMA polymers developed for drug delivery.

Methods: The general procedure consists of obtaining a structure as given by compound **1** in figure 1, where R is a linker which can be used to couple the compound to the carrier via amide coupling or copper-catalyzed click reactions. LG is a good leaving group for nucleophilic aromatic substitution such as Br, I, NO_2 or $N(CH_3)_3^+$. Additionally the position of this leaving group is activated for S_NAr by a strong electron withdrawing group (EWG) such as NO_2, CN, CF_3 or CHO.

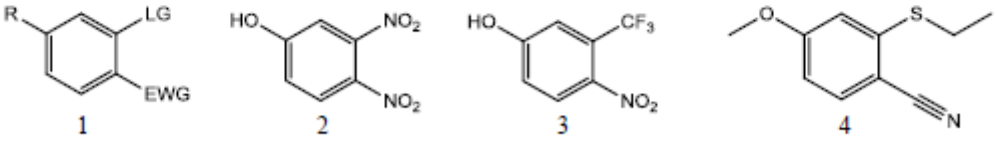

Figure 1: Showing the general principal and two example compounds, which have been labeled with ^{19}F for test purposes and an undesired side product.

Results: The feasibility of the concept was proven by ^{19}F test labeling of compounds **2** and **3** in figure 1. In figure 2 the general scheme used for these test labeling is shown, the labeling will be improved upon by testing various solvents and reaction conditions. Several compounds have shown to be very reactive and the leaving group could already be successfully replaced at room temperature. One of such reactive compounds is shown in figure 1, compound **4**, this side product was obtained in a high yield of 82% from the corresponding nitro derivative. Even though this specific product was undesired it clearly demonstrates the high reactivity of such activated systems.

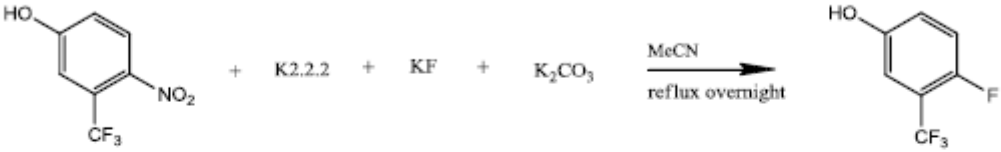

Figure 2: One of the compounds which was successfully test labeled with the reaction conditions

Conclusions: We successfully synthesized a number of highly activated aromatic systems for use in direct ^{18}F-labeling of molecular and supramolecular carriers. First test labeling results are promising and work is now underway to find the optimal reaction conditions. Furthermore a number of target compounds have already shown to be highly activated during synthesis and led to undesired side-products.

References: *J. Becaud_et al., Bioconjugate Chem. 2009, 20, 2254-2261; T.L. Ross et al. J. Am. Chem. Soc. 2007, 129, 8018-8025; B. Shen et al., J. Fluorine Chem. 2009, 130, 216-224.*

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Radiolabelling of different nanoparticles for dual-modality imaging

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Objectives:

Nanoparticles approved as potent diagnostic tools for tumour imaging and therapy. Especially, for a combination of PET (positron emission tomography) as a functional imaging method and MRT or CT as morphological imaging methods nanoparticles are ideal agents.

In this work we focus on inorganic particles based on $FeFe_3O_4$, $FePt@Fe_3O_4$, and TaO_x with different organic surfaces, especially with different amounts of free amino functionalities. We plan to label these particles with metallic positron emitting nuclides, mostly ^{68}Ga. Therefore, bifunctional chelating agents are needed which ensure a simultaneous complexation of the radiometal and a linkage to the particle. The advantage of these chelators is that one can vary between radionuclides with different properties (half-life, therapeutic or diagnostic radiation). This is of great interest especially for theranostics.

In this study we establish labelling methods for the ^{68}Ga-labelling of DOTA-coupled particles.

Various shells and coatings of particles, their labelling properties, their stabilities and their *in vivo* behaviour will be investigated.

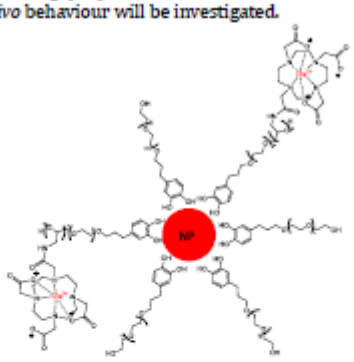

Figure 1: Molecular structure of an exemplary labelled nanoparticle.

Methods:

The various particles carry a PEG-surface containing free amino-functions.

DOTA-NHS-ester was coupled to $FePt@Fe_3O_4$ via amid bounding (see figure 1) at room temperature in phosphate buffer.

Purification of the crude reaction mixture was facilitated by dialysis with Float-A-Lyzer® G2 0.5-1 kDa.

The radiolabelling of DOTA-functionalized particles with ^{68}Ga was performed at 95 °C and low pH in 15 minutes.

Unspecific labelling of $FeFe_3O_4$ and TaO_x was determined under similar conditions.

Stability studies of the Janus particles were performed in $NaCl_{(aq)}$ (isotonic) and human serum albumin at 37 °C for 2 hours. Under similar conditions transchelating studies of $FeFe_3O_4$ and TaO_x were carried out against DTPA and apo-transferrin.

Purification of the labelling-mixture was done via size exclusion chromatography (SEC) using a PD-10 column.

Unspecific labelling of $FePt@Fe_3O_4$ was tested under identical conditions.

Results:

The coupling of DOTA-NHS-ester to $FePt@Fe_3O_4$ was accomplished successfully.

The labelling of this compound could be performed with radiochemical yields of up to 50% and the product could be isolated by SEC in good purities. The compound shows excellent stabilities in both isotonic NaCl and human serum albumin. The unspecific labelling of $FePt@Fe_3O_4$ was applicable low.

$FeFe_3O_4$ and TaO_x showed a very high unspecific labelling but poor stabilities against apo-transferrin and DTPA and thus are not convenient for our studies.

Conclusion and outlook

The labelling of $FePt@Fe_3O_4$ was performed successfully. The yields and purities give room for improvement. Its excellent stability in isotonic NaCl and human serum albumin are promising and suitable for future animal studies.

Due to the high amount of amino functions on their surface $FeFe_3O_4$ and TaO_x showed very high unspecific labelling without sufficient stability.

Further studies of the labelling behaviour of different particles (Janus particles and "normal" ones composed of metals for CT and MRI) with different surfaces are planned.

Moreover, *in vivo* studies for PET/MRI-imaging with ^{68}Ga-labelled $FePt@Fe_3O_4$ using tumour bearing mice are planned.

P14

Functionalized oligoproline as multivalent scaffold in tumor targeting

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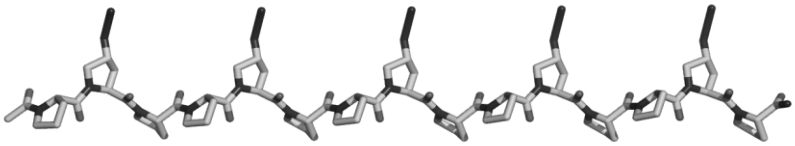
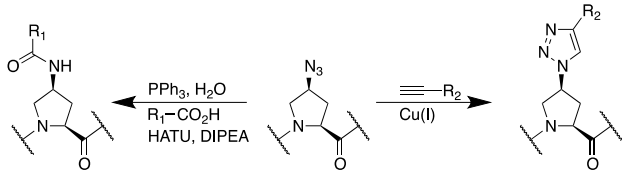
Oligoprolines adopt already at short chain lengths of six residues the conformationally well-defined polyproline II (PPII) helix in aqueous environments.[1] In this highly symmetrical left-handed secondary structure every third residue is stacked on top of each other at a distance of about 9.5Å (Scheme 1).[1] Oligoprolines change their conformation from PPII to the more compact right-handed polyproline I (PPI) helix in a more hydrophobic environment.[1]



Scheme 1 Azidoproline functionalized Oligoproline

Based on the structural integrity of the oligoproline scaffold, targeting vectors can be conjugated in well defined distances either via a copper-catalyzed azide-alkyne cycloaddition (CuAAC) by reacting the azides with alkynes or by reduction of the azides to amines followed by amidation (Scheme 2).[1,2]



Scheme 2 Possible functionalization of Azidoproline

Recent studies on oligoproline-conjugates bearing a bombesin agonist as well as an antagonist in the same molecule showed in vitro excellent binding and internalization compared to the established monovalent ligands.[3]

Neuroendocrine tumors overexpress somatostatin receptors leading to a high density of this receptor on the cell surface. So far they are targeted with monovalent DOTATOC. This principle is well established in diagnostics[4] as well as in therapeutics[5] of somatostatin-positive tumors.

Herein we present novel multivalent ligands based on azido- functionalized oligoprolines bearing the octreotide agonist Tyr3-Octreotide (TOC) as a tumor vector. Labelling with various radiometals allows for molecular imaging via PET. A series of molecules, differing in the distance between the targeting vectors as well as their number has been synthesized using microwave supported CuAAC on resin. A facile route to synthesize alkinylated Tyr3-Octreotide (TOC) has been therefore developed.

References

1. a) Kuemin, M; Sonntag, L.-S.; Wennemers, H. J. Am. Chem. Soc. 2007, 129 (3), 466. b) Erdmann, R.S.; Kuemin, M.; Wennemers, H. Chimia, 2009, 64(4), 19. c) Nagel, Y.A.; Kuemin, M.; Wennemers, H. Chimia, 2011, 65, 264.
2. Upert, G.; Bouillère, F.; Wennemers, H. Angew. Chem. Int. Ed. 2012, 51, 4231.
3. C. Kroll, R. Mansi, F. Braun, H. R. Maecke, H. Wennemers, manuscript submitted.
4. Gabriel, M.; Decristoforo, C.; Kendler, D.; Dobrozemsky, G.; Heute, D.; Uprimny, C.; Kovacs, P.; Von Guggenberg, E.; Bale, R.; Virgolini, I. J. J. Nucl Med, 2007, 48 508-518
5. Otte, A.; Jermann, E.; Behe M.; Goetze, M.; Bucher H. C.; Roser, H. W.; Heppeler, A.; Mueller-Brand, J.; Maecke, H. R. Eur J Nucl Med. 1997, 24 792-795

Complexation of [Gd(DTTA_Me) $(H_2O)_2$]$^-$ by F^- and its consequences to water exchange.

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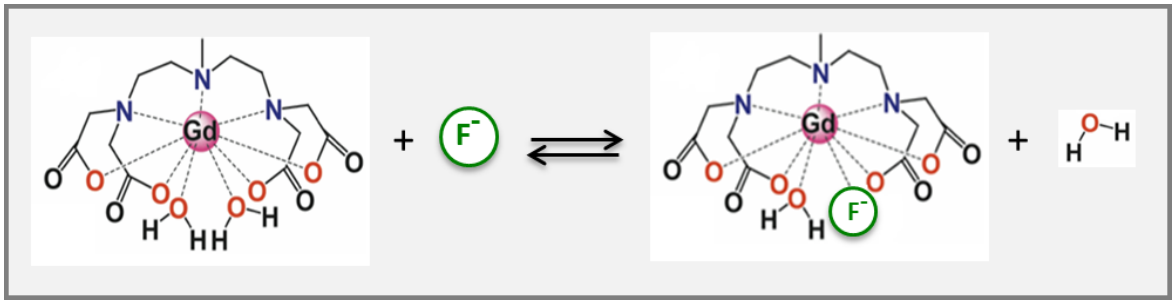
In this project, displacement of water molecules from the inner coordination sphere of [Gd(DTTA_Me) (H_2 O)_2]^- by fluoride anion was studied. Water replacement by an anion is investigated by monitoring the variation of the longitudinal water proton relaxation rate following the addition of increasing amount of fluoride to a solution of [Gd(DTTA_Me) (H_2 O)_2]^- complex. The relaxivity drops around 1.2 mM-1s-1 by increasing the fluoride concentration to 0.5 M. The data obtained allows the assessment of the affinity constant and accordingly entropy and enthalpy values. Finally, water exchange rates were obtained from variable-temperature 17O NMR measurements. The rate of water exchange was found to increase as the concentration of fluoride increased; [F^-]= 2 M (k_ex =87.5 x 106 s-1).



P16

TiO_2 nanoparticles as carries of $^{225}Ac/^{213}Bi$ and $^{212}Pb/^{212}Bi$ in vivo generators

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Alpha particle emitting isotopes are in considerable interest for radionuclide therapy because of their high cytotoxicity and short path length. Unfortunately, all available emitters have serious disadvantages: ^{211}At forms weak bond with carbon atoms in the biomolecule and in the case of ^{212}Bi, ^{213}Bi and ^{226}Th short half-life often limits the application of these radionuclides. However, the short half-life of ^{212}Bi and ^{213}Bi could be effectively lengthened by binding the parent radionuclide ^{212}Pb (t1/2 = 10.6 h) or ^{225}Ac (t1/2 = 10 d) to a biomolecule, thereby effectively extending the use of short half-life ^{212}Bi and ^{213}Bi. In addition, in vivo generators deliver much greater dose per unit of administered activity compared to ^{212}Bi and ^{213}Bi alone.

In our studies we investigated the use of TiO_2 nanoparticles as potential carriers for $^{225}Ac/^{213}Bi$ and $^{212}Pb/^{212}Bi$ in vivo generators. The TiO_2 nanoparticles have unique properties like: high specific surface, high affinity for multivalent cations and simple way of synthesis, which are useful in the process of labelling. Commercially available (e.g. P-25 Degussa) and synthesised in our laboratory nanoparticles were used in experiments. The nanoparticles were characterized by TEM, SEM, DLS and NanoSight techniques.

In our experiments we tested two different methods of labelling. The first one was based on the possibility of formation strong bonds with certain cations on the surface of the nanopraticles. In the second one, TiO_2 nanoparticles were doped with ^{225}Ac during the process of synthesis. In both cases we obtained high yields of labelling (>99%).

Afterwards, the stability of labelled nanoparticles was examined in 0.9 % NaCl, 10-3 M EDTA, solutions of biologically active substances (cysteine, glutathione) and human serum. In case of TiO_2 nanoparticles with ^{225}Ac, which was built in the crystalline structure, the leakage of ^{225}Ac and its daughter radionuclides was not significant in any of solutions, even when the incubation time was extended to 10 days. In the case of nanoparticles with adsorbed ^{225}Ac or ^{212}Pb on surface the leakage in serum was slightly higher, but still insignificant.

The obtained results show high stability of labelled nanoparticles and allow to further begin experiments, which are based on modification of the surface by silane compounds which enable binding TiO2 nanoparticles to the biomolecules.

P17

Exendin-4 labeled with ^{99m}Tc, ^{111}In and ^{68}Ga - a comparative biodistribution evaluation

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Glucagon-like peptide 1 (GLP-1) receptors are overexpressed in insulinoma and MTC and exendin-4 is a long-acting potent agonist of GLP-1 receptor. In the results of our earlier studies the diagnostic utility of ^{99m}Tc-labelled [Lys40-(Ahx-HYNIC)NH_2]Exendin-4 has been proved in a small group of patients with insulinoma [1] and MTC [2]. The aim of this work was to confirm the diagnostic potential of Exendin-4 labeled with ^{99m}Tc in comparison to other derivatives suitable for radiolabelling with ^{111}In and ^{68}Ga [3].

Comparative biodistribution of [Lys40-(Ahx-HYNIC-^{99m}Tc)NH_2]Exendin-4 (specific activity, SA, 57 GBq/mg), [Lys40-(Ahx-DOTA-^{111}In)NH2]Exendin-4 (SA 9 GBq/mg) and [Lys40-(Ahx-NODAGA-^{68}Ga)NH2]Exendin-4 (SA 11 GBq/mg) were studied in normal Wistar rats at 1 h post intravenous administration. In each case the radioactive preparation containing 0.15 μg of the radiolabelled peptide was injected into the tail vein of rat. In the parallel group of animals the radiolabelled peptides were co-injected with 100-times excess of the respective cold peptide (1.5 μg per animal) to block the GLP-1 receptors.

Similar biodistribution pattern was observed for all tested Exendin-4 analogues. In all cases the specificity of uptake in the organs normally expressing GLP-1 receptors (lungs, stomach, pancreas) was confirmed by blocking the receptors with cold peptide. Out of the three compared analogues, the ^{68}Ga-Exendin-4 seems to have the highest affinity to GLP-1 receptor (lung uptake: 25%ID/g vs. 17 and 8 %ID/g for ^{111}In- and ^{99m}Tc-labelled Exendin-4, respectively) and the lowest kidney uptake (22%ID/g vs. 29 and 32 %ID/g for ^{111}In- and ^{99m}Tc-labelled Exendin-4, respectively).

References

1. A. Sowa-Staszczak, D. Pach, R. Mikołajczak, H. Mäcke, et al. Eur. J. Nucl. Med. Mol. Imaging 2013 DOI 10.1007/s00259-012-2299-1
2. D. Pach, A. Sowa-Staszczak, A. Jabrocka-Hybel, A. Stefańska et al. Int. J of Endocrinology 2013 DOI 10.1155/2013/384508
3. D. Wild, A. Wicki, R. Mansi, M. Béhé, B. Keil, P. Bernhardt et al. Exendin-4-based radiopharmaceuticals for glucagonlike peptide-1 receptor PET/CT and SPECT/CT J Nucl Med, 2010 (51). 1059–1067

P18

Near-infrared Luminescent Dendrimer Lanthanide Complexes for Biological Imagery

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Luminescent lanthanides probes are emerging in optical imaging. They have several advantages for the study and analysis of biological problems. Lanthanide ions possess long emission lifetimes, a good resistance to photodecomposition and sharp emission bands that allow spectral discrimination.
Several lanthanides (Yb^{3+}, Nd^{3+}, Ho^{3+}, $Tm^{3+,}$ Pr^{3+}) emit in the near infrared (NIR) making them very attractive for *in vivo* imaging. NIR light can cross important depths of biological tissue and allow detection in a wavelength range almost free of autofluorescence, increasing signal-to-noise and detection sensitivity.

Free lanthanide ions possess very low absorption in the UV and visible regions. Therefore, they need to be sensitized by the “antenna effect”. The lanthanide ion must be located in a sufficiently close proximity to a chromophoric antenna in a stable scaffold in order to prevent the release of free lanthanide in cells and biological systems to avoid any toxic effect.
We have developed new generation-3 polyamidoamine dendrimers which can sensitize 8 lanthanide ions (Eu^{3+}, Nd^{3+},Yb^{3+}) and possess 32 antennae attached to the end of the branches. This strategy allows to generate a high number of photons per unit volume leading to enhanced sensitive detection. For the same dendrimer ligand, we can choose the emission wavelength by the choice of the appropriate lanthanide ion: Eu^{3+} (614nm), Yb^{3+} (980nm) or Nd^{3+} (1064nm).
The aim of this work is to study the behavior of these new compounds *in cellulo* before an evaluation *in vivo* (mice) for tumor labeling.

We have shown that our prototypes molecules are not cytotoxic and present a good cellular uptake despite the high molecular weight (approximately 29 kDa). We have demonstrated that these compounds are highly stable in biological environment. Near Infrared microscopic studies showed perinuclear localization and a good transfer to daughter cells during cell divisions.
The emission of our dendrimer complexes is easily observable in the NIR (980nm) *in vivo* thanks to its intensity and the absence of autofluorescence. These results indicate that dendrimer complexes of this family are promising candidate for tumor imaging.

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A R_{2p}/R_{1p} ratiometric approach to visualize Matrix Metalloproteinase-2 activity by MRI

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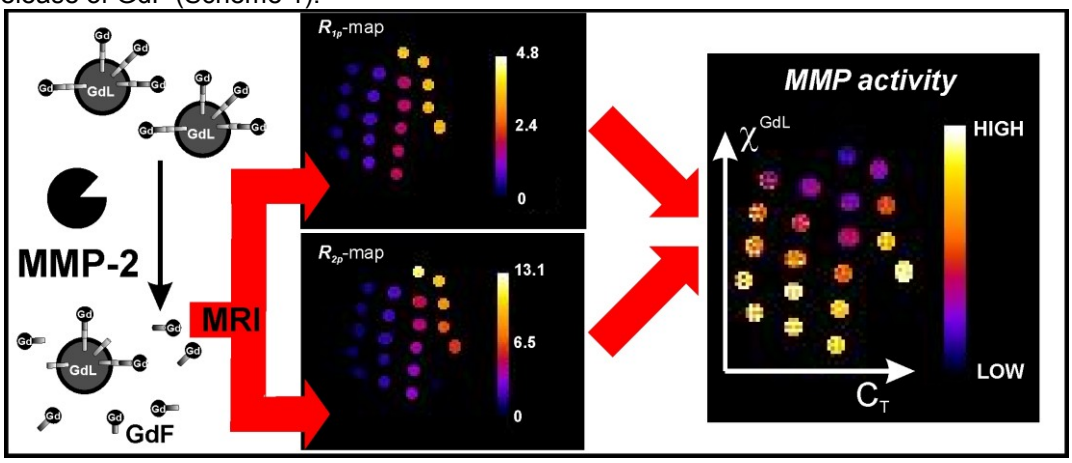
Responsive MRI probes to assess enzyme activity in tissue typically rely on enzyme-cleavable probes whose relaxivity changes upon enzymatic processing.[1] However, such an approach requires the knowledge of the total tissue concentration of Gd (C_T) to unambiguously translate image contrast enhancement into enzyme activity maps. A possible solution to this problem is provided by the R_{2p}/R_{1p} ratiometric approach, which is based on the measurement of the ratio between R_{2p} and R_{1p} (with $R_{ip}=1/T_{ip}$, i=1,2). As a matter of facts, R_{2p}/R_{1p} ratiometric maps yield the molar ratio χ^{GdL} between the processed and unprocessed forms of the probe in a C_T independent manner.[2]

We have developed a paramagnetic liposome for the ratiometric detection of the activity of Matrix Metalloproteinase-2 (MMP-2). An amphiphilic MMP-2 cleavable peptide has been conjugated with Gd-DOTA at the N-terminus and inserted into a stealth liposome, to obtain the paramagnetic probe GdL. Cleavage by MMP-2 yields the release of GdF (Scheme 1).



Scheme 1. *The R_{2p}/ R_{1p} ratiometric approach to the assessment of MMP activity*

At the optimal magnetic field strength of 7 T, the transverse millimolar relaxivities of GdL and GdF in 1% agar were 46.1 and 9.7 $mM^{-1}s^{-1}$ respectively, while the longitudinal relaxivity were similar (4.8 $mM^{-1}s^{-1}$). Parametric R_{2p}/R_{1p} images of agar phantoms under a MRI setting showed image contrast which was responsive to the GdL-to-GdF molar ratio (χ^{GdL}) and independent from C_T within the 0.125-1 mM range. The detection limit was 120 μM. Strategies for the optimization of the paramagnetic vesicle as a MMP-2 responsive ratiometric probe are finally described.

Acknowledgements

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References

1. *a)* R. Lebel et al. Magn. Reson. Med. 2008, 60, 1056–1065; *b)* E. S. Olson et al. PNAS 2010, 107, 4311–4316; *c)* C. V. Gringeri et al. Contrast Media Mol. Imaging 2012, 7, 175–184.
2. V. Catanzaro et al. Angew. Chemie Int. Ed., 2013, 52, 3926–3930.

P20

Is the radiolabeling of DOTA-based phosphonic acids with 44Sc efficient as NOTA-based phosphonic acids with 68Ga ?

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^{44}Sc as a positron emitter can be an interesting alternative to ^{68}Ga (T1/2=67.71min) due to its longer half-life ($T_{1/2}$=3.97h). It has been already proposed as a PET radionuclide for studying bone disease and is already available as a ^{44}Ti/^{44}Sc generator. ^{44}Sc has an isomeric state, ^{44m}Sc ($T_{1/2}$=58.6 h), which is co-produced with ^{44}Sc and that is expected to be used as an in vivo PET generator ^{44m}Sc/^{44}Sc. It would thus allow to monitor the kinetics over long periods of time.The ^{44}Sc is a trivalent metal cation and should be suitable for complexation with chelators alone or with bifunctional ligands conjugated to peptides or other molecular targeting vectors. The DOTA macrocyclics ligand form stable complexes with many cations besides scandium. The aim of this work was to evaluate the phosphonic acid DOTA derivatives, new ligands which can be applied for labeling biomolecules with Scandium radionuclides.

Potentiometric studies showed that the ligands have a similar basicity to the parent H4dota and the stability constants of their complexes with Sodium (I) and selected lanthanide (III) ions are also similar. It was found that the phosphonic acid derivatives formed complexes relatively fast [1]. It is also known that the phosphorus acid derivatives and their complexes have some different properties (size and shape of the coordinating group, overall hydrophilicity or acidity/basicity of the ligands) if compared to their acetate analogs. [2], [3]. It was proved that the monophosphorus acid analogs of H4dota form thermodynamically very stable and kinetically inert complexes with Ce^{3+} and Gd^{3+}[4] as well as Y^{3+}.[5]

The influence of various reaction parameters and conditions on radiolabelling efficiency, such as the amount of ligand, pH range, reaction time and reaction temperature, were investigated and optimized in order to maximize the radiochemical yield. Comparison between the results obtained with ^{44}Ti/^{44}Sc generator and ^{44m}Sc/^{44}Sc generator will be presented.

References

1. M. Försterová, Z. Jandurová, F. Marques, L. Gano, P. Lubal, J. Vaněk, P. Hermann and I. Santos, Chemical and biological evaluation of ^{153}Sm and ^{166}Ho complexes of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylphosphonic acid monoethylester) (H4dotpOEt). J. Inorg. Biochem., 2008, 102, 1531–1540.
2. Lukeš, J. Kotek, P. Vojtíšek and P. Hermann, Complexes of tetraazacycles bearing methylphosphinic/phosphonic acid pendant arms with copper(II), zinc(II) and lanthanides(III). A comparison with their acetic acid analogues. Coord. Chem. Rev., 2001, 216–217, 287–312.
3. R. Delgado, V. Félix, L.M.P. Lima and D.W. Price, Metal complexes of cyclen and cyclam derivatives useful for medical applications: a discussion based on thermodynamic stability constants and structural data. Dalton Trans., 2007, 2734–2745.
4. Táborský, P. Lubal, J. Havel, J. Kotek, P. Hermann and I. Lukeš, Thermodynamic and kinetic studies of lanthanide(III) complexes with H5do3ap (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic-10-(methylphosphonicacid)), a monophosphonate analogue of H4dota. Collect. Czech. Chem. Commun., 2005, 70, 1909–1942.
5. M. Försterová, I. Svobodová, P. Lubal, P. Táborský, J. Kotek, P. Hermann and I. Lukeš, Thermodynamic study of lanthanide(III) complexes with bifunctional monophosphinic acid analogues of H4dota and comparative kinetic study of yttrium(III) complexes. Dalton Trans., 2007, 535–549.

P21

Synthesis of High Affinity Contrast Agents for Targeted MR Neuroimaging

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Magnetic resonance imaging (MRI) has become one of the essential noninvasive diagnostic techniques for soft tissues and diseases. Contrast agents have been developed to produce additional contrast and increase the signal intensity for MRI [1]. Here we report on the synthesis development of target specific contrast agents for MR neuroimaging. Monomeric and dendrimeric targeted contrast agents (TCA) were synthesized taking advantage of the highly specific interaction of the protein avidin with its ligand biotin [2]. The classical monomeric CAs have disadvantages such as non-specificity, fast renal excretion, low contrast efficiency and therefore they require a high dosage. To overcome this problem, we use multivalent, highly-branched dendrimeric molecules that are capable of carrying large number of CAs and hence the MRI amplification [3].

The TCAs are additionally labeled with a fluorescent dye, for to achieve their multimodal detection by means of optical and MR-based techniques. Upon their preparation, the biotinylated TCAs are suitable for labeling genetically engineered cell surface receptors that contain avidin. [4]. The preliminary experiments showed that TCAs bind specifically to avidin-coated beads (Figure 1). Their further characterization ensures exciting progress in neuroimaging enabling high resolution MRI of specific neuronal populations.

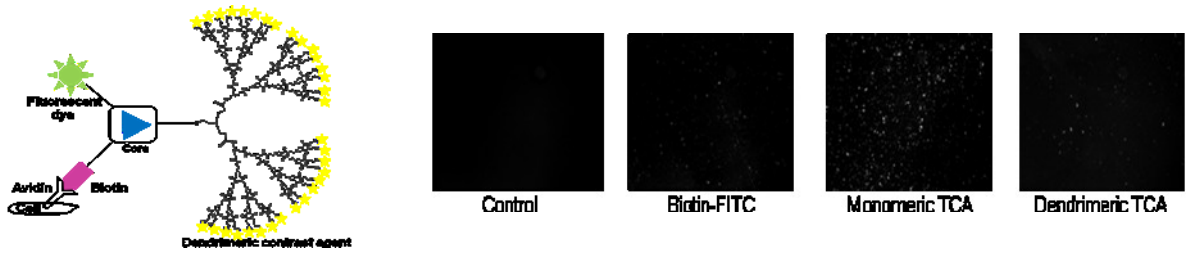

Figure 1: The structure of dendrimeric TCA (left) and fluorescent microscopy of prepared TCAs with avidin-coated beads (right)

References

1. R. B. Lauffer, Chem. Rev. 1987, 87, 901–927.
2. H. Xu, C. A. S. Regino, Y. Koyama, Y. Hama, A. J. Gunn, M. Bernardo, H. Kobayashi, P. L. Choyke, M. W. Brechbiel, Bioconjugate Chem. 2007, 18, 1474-1482.
3. S. Langereis, A. Dirksen, T. M. Hackeng, M. H. P. van Genderen, E. W. Meijer, New J. Chem. 2007, 31, 1152–1160.
4. H. Kobayashi, S. Kawamoto, T. Saga, N. Sato, T. Ishimori, J. Konishi, K. Ono, K. Togashi, M. W. Brechbiel, Bioconjugate Chem. 2001, 12, 587-593.

P22

New macrocyclic imaging agents targeting chemokine receptor CXCR4

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CXCR4 is a chemokine receptor that functions as a co-receptor for human immunodeficiency virus (HIV). It is also overexpressed in numerous cancer types and plays a key role in invasion and metastases. For this reason, CXCR4 appears as an interesting biomarker for the development of anti-HIV drugs and for the field of diagnostic oncology. CXCR4 targeting has notably gained interest for application in molecular imaging.[1] In the past few years, both peptide-based and small molecules have been developed as CXCR4 inhibitors.[2] Among all existing organic molecules, cyclam derivatives AMD3100 and AMD3465 were described as strong CXCR4 inhibitors. To image CXCR4 expression in tumor models, macromolecular agents such as ^{111}In- and ^{18}F-labeled peptides and 125I-labeled monoclonal antibodies have been investigated using either SPECT (Single Photon Emission Computed Tomography) or PET (Positron Emission Tomography).[3] Recently, AMD3100 and AMD3465 were directly radiolabeled with ^{99m}Tc and ^{64}Cu, but these complexes have a low in vivo stability leading to the decomplexation of the metal.[4][5] In this context, we decided to use the high affinity of these molecules for CXCR4 and to attach to these systems an optimized chelating agent in order to image CXCR4 by SPECT or PET imaging using different radiometals such as ^{111}In, ^{64}Cu, or ^{68}Ga. This route also offers the possibility of attaching a fluorescent tag for optical imaging or a bimodal system for both radio-(PET/SPECT) and fluorescence imaging.

Here, we present how AMD3100 and AMD3465 were modified in order to introduce an adapted chelator and the synthetic pathway toward our new derivatives for nuclear imaging. Preliminary biological studies on eleven new potent CXCR4 inhibitors were performed to validate our concept. Flow cytometry and invasion assays were carried out and the first results will be shown. Two promising compounds were selected and are going to be used for in-vivo imaging.

With an optimized chelator for copper (II) and a high CXCR4 inhibitor, our compounds could also be used either for PET imaging (^{64}Cu) and for therapy (^{67}Cu).

Acknowledgements

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References

1. J. Kuil, T. Buckle, F. W. B. Van Leeuwen, Chem. Soc. Rev. 2012, 41, 5239-5261.
2. E. De Clercq, Pharmacol. Ther. 2010, 128, 509-518.
3. S. Nimmagadda, M. Pullambhatla, M. G. Pomper, J. Nucl. Med. 2009, 50, 1124-1130.
4. O. Jacobson, I. D. Weiss, L. Szajek, J. M. Farber, D. O. Kiesewetter, Bioorg. Med. Chem. 2009, 17, 1486-1493.
5. R. A. De Silva, K. Peyre, M. Pullambhatla, J. J. Fox, M. G. Pomper, S. Nimmagadda, J. Nucl. Med. 2011, 52, 986-993.

P23

Oligoprolines as Scaffolds for Tumor Targeting with Hybrid Bombesin Analogues

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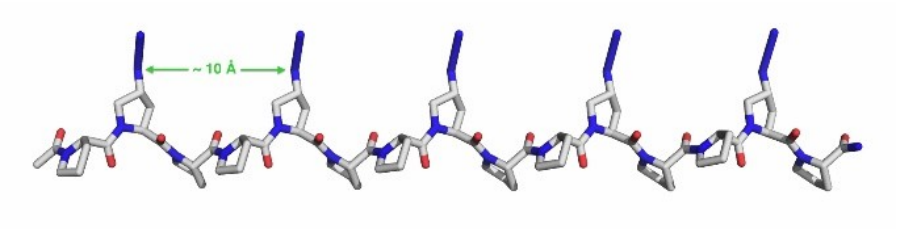
In aqueous environments oligoprolines adopt the well-defined polyproline II (PPII) helix already at chain lengths as short as six residues.[1] Within this left-handed secondary structure every third proline residues is stacked on top of each other in a distance of approximately 1 nm (Scheme 1).[1] Incorporation of 4-azidoproline (Azp) residues into this helix allows for installing sites in desired distances towards each other that can easily be functionalized by Cu(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition (click reaction) with terminal alkynes or Staudinger reduction followed by acylation.[2]



Scheme 1: Oligoproline with 4-azidoproline (Azp) residues in every repeating position.[2]

Previous studies within our group showed that hybrid ligands consisting of an oligoproline scaffold equipped with a bombesin-based agonist and antagonist as recognition motives exhibit extraordinary tumor uptake properties.[3] The hybrid ligands showed significantly higher tumor uptakes in vitro and in vivo compared to not only monovalent but also divalent controls. Notably, the defined distance between the recognition motives proved to be important for high tumor uptakes. We are currently designing ligands with yet higher tumor uptake properties as well as ligands to gain a deeper understanding of how the high tumor uptake is achieved.

References

1. a) F. Rabanal, L. M. Pons, E. Giralt, Biopolymers 1993, 33,1019. b) S. Kakinoki, Y. Hirano, M. Oka, Polym. Bull. 2005, 53, 109.
2. a) M. Kuemin, L.-S. Sonntag, H. Wennemers, J. Am. Chem. Soc. 2007, 129, 466. b) R. S. Erdmann, M. Kuemin, H. Wennemers, Chimia 2009, 64, 19. c) Y. A. Nagel, M. Kuemin, H. Wennemers, Chimia 2011, 65, 264. d) G. Upert, F. Bouillère, H. Wennemers, Angew. Chem. Int. Ed. 2012, 51, 4231.
3. C. Kroll, R. Mansi, F. Braun, H. R. Maecke, H. Wennemers, manuscript submitted.

P24

Theranostic nanoparticles for MRI-guided thermoablation of tumors

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Introduction

Theranostic ferromagnetic nanoparticles with a low Curie temperature and high relaxivity represent a suitable agent for MRI-guided magnetic fluid hyperthermia applicable for tumor treatment. The nanoparticles deposited in the tissue may be heated by exposure to a high frequency (HF) electromagnetic field, thus inducing apoptosis in their vicinity. The aim of our study was to verify the possibility of tumor thermoablation using ferromagnetic particles in animal experiments in vivo.

Methods

Tumor model: glioblastoma cells were implanted subcutaneously in 13 rats. After two weeks the tumors reached a size of approximately 1 cm in diameter.

A suspension of perovskite nanoparticles (La1-xSrxMnO3), coated by SiO2 (50μL/4mM), was injected into the tumors of 7 rats. Sagittal and transversal T2-weighted MR images were obtained before and immediately after the nanoparticle injection. The rats were exposed to a HF electromagnetic field (480kHz/11mT) for 30 minutes. The temperature was measured in the tumors by a fluorescent probe on an optical fiber. The animals were sacrificed 1 week after ablation. Apoptosis in the tumors was assessed by the TUNEL assay.

Five control animals with tumors underwent either HF field exposure without nanoparticles or the application of nanoparticles without HF field exposure to eliminate any influence of the HF field or the nanoparticles themselves.

Results

MRI confirmed the presence and distribution of the nanoparticles in the tumors. The temperature in the tumors with injected nanoparticles increased to 41.5°C during exposure to the HF field. The TUNEL assay confirmed substantially greater apoptosis in the tumors of animals with injected nanoparticles after exposure to the HF field.

Discussion/Conclusion

The distribution of nanoparticles can be easily tracked using MRI. Increased temperature in the tissue was found during exposition to HF field in animals with injected nanoparticles. Local thermoablation was confirmed by the TUNEL assay, which revealed apoptosis in the tumors.

The tested particles potentially represent a powerful tool for non-invasive tumor ablation.

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References

Pollert E et al.2010,Phil.Trans.R.Soc.368:4389-4405

P25

Labeling of HPMA-based, functionalized polymer-systems using metallic radionuclides

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Objectives

The aim of this study is the synthesis and evaluation of several different DOTA-spacer-systems for functionalization of HPMA-based polymers. By introducing the bifunctional chelator DOTA, a broad pool of radionuclides becomes available for labeling. These possible variations allow the visualization of the biological behavior over different periods of time depending on the half-life of the used radionuclide (68Ga T1/2 = 68 min; 44Sc T1/2= 3,9 h). Additionally, the usage of different imaging modalities or endoradiotherapy can be applied depending on the employed radionuclide (68Ga (PET); 111In (SPECT); Gd (MRT); 90Y/177Lu (Therapy).

Methods

Coupling between chelator and polymers was facilitated via several spacer units with variable length and structure. The spacers carry two amino-functions, in α- and ω-position, and thus can be coupled to the polymer and the chelator via amid-formation with activated esters. Reductive removal of the tBu-groups lead to the final labeling precursor.

The DOTA-functionalized polymer-systems were labeled with 68Ga, 44Sc and 177Lu. Stability studies were performed with NaCl, EDTA, DTPA, Fe3+, Ca2+, Mg2+ and human serum with the 68Ga labeled compounds.

Results

The spacer synthesis, the coupling to DOTA-tris(tBu)-ester and the reductive deprotection were successfully accomplished and optimized. We obtained, depending on the used spacer, 50-70% of the corresponding DOTA-spacer-system. These systems were coupled to HPMA-based polymers via amid formation and deprotected with TFA quantitatively. The achieved DOTA-functionalized polymer-systems were effectively labeled with the different radionuclides at 95 °C by 15 – 30 min heating.

Conclusion

Different chelator-spacer-systems could be successfully purified, coupled to the polymer and deprotected. The conjugates show high stability against transmetallation or other chelators in in vitro studies. The functionalized HPMA-polymers will be evaluated pharmacologically in in vivo µ-PET studies.

P26

Ultrasound-enhanced accumulation in fat and efficient cellular uptake of hydrophobic drugs using a novel nanoparticle-microbubble platform.

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Successful cancer therapy requires efficient delivery of the therapeutic agents to the target cells and minimal toxicity toward normal tissue. Optimal delivery implies long circulation time, efficient extravasation into and throughout the extracellular matrix and finally high accumulation into the cells and to the intracellular target. Several promising chemotherapeutics are hydrophobic drugs, for instance taxane, having very low solubility in water. Such drugs require a delivery system. We have developed a novel multimodal, multifunctional drug delivery system consisting of microbubbles (MBs) stabilized by polymeric nanoparticles (NPs). Miniemulsion polymerization was used to prepare NPs of the biocompatible and biodegradable polymer poly(butyl-2-cyanoacrylate) (PBCA). The NPs were coated with PEG to improve the circulation time and biodistribution. Encapsulated into the NPs was the hydrophobic fluorescent dye nile red, which changes its spectral characteristics depending on the lipophilic environment, a property that was used to indicate what nile red was binding to. MBs stabilized by these NPs were prepared by mixing the NP-dispersion with stabilizing proteins and air using an ultra-turrax.

The NP-MBs were injected i.v. in athymic mice bearing subcutaneous prostate tumours and within 30 sec the tumour was exposed to 1 MHz ultrasound (MI=0.4). Blood vessels were visualized by injecting fluorescein-labelled lectin binding to endothelial cells. The micro distribution of nile red was studied on frozen sections from the tumour. Nile red fluorescence was much brighter in ultrasound-treated tumours, compared to tumours only receiving NPs, with enhanced staining of tumour fat tissue.

Cellular uptake of nile red encapsulated in NPs (without MB and ultrasound) in vitro was measured by flow cytometry at 4°C and 37°C to distinguish between cellular uptake by diffusion or endocytosis. Spectral analysis of confocal microscopy images was used for intracellular localization of nile red. Nile red was internalized at 4°C, demonstrating that diffusion and not endocytosis was the major uptake mechanism, thus the fluorescence was caused by nile red released from the NPs rather than internalized NP. Moreover, the uptake of NP encapsulated nile red was more efficient than uptake of nile red dissolved directly into the cell culturing medium. We found that NPs increase nile red uptake by direct contact between the NP and plasma membrane and to some extent by release into the medium, followed by cellular uptake. The low uptake of free nile red is probably due to nile red binding to serum proteins or aggregation of the dye in aqueous environment.

Thus, our new technology platform consisting of NP-stabilized MBs show great promise for enhanced ultrasound-mediated delivery of hydrophobic drugs, with efficient transfer of hydrophobic drugs from NPs into cells.

P27

Targeted-receptor Bimodal Probe for Sentinel lymph Node Detection

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The availability of bimodal probes might improve the clinical procedure for the sentinel lymph node detection (SLND) allowing fast and accurate localization of the first lymph node involved in metastization process. Indeed, the synergistic effect of a dual imaging reporter could enable real-time SLND by radio- or near-infrared fluorescent (NIRF) guided surgery as well as fluorescent histology [1, 2]

Therefore, aiming at the design of a bimodal probe for specific targeting of sentinel lymph node (SLN) by nuclear and optical imaging techniques, we have synthesized dextran derivatives containing three components: mannose units for specific receptor targeting, a bifunctional chelator suitable to stabilize the radiometal (^{68}Ga) and a NIRF dye.

The final compounds were characterized by the usual analytical techniques in chemistry, including SEC- and RP-HPLC, and NMR spectroscopy.

The polymeric compounds were labeled with ^{68}Ga in high yield and radiochemical purity. Ligand challenge experiments have shown that both radiolabelled polymers present high in vitro stability towards transchelation.

In this communication we report on the synthesis, characterization and biological evaluation of fluorescent ^{68}Ga-labeled mannosylated dextran that would enable the preoperative visualization of SLN by Positron Emission Tomography (PET) as well as intraoperative real-time guidance for surgical excision by optical imaging in the near infrared (NIR) field.

Acknowledgements

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References

1. Ravizzini, G.; Turkbey, B.; Barrett, T.; Kobayashi, H.; Choyke, P. L. Nanoparticles in sentinel lymph node mapping. Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology 2009, 1, 610-623.
2. Sharma, R.; Wendt, J. A.; Rasmussen, J. C.; Adams, K. E.; Marshall, M. V.; Sevick-Muraca, E. M. New horizons for imaging lymphatic function. Lymphatic Continuum Revisited 2008, 1131, 13-36.

P28

Multimodal contrast agent for the imaging of pancreatic amyloids in type 2 diabetes

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Type 2 diabetes (T2D) is a pathology, in which the peripheral resistance to insulin triggers an increase need for this hormone. This results in pancreatic beta cells exhaustion, leading to a decrease in insulin secretion and eventually to beta cells loss. Hyperglycemia usually appears when 50 % of the insulin producing cells has disappeared. The reasons of the cellular death are not yet understood. A common feature in most T2D patients is the presence of extra-cellular amyloids within the pancreas. Amyloids are aggregates of proteins which are present in many degenerative diseases, including Alzheimer's and Parkinson's. Their role in the pathology is not fully elucidated. The formation of amyloids could be a way for protecting the organs against an increase production of certain proteins, resulting in the formation of toxic oligomers [1]. Amyloids are also found after a while in transplanted islets, leading to the graft failure [2].

Our purpose is to develop a multimodal contrast agent in order to visualize the pancreatic amyloids in vivo. To do so, silica-based nanoparticles have been synthesized [3]. Their small size (< 10 nm) allows renal excretion. The nanoparticles carry chelated gadolinium and cyanin 5.5 for imaging by magnetic resonance (MRI) and fluorescence. They are functionalized with short peptides or camelid antibodies fragments (nanobodies) in order to ensure the targeting to the amyloid plaques.

In vitro experiments in water and plasma have assessed that the limit of detection of the synthesized particles is below 0.5 µM in fluorescence and at 50 µM in MRI. The pharmacokinetics of the nanoparticles has been determined in FVB/N mice (which do not present pancreatic amyloids). The observed blood half-life of the contrast agents was of 10.7 ± 4.1 minutes. The sampling of the organs has shown that a large quantity of nanoparticles is present in the kidneys. This was expected due to the hydrophilicity of the constructs and their relatively low molecular weight (Mw <20'000) which result in renal secretion. There was no uptake in the liver, pancreas, lungs and spleen.

The next experiments will consist in the imaging of the animals by fluorescence and MRI to assess the ability of the nanoparticles to provide a good contrast in vivo. A further step will be the use of transgenic animals developing pancreatic amyloids to assess the efficacy of the targeting.

References

1. Haataja, L., et al., Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocr Rev, 2008. 29(3): p. 303-16.
2. Abedini, A. and A.M. Schmidt, Mechanisms of islet amyloidosis toxicity in type 2 diabetes. FEBS Lett, 2013.
3. Lux, F., et al., Ultrasmall rigid particles as multimodal probes for medical applications. Angew Chem Int Ed Engl, 2011. 50(51): p. 12299-303.

P29

A tyrosine-based amphiphilic chelating molecule for magnetic resonance imaging, Synthesis steps and characterization.

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Magnetic Resonance Imaging (MRI) is a powerful non-invasive modality for clinical diagnostic procedures, frequently carried out after injection of formulations of contrast agents containing a paramagnetic gadolinium complex. There is a great need for contrast agents that could be used for molecular imaging. Ideally these structures should provide a strong MR signal after accumulation at the target site. One way toward this goal is to formulate supramolecular contrast agents containing a large number of gadolinium chelates, tailored blood circulation properties and the possibility to add targeting moieties. The aim of the study was the synthesis of a novel amphiphilic molecule as part of a micellar MRI contrast agent.

The synthesis relies on the use of a (L)-tyrosine-OH 1 as starting material [1]. This amino-acid displays three functional sites that were selectively grafted. The carboxylic acid, the amino and the hydroxyl moieties were substituted by a hydrophobic NH-(C18)2 chain, a DO3A coordinating metal centre separated by a benzyl spacer, and a hydrophilic methoxy poly(ethyleneglycol)2000 (M-PEG2000), respectively. This multi-step synthesis leads to compound 2 as presented in figure 1.

After each synthesis step, the molecules were purified and characterized (NMR, MS). The assignments of each intermediate proved the coupling of the hydrophobic tail, the spacer, and the chelate. The final step of attaching M-PEG2000 leads to the amphiphilic compound. A characteristic of the latter is the various signals observed on NMR spectra depending on the solvent used for the analysis [2]. Further work will be focused on the formulation of the micelles and the characterisation of the complex as MRI contrast agent.

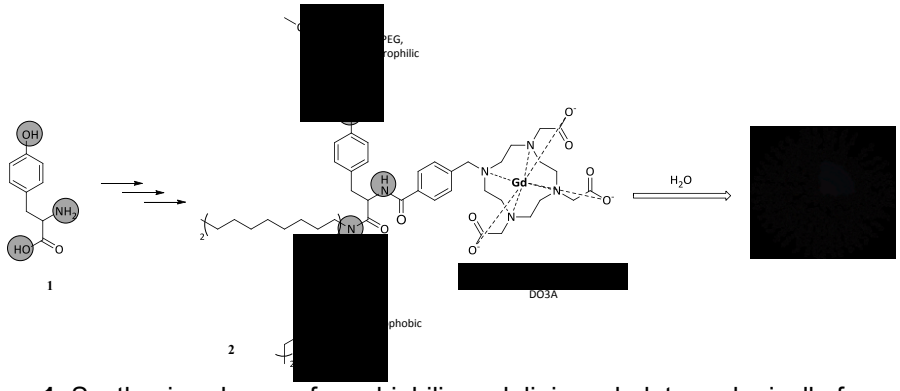

Figure 1. Synthesis scheme of amphiphilic gadolinium chelate and micelle formation

References

1. A. Beilvert, et al. "Tyrosine Polyethylene Glycol (PEG)-Micelle Magnetic Resonance Contrast Agent for the Detection of Lipid Rich Areas in Atherosclerotic Plaque" Magnetic Resonance in Medicine, 62(5) 2009 1195-1201
2. A. Accardo, et al. "Physicochemical properties of mixed micellar aggregates containing CCK peptides and Gd complexes designed as tumor specific contrast agents in MRI" Journal of the American Chemical Society, 126(10) 2004 3097-3107

P30

In vitro cell interaction and in vivo biodistribution of poly (dl-lactide-co-glycolide) nanospheres with encapsulated selenium nanoparticles for the treatment of liver diseases

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The role of selenium as a chemopreventive and chemotherapeutic agent has been supported by a large number of epidemiological, preclinical, and clinical trials [1, 2] suggesting that anti-tumor effect mechanisms of selenium include induction of apoptosis, inhibition of cell proliferation, protection against oxidative stress, and stimulation of immune system.

Herein we demonstrate a simple and quick synthesis of uniform, stable, amorphous selenium nanoparticles (SeNps), using ascorbic acid as the reduction agent. The choice of an appropriate stabilizer and reducing agent for preparation of stable selenium nanoparticles is very important. We used bovine serum albumin (BSA) as an organic layer for selenium nanoparticles, i.e., as a capping agent to make them more biocompatibile and protect them from agglomeration in the suspension medium.

SeNps were additionally encapsulated within spherical PLGA particles (PLGA/SeNps). One of the most important requirements for the controlled and balanced release of the drug in the body is ideal spherical shape of the particles and narrow distribution of their sizes. The morphology (size and shape) of the particles plays key role in their adhesion and interaction with the cell.

The influence of PLGA/SeNps on cell viability, ROS generation in HepG2 cells, as well as anticancer activity against epithelial tumor cells was investigated. Synthesized nanoparticles were characterized by FTIR spectroscopy, FESEM, TEM, HRTEM, and Zeta potential measurements. As a part of this study, we have also performed in vivo dynamic imaging studies in normal mice, using SPECT imaging and a high resolution gamma camera. The PLGA/SeNps nanoparticles have been radiolabelled with Tc-99m, by applying the direct labeling method [3]. Ex vivo biodistribution measurements, as well as in vivo dynamic studies up to 1h p.i. and at 24h were performed, showing increased concentration in liver and spleen.

Acknowledgements

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References

1. Popova, N. V. Cancer Lett. 2002, 179, 39–42.
2. Li, S.; Zhou, Y.; Wang, R.; Zhang, H.; Dong, Y.; Ip, C. Mol. Cancer Ther. 2007, 6, 1031–1038.
3. Psimadas, D.; Baldi, G.; Ravagli, C.; Bouziotis, P.; Xanthopoulos, S.; Francini, M.; Georgoulias, P.; Loudos, G.; J. Biom. Nan., 2012, 8, 4, 575-585.

P31

Polymeric nanocapsule for triple-modal imaging as a theranostic system in cancer therapy

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Nanoparticles are promising anti-cancer drug carriers and have been attracting great attention in recent years. They tend to show high accumulation at the tumour site due to the enhanced permeability and retention effect (EPR) which provides a passive tumour-targeting effect and therefore are widely used for cancer therapy [1]. Apart from their therapeutic applications, extensive studies have been performed to utilise them for theranostic approaches such as the non-invasive assessment of organ biodistribution and accumulation of anti-cancer drugs in target tissues [2]. We have developed a nano-theranostic drug delivery system based on polymeric nanocapsules encapsulating multimodal imaging probes for cancer imaging and therapy. To achieve triple imaging and therapeutic capabilities, the superparamagnetic iron oxide nanoparticles (SPIONs), a near infrared (NIR) dye, indocyanine green (ICG) and the gamma emitter Indium 111 were encapsulated into the nanocapsules for magnetic resonance imaging (MRI), optical fluorescence and nuclear imaging, respectively. In addition, SPIONs were also used for the magnetic hyperthermia with the potential to be combined with anti-cancer drug therapy in the future to achieve synergistic effect in cancer treatment. Poly (lactic-co-glycolic acid) (PLGA) was used to formulate the nanocapsules due to its biodegradability and biocompatibility. PEGylated PLGA (PLGA-PEG) was used to further prolong blood circulation profile thus enhancing tumour accumulation in vivo. The multifunctional nanocapsules were prepared by two methods, namely nano-precipitation and emulsification/ solvent evaporation method and the results were compared. The photo-, thermal- and aqueous-stability and in vitro release profiles of the ICG from the nanocapsules were determined. The MRI contrast properties (T2 relaxation time) and hyperthermia properties (intrinsic loss power) of SIPONs with different diameters and coatings were characterised in order to find the most suitable SPIONs for this study. The co-encapsulation of SPIONs and ICG and the stable labeling with a gamma emitter was confirmed in vivo by MRI imaging, nuclear imaging (SPECT/CT) and florescence imaging (IVIS Lumina III). Major organs and tumour tissues were histologically analysed using H&E and Perl's staining, and the iron content was quantified by superconducting quantum interference device (SQUID). As a result, the theranostic polymeric nanocapsule system prepared here exhibited triple imaging properties with great potential to be combined with hyperthermia for for cancer therapy.

Acknowledgements

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References

1. H. Maeda, H. Nakamura, J. Fang, The EPR effect for macromolecular drug delivery to solid tumours: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo, Adv Drug Deliv Rev, 2013, 65(1), 71-9.
2. T. Lammers, F. Kiessling, W.E. Hennink, G. Storm, Nanotheranostics and image-guided drug delivery: current concepts and future directions, Mol Pharm, 7(6):1899-912.

P32

Small-core Gold Nanoparticles Stabilized with a Thiolated DOTA–based Ligand

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Gold nanoparticles (AuNPs) show great promise as diagnosis and therapeutic agents for cancer. Nanoparticles can be loaded with a wide range of distinct functionalizing molecules, from target specific biomolecules to therapeutic drugs, aiming to enhance the target specificity and diagnostic/therapeutic payloads. They also provide attractive platforms for the development of theranostic agents since a single nanoparticle can have both imaging and therapeutic modalities.

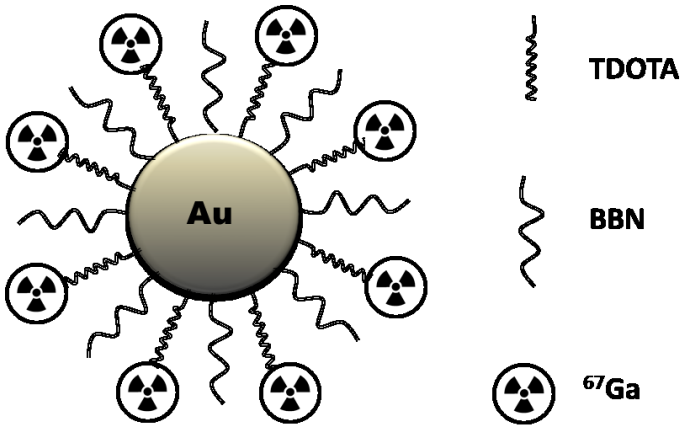

Figure 1

In this work we describe the synthesis, characterization and biological evaluation of small core (3-5 nm) gold nanoparticles (AuNP-TDOTA) stabilized with a new DOTA- based ligand (TDOTA) through thiol-to-gold covalent bonds. These nanoparticles were successfully decorated with a bombesin (BBN) derivative as a target specific vector. In this communication, we will report on the labeling of these nanoparticles (AuNP-TDOTA and BBN-AuNP-TDOTA) with 67Ga (Figure 1). Herein, we will also report on the in vitro stability of these 67Ga-labelled AuNPs, as well as on the evaluation of their cell uptake in PC-3 human tumor cells and their biodistribution in PC-3 tumor-bearing mice.

P33

Polyglutamic acid-PEG block copolymer nanocapsules: biodistribution study following two administration routes

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The lymphathic system is an important route for metastatic spread (1). Our laboratory is currently involved in the design of new nanomedicines with efficient biodistribution to the lymphatic system. Results have shown that polyglutamic acid-polyethyleneglycol block copolymer nanocapsules (PGA-PEG NCs) can load a variety of drugs and prolong their presence in plasma (2). The goal of this study was to analyze the biodistribution of PGA-PEG NCs of 100 and 200 nm of particle size as a new potential lymphatic carrier platform following subcutaneous and intravenous administration.

PGA-PEG nanocapsules were prepared by a solvent displacement technique; different rates of dispersed phase addition led to nanocapsule suspensions of two distinct particle sizes, 100 and 200 nm. In both cases, photon correlation spectroscopy analysis indicated that PGA-PEG NCs present a monomodal particle population centred on 100 and 200 nm and with negative zeta potential (-20 mV). By comparing the zeta potential to non-PEGylated PGA nanocapsules we could conclude that an effective polymer coating had been achieved. Successful PEGylation of the surface of PGA-PEG NCs is important for the system to maintain its stability in physiological media.

PGA-PEG nanocapsules could be effectively loaded with a fluorescent label, and this fluorescent suspension was used for biodistribution studies. The biodistribution studies show that PGA-PEG NCs injected intravenously have a preferential uptake by the liver and some minor uptake in hearth, spleen and lungs. Some distribution to the axillary, cervical, and mediastinal lymph nodes was noted. Subcutaneous injection of PGA-PEG NCs results in lymphatic accumulation similar to that observed following IV administration, but nanocarrier accumulation in internal organs was reduced while a significant fraction of particles were retained at the injection site. Smaller NCs reach the lymphatic nodes and the periferic organs slightly faster than larger particles, but after some time similar levels of particle biodistribution had been achieved.

Acknowledgements

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References

1. D. Hanahan, R. Weinberg, Halmarks of cancer: the next generation, Cell 144(5), 646-674, 2011.
2. T. Gonzalo, G. Lollo, M. Garcia-Fuentes, D. Torres, J. Correa, R. Riguera, E. Fernandez-Megia, P. Calvo, P. Aviles, M.J. Guillen, M.J. Alonso, A new potential nano-oncological therapy based on polyamino acid nanocapsules, J. Control. Release 169, 10-16, 2013.

P34

A phase-shift concept for ultrasound mediated drug delivery

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Ultrasound triggered release of compounds from particles are a promising area in drug delivery, allowing spatially localised drug delivery under ultrasound imaging control with reduced systemic exposure to other tissues potentially reducing side effects [1]. These technologies also open the possibility of delivering drugs, such as highly lipophilic compounds, that are difficult to formulate via other means.

Phoenix Solutions AS is developing a technology based on ultrasound mediated release from a bi-phasic (two component) micro-particle system engineered to phase shift in a controlled and reproducible manner. Drug is incorporated into low boiling point, micron sized oil droplets stabilized with a positively charged phospholipid membrane. Before administration, the drug loaded droplets are mixed with micron sized bubbles consisting of a low solubility perfluorocabon gas core stabilized with a negatively charged phospholipid membrane. Upon mixing the formulation is designed to allow the formation of small clusters of micro-bubbles and oil droplets. When exposed to ultrasound (standard medical imaging frequency and intensity) at the targeted pathology, the micro-bubble transfers acoustic energy to the attached oil droplets and acts as a 'seed' for the oil to undergo a liquid-to-gas phase shift (vaporisation). During this process the drug load is instantly released from the oil phase. The resulting bubble undergoes a rapid expansion to approximately 30 µm and temporarily blocks the microcirculation (capillary network), transiently stopping blood flow for approximately 1 to 3 minutes, keeping the released drug at high concentration and close proximity to the target pathology.

Compared to the existing state of the art alternative approaches the proposed concept offers a number of unique attributes which may enhance clinical utility significantly;

- Greatly increased loading capacity as the concept utilizes the volume of the micro-particle and not the surface membrane only.
- Deposit properties, transiently trapping the drug within the targeted pathology and avoiding rapid wash out after release.
- Burst release of non-modified drug. No linker technology or chemical modification is necessary – solubility in the oil phase is the only criterion.

Acknowledgements

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References

1. Castle J., Butts M., Healey A., Kent K., Marino M., Feinstein S. B., "Ultrasound Mediated Targeted Drug Delivery: Recent success and remaining challenges", Am J Physiol Heart Circ Physiol vol. 304 no. 3 H350-H357, 2013.

Initial in vitro and in vivo assessment of Au@DTDTPA-RGD nanoparticles labeled with Ga-68

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Introduction

Au@DTDTPA gold nanoparticles can be applied as contrast agents for both in vivo X-ray and magnetic resonance imaging. These particles are obtained by encapsulating gold cores within a multilayered organic shell which is composed of gadolinium chelates bound to each other through disulfide bonds [1]. In this work, our aim was to radiolabel and evaluate a gold nanoparticle, which was conjugated to a MRI contrast agent (gadolinium ions) via a dithiolated derivative of DTPA (Au@DTDTPA). Free DTDTPA moieties can be used for the entrapment of radiometals (in this case Ga-68), in order to produce a dual PET/MRI imaging probe that also has therapeutic capabilities.

Materials and Methods

For a typical preparation of ^{68}Ga-labeled nanoparticles, the Au@DTDTPA nanoparticles (Au@DTDTPA/Au@DTDTPA-RGD) were mixed with ammonium acetate buffer, pH 5 and 200 μL of ^{68}Ga eluate were consequently added. The mixture was then incubated for 45 min at 65 °C. Radiochemical purity was determined by ITLC, using KCL 0.2M as the mobile phase. In vitro stability of both radiolabeled species was assessed in saline and serum, up to 3 h. In vitro cell binding experiments were performed on integrin $\alpha_v\beta_3$ receptor-positive U87MG cancer cells, in order to assess the targeting capability of ^{68}Ga-labeled c-RGD-functionalized Au@DTDTPA. The non-specific Au@DTDTPA was used for comparison. In vivo biodistribution studies in U87MG tumor-bearing SCID mice followed.

Results

The Au@DTDTPA nanoparticles were successfully labeled with Gallium-68 at high radiochemical yield (>95%). Both ^{68}Ga-Au@DTDTPA and ^{68}Ga-cRGD-Au@DTDTPA nanoparticles were stable at RT up to 3h, as well as in the presence of serum for up to 3 h, at 37 °C. The cell binding assay on U87MG glioma cells proved that ^{68}Ga-cRGD-Au@DTDTPA had specific recognition, because the αvβ3 receptor-positive cell uptake was significantly higher with respect to that of non-specific ^{68}Ga-Au@DTDTPA. Biodistribution studies in U87MG tumor-bearing SCID mice showed that the radiotracer accumulates in the tumor at 1 h p.i. (1.85 ± 0.54%) and increased at 2 h p.i. (2.37 ± 0.07%).

Conclusions

The preliminary results of this study warrant the need for further development of Au@DTDTPA nanoparticles radiolabeled with Ga-68, as a possible dual-modality PET/MRI imaging agent.

References

1. Gadolinium Chelate Coated Gold Nanoparticles As Contrast Agents for Both X-ray Computed Tomography and Magnetic Resonance Imaging, C. Alric, J. Taleb, G. Le Duc, C. Mandon, C. Billotey, A. Le Meur-Herland, T. Brochard, F. V., M. Janier, P. Perriat, S. Roux, and O. Tillement

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Polymer-grafted USPIO as Thermosensitive Contrast Agents for MRI

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This study reports the synthesis of thermosensitive polymer-grafted ultra-small superparamagnetic iron oxide nanoparticles (USPIO) with temperature-dependent magnetic resonance imaging (MRI) signal at low field.

A thermosensitive polymer characterized by a lower critical solution temperature (LCST) in water, Jeffamine® M-2005 (PEO5-st-PPO37), was grafted onto the surface of USPIO by a silanization reaction and an amide-bond coupling.

A 1H NMR spectroscopic method was used to determine the LCST of Jeffamine M-2005® and indicated a transition temperature near 25°C.

USPIO surrounded by a thermosensitive polymer exhibit a temperature-responsive behavior, their surface reversibly changing from hydrophilic below LCST to hydrophobic above it. This phenomenon was used to prove that relaxivity of iron oxide nanoparticles is influenced by the hydrophobicity of their surface [1]. Transverse relaxivity (r2) of the USPIO@PEO5-st-PPO37 core-shell nanoparticles was measured at frequencies 8.25, 20, 60, and 300 MHz and longitudinal relaxivity (r1) was acquired between 0.01 and 300 MHz at temperatures ranging from 15 to 50°C. A decrease of these relaxivities vs. temperature with an inflection point at the LCST was observed at low field (<60MHz).

To illustrate the interest of such nanoparticles for their use as smart contrast agents, MR images were realized at low field (8.25 MHz) with either T1-or T2-weighted spin echo sequences. USPIO@PEO5-st-PPO37 shows a perfect linearity of the signal with temperature with a change of contrast from negative below the LCST to positive above it with the T2-weighted sequence.

The decrease of relaxivities above the LCST of the thermosensitive polymer-coated USPIO clearly shows the influence of iron oxide nanoparticles surface's hydrophobicity on relaxivity.

Combining a USPIO core with a thermosensitive shell offers the possibility of modulate the MRI contrast with temperature. The perfect linearity of the signal with temperature with a T2-weighted sequence at low field demonstrated unequivocally the interest of such nanosystems for the design of a temperature responsive contrast agent.

Acknowledgements

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References

1. S. Pinho et al., 2012, Phys. Chem. C, 2285-2291.

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AGuIX® nanoparticles vectorization for an apoptosis targeting

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Small and rigid platforms of polysiloxane, called AGuIX®, are a new material recently used in various medical imaging applications. They are formed after the dissolution in an aqueous solution of the gadolinium oxide core of nanoparticles with a core/shell structure. The shell is composed of polysiloxane and has several amine functions on its surface for attachment of various ligand as complexing molecules like DOTA or DTPA. The dissolution of Gd2O3 in nanoparticles is followed by the complexation of the Gd3+ ions thanks to the ligand grafted on their surface [1].

Polysiloxane platforms are considered for oncology applications. A passive tumor targeting by EPR effect (enhanced permeability and retention effect) of these particles was already observed. These particles are already tested as multimodal and theragnostic contrast agent. These nanoparticles have the advantage of combining multi-modal properties (MRI, scintigraphy, fluorescence) while maintaining a small size (less than 5 nm), which allows their elimination by the kidney [2]. The targeting can be enhanced by the attachment of an active targeting agent (e.g.: a peptide) on their surface. Grafted particles can be used to improve the diagnosis and the therapy effectiveness. The targeting agent TLVSSL peptide is proposed for his specificity for phosphatidylserine, a phospholipid overexpressed on apoptotic cells membranes. An active targeting of apoptotic cells would be useful for example to following-up an anti-tumoral therapy.

The optimization of peptide synthesis and polysiloxane platform vectorization with a coupling using EDC and NHS is the first objective of this work. Platforms were also grafted with cyanine 5.5 to give them fluorescent and bimodal properties. They were analyzed by HPLC, relaxometry, Infra-red spectroscopy and PCS (photon scattered spectroscopy). Different characterization allows determining an efficient coupling of the peptide. An increase of relaxivity and stability of AGuIX® nanoparticles is also observed after them vectorization.

Moreover, previous in vitro findings have shown the relative effectiveness of platforms vectorized by E3 peptides in targeting apoptotic cells. These tests were performed on Jurkat (lymphoma) cells incubated with anti-fas to induce apoptosis.

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References

1. Anna Mignot et Al. , Chem. Eur. J., 2013, 19, 6122–6136.
2. G. Le Duc et Al., ACS Nano, 2011, 5, 9566-9574.

Development of a stable superparamagnetic platform for biomedical applications

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Background: Superparamagnetic iron oxide NPs (SIONPs) have great potential for various biotechnologies and medical applications including cell labelling, drug delivery, hyperthemia and magnetic resonance imaging (MRI). For all biomedical applications, the control of the surface chemistry of SIONPs is required. In this work, we successfully developed a new superparamagnetic platform electrostatically stabilized by a thin polysiloxan shell exhibiting carboxylate functions. Various biomedical applications related these carboxy-silane coated NPs, ranging from cell labeling to in vivo bimodal imaging, are presented.

Procedure details: The synthesis protocol used allowed us to obtain iron oxide nanoparticles in huge quantity (multi-gram scale) and characterized by narrow size distribution. The coating of their surface using the appropriate organosilane resulted in the formation of thin layer (≈1nm) of polysiloxane shell ensuring the colloidal stability without affecting the relaxometric properties of the iron oxyde core (i.e. their efficiency). The as-obtained particles have been covalently decorated by rhodamin and the resulted ferrofluid has been administrated to a mice suffering from a tumor. As hoped, the bimodal probe could be localized by MRI and optical imaging.

Conclusion: The as-developed nanosystems have been successfully used in different biomedical applications such as medical imaging or cell labeling. The possibility of grafting has been demonstrated by the introduction of a fluorophore on these nanoparticles. The introduction of biological vectors in order to make these systems useful for molecular imaging applications is under development in our laboratory.

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Development and characterization of novel multimodal nanoplatforms of diamond for medical imaging

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Nanotechnology is a multidisciplinary research field involving the use of devices reduced to the nanoscale. These nanomaterials are new objects whose reactivity and properties are different from those observed at the micro- or macroscopic scale. These "nano-objects" found many applications in various domains including biomedical research.[1]

Recently, the nanoparticles of diamond have emerged as a new class of promising materials in nanotechnology. The combination of their advantageous properties is adapted for bio-applications including labelling and monitoring of cellular processes, the targeted drug delivery or the adsorption of biomolecules.[2-3]

The objective of this work is to develop and characterize a multimodal nanodiamond-based probe effective not only in magnetic resonance imaging (MRI) but also in optical imaging (OI). The nanoparticles of diamond have been studied by different characterization techniques (size, morphology and stability) before being submitted to an oxidation reaction and coupled with agents of interest. The functionalization of the nanodiamond platform involves the grafting of an apoptosis-specific vector (TLVSSL or E3 peptide), an optical agent (fluorescein trifluoroacetate or methyl violet) and a paramagnetic complex.

The last step of the work has been dedicated to the characterization of the new multimodal probes. Two samples of oxidized diamond nanoparticles have been reserved for the grafting of the apoptosis-specific vector and one of the optical agents (fluorescein trifluoroacetate or methyl violet). The coupling of the platform with fluorescein has been demonstrated by fluorescence spectroscopy. In addition, optical microscopy study conducted on cell cultures gave evidence of the efficiency of graftings (peptide and fluorophores) through the affinity of nanoparticles with Jurkat cells stimulated by anti-Fas. A third sample of oxidized nanoparticles has been reserved for the grafting of the MRI contrast agent previously synthesized and characterized. The designed multifunctional probe showed promising relaxation rate for MRI applications.

Results demonstrated clearly the effectiveness of the MRI nanodiamond-based probe for medical imaging.

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References

1. Chuto G., Chaumet-Riffaud P., J.Med Nucl, Vol 34, 2010, 6:370-376.
2. Mitura S., JAMME, 2007, 24: 166-171.
3. Shenderova O. A., Ciftan Hens, S. A., ITC, 2010, 79-116.

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Targeting Immuno-liposomes using TCR-like antibodies directed against melanoma MAGE-A1 antigen

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Nano-carriers provide an attractive alternative to free cytotoxic drug in the treatment of solid tumors as they reduce drug-related toxicity and promote drug accumulation in tumors. When coupled to a ligand moiety, nano-carriers can be selectively internalized and specifically targeted to tumor cells. Here, we aim to improve liposomal chemotherapy by development of drug-loaded liposomes that specifically target cell surface-expressed peptide-Major Histocompatibility Complexes (pMHC), which constitute natural targets for T-cells and, in defined cases, are uniquely expressed by melanoma cells. The target pMHC is the HLA-A1-restricted, MAGE-A1 peptide EADPTGHSY, which belongs to the family of Cancer Testis Antigens, is not present on normal tissue and has proven to be an effective in vitro target for melanoma treatment with T cells.

Single chain Fvs were selected from a phage-display library that are pMHC-specific and have different affinities (KD: 250 and 14 nM). To allow cysteine-conjugation to liposomes, scFvs were cloned in a modified vector pABC4, after which scFv were produced in the periplasmic fractions of BL21 bacteria and extracted by immobilized metal ion affinity chromatography. Liposomes were prepared by film hydration and extrusion methods, and characterized for size, polydispersity and lipid concentration prior and following coupling to scFv. The coupling of scFv to liposomes has been optimized and demonstratespMHC-specific recognition by ELISA and flow cytometry. We are currently further evaluating these immuno-conjugates in vitro and will move to in vivo testing in relevant tumor models.

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References

1. Messerschmidt SKE, Beuttler J, Rothdiener M, 2010, Immunoliposomes, Antibody Engineering, Part IV, 401.
2. Chames P, Hufton SE, Coulie PG, Uchanska-Ziegler B, Hoogenboom HR, 2000, Direct selection of a human antibody fragment directed against the tumor T-cell epitope HLA-A1-MAGE-A1 from a nonimmunized phage-Fab library. Proc Natl Acad Sci USA 97: 7969.
3. Chames P, Willemsen RA, Rojas G, Dieckmann D, Rem L, Schuler G, Bolhuis RL, Hoogenboom HR, 2002, TCR-like human antibodies expressed on human CTLs mediate antibody affinity-dependent cytolytic activity. J Immunol 169 : 1110.
4. Willemsen R, Chames P, Schooten E, Gratama JW, Debets R, 2008, Selection of human antibody fragments directed against tumor T-cell epitopes for adoptive T cell therapy. Cytometry A 73: 1093.
5. Straetemans T, van Brakel M, van Steenbergen S, Broertjes M, Drexhage J, Hegmans J, Lambrecht BN, Lamers C, van der Bruggen P, Coullie PG, Debets R, 2012, TCR gene transfer: MAGE-C2/HLA-A2 and MAGE-A3/HLA-DP4 epitopes as melanoma-specific immune targets. Clin.Dev.Immunol. 2012: 586314.

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Identification of new bio-effects of ultrasound and microbubbles assisted drug delivery

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Upon ultrasound exposure, gas microbubbles can be expanded, moved and even destroyed. These properties offer the opportunity of site-specific local drug/gene delivery. Activation of microbubbles under specific ultrasound beams induces a transient cell membrane permeabilization with a process known as sonoporation. Transient pores formed at the plasma membrane are supposed to be responsible for inward and outward transports of molecules [1]. A key to success of this technique lies in understanding mechanisms governing microbubble-cell interactions. Improving our knowledge will allow us to fully exploit this method for therapy.

Here, we investigate how microbubbles and ultrasound behave towards cells upon sonoporation at 1 MHz of frequency in presence of MicromarkerTM microbubbles. In the context of gene delivery in HeLa cells, optimal transgene expression was obtained with the following parameters: 150 kPa, 40% duty cycle and 60 sec of stimulation time. In addition to cellular massage and shear stress, some microbubbles were translated inside cells under this condition, as shown previously [2]. We assessed which cellular bio-effects are induced by these physical stresses. Actin stress fibers formation, an increase of intracellular calcium concentration and ROS production were observed as already reported by others in the context of ultrasound stimulation [3]. Interestingly, we found that the nucleus was also affected. In line with data obtained by Furosawa et al., DNA damage sensor as γ-H2AX was transiently detected [4]; the level was dependent on ultrasound dose and the cell type. The novelty of this work was the determination of ultrasound effect on chromatin compaction by using HeLa cells stably co-expressing histones H2B-GFP and H2B-Cherry [5]. FLIM-FRET experiments allowed us concluding that sonoporation was able to reverse the chromatin compaction during mitosis or induced by ATP depletion and 1 mM MgCl2 treatments. This has been validated by an increase of phosphorylated H2B in treated cells. Since Adenosine Monophosphate-activated Protein Kinase (AMPK), a mechanical and energy sensor molecule, is acting upstream of pathways involving H2B and H2AX phosphorylation, we evaluated its activation [6]. Indeed, AMPK phosphorylation was dependent on ultrasound intensity. The downstream effect of AMPK activation is currently investigated in our laboratory. Knowing that AMPK can be linked to protein expression, cell survival and cell cycle regulation, a deep knowledge on the signaling pathway(s) involved here will be of interest for a safe and fine use of sonoporation for drug/gene delivery.

References

1. Delalande, A., et al., Ultrasound-assisted gene delivery: recent advances and ongoing challenges. Therapeutic delivery, 2012. in press.
2. Delalande, A., et al., Sonoporation at a low mechanical index. Bubble science, Engineering and Technology, 2011. 3(1): p. 3-11.
3. Juffermans, L.J., et al., Ultrasound and microbubble-induced intra- and intercellular bioeffects in primary endothelial cells. Ultrasound Med Biol, 2009. 35(11): p. 1917-27.
4. Furusawa, Y., et al., DNA double-strand breaks induced by cavitational mechanical effects of ultrasound in cancer cell lines. PloS one, 2012. 7(1): p. e29012.
5. Lleres, D., et al., Quantitative analysis of chromatin compaction in living cells using FLIM-FRET. J Cell Biol, 2009. 187(4): p. 481-96.
6. Bungard, D., et al., Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. Science, 2010. 329(5996): p. 1201-5.

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Superparamagnetically grafted multi-walled carbon nanotubes for dual-modality SPECT/MR biomedical imaging

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The present study reported radio-labelled iron oxide decorated multi-walled carbon nanotubes (MWNT) for dual magnetic resonance (MR) and single photon emission computed tomography (SPECT) imaging. Superparamagnetic iron oxide nanoparticles (SPION) grafted MWNTs were synthesised, followed by characterisations of the physicochemical and magnetic properties. High r2 relaxivities were obtained in both phantom and in vivo MRI. A functionalized bisphosphonate was used to bridge technetium-99m and SPION. The obtained radio-labelled hybrids were intravenously injected to mice for further whole body 3D SPECT/CT imaging and organ biodistribution studies by γ-scintigraphy. No signs of abnormality were observed on H&E stained tissue sections and the presence of SPION and MWNT were identified by Perls stain and Neutral Red stain, respectively. TEM images of liver and spleen tissues denoted the co-localization of SPION and MWNT within the same intracellular vesicles, indicating the good in vivo stability of the hybrids. Collectively, the synthesized magnetic yet radioactive MWNT-SPION hybrids demonstrated the capability as dual MRI-SPECT contrast agents.

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Comparative in vitro and in vivo evaluation of nanosized Liposome appropriately modified for being labelled with Tc-99m by two different radiolabelling approaches

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Liposomes are drug delivery agents with a well recognised application in cancer therapy [1]. Radiolabelling of liposomes with the γ-radiation emitter 99mTc is an extensively investigated concept for tracking their in vivo fate [2,3]. The aim of this study was to radiolabel and to perform a comparative evaluation of the in vitro and in vivo behavior of two liposomes: LP (I), that was consisted of distearoylphosphatidylcholine (DSPC), cholesterol, PEG-distearoylphosphatidylethanolamine (PEG-DSPE) and carboxylated PEG2000-distearoylphosphatidylethanolamine (DSPE-PEG2000-COOH) and was radiolabelled by direct labeling methodology [4] and LP (II), that contained the same lipid components as LP (I) with the exception that DSPE-PEG2000-COOH was modified with the ligand pyridyl-ethyl cysteine (PEC-2) for being complexed with 99mTc via the carbonyl labelling approach [5].

Liposomes were prepared by the well-established lipid film hydration technique followed by extrusion. They were characterized by dynamic light scattering (DLS) measurements prior to and after their radiolabelling with 99mTc.Their labelling efficiency and their in vitro stability were determined by instant thin layer chromatography ITLC-SA and Whatman paper chromatography. Biodistribution studies were conducted by intravenous injection (i.v.) in normal Swiss mice. For the imaging a custom Single Photon Emission Computed Tomography (SPECT) system with 1.5mm spatial resolution was used and the results were correlated to the biodistribution data.

The size of LP (I) was 139.1± 45.77 nm and of LP (II) was 131.2± 45.64 nm. The applied radiolabeling conditions didn't seem to affect the liposomes size. LP (I) was obtained in high radiolabelling yield and in radiochemical purity, while the labelling of LP (II) was relatively low although of higher radiochemical stability following its purification compared to LP (I) in relation to time. LP (I) was less stable both in the presence of competitive for ^{99m}Tc ligands and in serum than LP (II). The different radiolabelling methods seemed to affect the biodistribution pattern, with the LP (II) showing prolonged circulation in blood and being recognised by the Mononuclear Phagocyte System (MPS) to a lower extent than LP (I). These findings were also confirmed by in vivo scintigraphic imaging.

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References

1. Perche F, Torchilin VP. Recent trends in multifunctional liposomal nanocarriers for enhanced tumor targeting. Journal of drug delivery. 2013; Available at: http://dx.doi.org/10.1155/2013/705265
2. Petersen AL, Hansen AE, Gabizon A, Andresen TL. Liposome imaging agents in personalized medicine. Advanced Drug Delivery Reviews. 2012;64(13):1417–1435.
3. Goins B a. Radiolabeled lipid nanoparticles for diagnostic imaging. Expert opinion on medical diagnostics. 2008;2(7):853–73.
4. Psimadas D, Oliveira H, Thevenot J et al, Polymeric micelles and vesicles: biological behavior evaluation using radiolabeling techniques. Pharmaceutical development and technology. 2013;7450:1–5.
5. Karagiorgou O, Patsis G, Pelecanou M et al, (S)-(2-(2′-Pyridyl)ethyl)cysteamine and (S)-(2-(2′-Pyridyl)ethyl)-D,L-homocysteine as Ligands for the "fac-[M(CO)3]+"(M=Re, 99mTc) Core. Inorganic Chemistry 2005; 44(12):4118–4120.

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USPIO-labeled Collagen Scaffolds for Non-invasive MR Imaging in Tissue Engineering

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Non-invasive imaging holds significant potential for implementation in tissue engineering. It can be used to monitor the localization and function of tissue-engineered implants, as well as their resorption and remodelling. Thus far, however, the vast majority of effort in this area of research have focused on the use of ultrasmall super-paramagnetic iron oxide (USPIO) nanoparticle-labeled cells, colonizing the scaffolds, to indirectly image the implant material. Reasoning that directly labeling scaffold materials might be more beneficial (enabling imaging also in the case of non-cellularized implants), more informative (enabling the noninvasive visualization and quantification of scaffold degradation), and easier to translate into the clinic (cell-free materials are less complex from a regulatory point-of-view), three different types of USPIO nanoparticles are prepared and incorporated both passively and actively (via chemical conjugation; during collagen crosslinking) into collagen-based scaffold materials. The amount of USPIO incorporated into the scaffolds is optimized, and correlated with MR signal intensity, showing that the labeled scaffolds are highly biocompatible, and that scaffold degradation can be visualized using MRI. This provides an initial proof-of-principle for the in vivo visualization of the scaffolds. Consequently, USPIO-labeled scaffold materials seem to be highly suitable for image-guided tissue engineering applications.

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References

Mertens M, Herrmann A, Bóhren A, Olde-Damink L, Moeckel D, Gremse F, Ehling J, Kiessling F, Lammers T. USPIO-labeled Collagen Scaffolds for Non-invasive MR Imaging in Tissue Engineering. *Adv Funct Mater (in press)*

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^{99m}Tc-Labeled aminosilane-coated iron oxide nanoparticles as dual modality imaging agents of tumor angiogenesis and in vivo hyperthermia evaluation

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Introduction: Cancer treatment using magnetic nanoparticles is attracting increased attention since hyperthermia is one of the promising approaches in cancer therapy. In addition, magnetic nanoparticles have become important tools for diagnostic applications. We developed and evaluated as a tumor imaging agent in nuclear medicine, radiolabeled iron oxide nanoparticles (IONPs). Moreover, we proceeded with the evaluation of a multimeric system of targeted ^{99m}Tc-labeled nanoparticles (IONPs) conjugated with a new RGD derivate (cRGDfK-Orn3-CGG), providing an excellent system for target-specific molecular recognition.

Materials & Methods: We developed aminosilane coated Fe_3O_4 (10±2 nm) through 3-aminopropyltriethoxysilane (APTES) and RGD derivative functionalization as well. Transmission Electron Microscopy (TEM) and spectroscopy techniques were used to characterize the NPs indicating that they get functionalized with peptides. Both radiolabeled IONPs (targeted, non-targeted) are evaluated with regard to their radiochemical, radiobiological and imaging characteristics. In vivo studies were performed in normal and *αvb3*–positive bearing mice. Additionally, we performed a preliminary in vivo evaluation of the magnetic biomaterial in hyperthermia therapy.

Results: Both radiolabeled IONPs were obtained in high radiochemical yield (>98%) and proved stable in vitro. The in vivo and biodistribution studies for both IONPs have shown significant liver and spleen uptake at all examined time points in normal and U87MG glioblastoma tumor-bearing mice, due to their colloidal nature. We have administrated in vivo and from biodistribution studies we confirmed that the non-targeted ^{99m}Tc-NPs obtaining tumor uptake of about 1.05 D/g at 1 h p.i.. Biodistribution evaluation of targeted ^{99m}Tc-NPs-RGD, presents significantly higher tumor uptake of about 9.01 ID/g at 1 h p.i.. Accumulation in other organs was negligible, for both IONPs. Blocking experiments indicated target specificity for integrin receptors in U87MG glioblastoma cells. The preliminary in vivo heating efficiency experiment showed that inducing hyperthermia by using iron oxide NPs was feasible.

Conclusions: The non-targeted ^{99m}Tc-NPs present suitable characteristics as an imaging agent. Moreover, ^{99m}Tc-NPs-RGD demonstrate properties suitable for use as a target-specific agent for molecular imaging of *avb3* expression in tumor angiogenesis. In conclusion, the above preliminary results on IONPs justify further investigation towards potential hyperthermia application.

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