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Book of Abstracts

ICONAN 2019

Bridging BASIC BIO-NANO-SCIENCE
and CLINICAL TRANSLATION

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My 27 Years of Failure (Trying to Cure Lung and LIver Metastases)

Wednesday, 16th October - 09:05: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 266

Dr. mauro ferrari [1]

1. *University of St Thomas*

Nanomedicine has been a great success, in research laboratories, and in the clinic, since its emergence in medicine about 25 years ago. Yet, it has not been able to yield a general cure for metastatic disease to lungs and liver - which unfortunately are responsible for the vast majority of cancer deaths. nenomedicine is not alone in this “failure” - all other approaches have similarly failed, to date, including chemotherapy, molecularly targeted biotherapeutics, and immunotherapy. In my talk, I will give an overview of my long and rather colorful list of personal failures in attempting to do just that: To cure visceral metastases, regardless of their primary cancer site of origin. Along the way, I found myself in the early, formative stages of nanomedicine - and we have continued to share the journey until now. Post-nanomedicine I also developed a number of different approaches, which employed nanotechnology as one of the components of the attempted solutions. This gave rise to other fields, such as multi-stage vectors (MSV), transport oncophysics (TOP), and injectable nanoparticle generators (iNPG). A combination of these is now giving me new hope that a cure for metastatic disease to lung and liver for many may actually be reachable, soon. We have developed a new drug, regulatory codename ML-016, (scientific name iNPG-pDox), which has shown unprecedented curative results in preclinical models, and now we are taking it to the clinic. We have developed and scaled up good manufacturing techniques, build a specialized facility, progressed through toxicity studies with exemplary results, and had independent verification of of efficacy results. We established a company (BrYet, LLC) and secured the portfolio of issued and pending patents supporting ML-016. Independent clinical trials on triple-negative breast cancer with visceral metastases are scheduled to start in June at Houston Methodist hospital, with support from the Department of Defense of the USA, under the clinical leadership of Dr Jenny Chang. BrYet is looking at starting its all-comer visceral metastases Phase I/Ib later in 2020. in this talk, I will focus on recent scientific developments of importance for for ML-016, and namely novel validations for its postulated, transport-based MOA (primary authors: Shreya Goel, Haifa Shen); and new discoveries on the modalities of uptake of particulate drugs (more in general than ML-016) by the liver and other biological barriers (primary author: Sara Nizzero). Looking forward to discussing it all with you! My most cordial regards - Mauro

A cancer survivor’s journey; A patient’s perspective

Wednesday, 16th October - 09:33: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 277

Mrs. Lora Kelly [1]

1. *Director of Clinical Nursing Education (HACC) & Chair of Central Pennsylvania National Pancreas Foundation*

Abstract (Summary of nonacademic Lecture). Lora Kelly is a healthy pancreatic cancer survivor who shares her journey of diagnosis and treatment over the past several years in a truthful, vulnerable manner to compel the listener to rightly begin to understand what cancer patients endure. Lora’s story is one of hope, resilience, and positivity. Lora demonstrates great appreciation for the scientific community while equally urging cancer research scientists to gain a sense of immediacy to develop better therapies for cancer patients. Lora shares her story in such a personal way to both honor any person touched by cancer as well as reallyhelp the scientific community understand the great value and necessity of their work through the eyes of a cancer survivor.

Polypeptide-based Conjugates as Versatile Therapeutics

Wednesday, 16th October - 10:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 83

Dr. María J. Vicent [1]

1. *Polymer Therapeutics Lab. Prince Felipe Research Center*

Polypeptides are already playing a major role on a number of different relevant areas such as nanomedicine [1]. The physico-chemical parameters of a polypeptide-conjugate, and hence its biological performance, are defined by an intricate interplay of multiple structural factors. This highlights the need for detailed structure-activity relationship studies to develop the hierarchical strategies of polypeptide conjugate design. However, structural complexity also represents a unique opportunity, since small changes at the structural level might endow nanomedicines with outstanding and unexpected biological performance [2].

In our group, we have overcome the main classical limitations for the synthesis of defined polypeptides using precise controlled reactions followed by an adequate characterization yielding to well-defined polypeptidic architectures (including stars, graft and block-copolymers) by NCA polymerization techniques [3]. In addition, post-polymerization techniques allow us the introduction of a variety of functionalities yielding a set of orthogonal reactive attachment sides [4]. Using these techniques and following a bottom-up strategy we have been able to obtain star-based polypeptide architectures with the capacity to self-assemble yielding supramolecular nanostructures with interesting properties [5]. This strategy together with an adequate polymer-drug linker design [6] enabled *in vitro* and *in vivo*evaluation, revealing a lack of toxicity, an enhanced *in vitro* cell internalization rate and significantly greater terminal and accumulation half-life *in vivo* together with a significant lymph node accumulation [5]. These results allow us to envisage these systems as promising nanocarriers for therapeutic or diagnostic applications, especially in anti-cancer treatments including lymph node metastasis and cancer immunotherapy. Proof of Concept for metastatic breast cancer [6] and for immunotherapy design in melanoma will be also shown as well as the use of this self-assembled architectures in applications such us neurodegenerative disorders or acute kidney injury.

References

[1] a) Duro-Castano A., Conejos-Sánchez I., Vicent M.J. Polymers 2014, 6, 515-551; b) Duro-Castaño A,Movellan J, Vicent MJ Biomater. Sci., 2015,**3**, 1321-1334

[2] Zagorodko O., Arroyo-Crespo, J.J., Nebot, V.J., and Vicent, M.J.Macromolecular Bioscience 2017, 17, 1600316-n/a.

[3] a) Duro-Castano A., England, R.M., Razola, D., Romero, E., Oteo-Vives, M., Morcillo, M.A., and Vicent, M.J. Molecular Pharmaceutics 2015, 12, 3639-3649; b) Conejos-Sánchez I. Duro-Castano, A., Birke, A., Barz, M., and Vicent, M.J. Polymer chemistry 2013, 4, 3182-3186.

[4] Barz M., Duro-Castano A., Vicent M.J. Polymer Chemistry 2013, 4, 2989-2994

[5] Duro-Casaño Nebot, V. J., Niño-Pariente, A., Armiñán, A., Arroyo-Crespo, J. J., Paul, A., Feiner-Gracia, N., Albertazzi, L. and Vicent, M. J. Advanced Materials 2017, doi. 10.1002/adma.201702888

[6] a) Arroyo-Crespo, J.J, Armiñán A., Charbonnier D:, Balzano-Nogueira L., Huertas-López F., Martí C., Tarazona S., Forteza J., Conesa A., Vicent M.J. Biomaterials 2018, 186, 8-21; b) Arroyo□Crespo, J.J., Deladriere, C. et al. Adv. Func. Mat. 2018. 28(22): p. 1800931

Acknowledgments

Spanish Ministry of Economy and Competitiveness (SAF2016-80427-R) and the European Research Council (Grant ERC-CoG-2014-648831 MyNano, Grant ERC-PoC-2018-825798 Polymmune) for financial support. Part of the equipment employed in this work has been funded by Generalitat Valenciana and co-financed with FEDER funds (PO FEDER of Comunitat Valenciana 2014-2020).

Squalene lipid prodrugs: a unique biomimetic approach exploiting endogenous lipoproteins for drug delivery

Wednesday, 16th October - 11:00: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 10

Dr. Simona Mura [1]

1. *University of Paris-Sud*

Introduction. An amphiphilic prodrug of gemcitabine (Gem) has been synthesized by its covalent conjugation to the squalene (SQ), a natural lipid, precursor in the cholesterol biosynthesis. Compared to the free drug, the nanoparticles made of this bioconjugate (SQGem NPs) revealed higher anticancer activity in various animal tumor models.[1] However, in absence of any specific ligand targeted towards cancer cells, the exact mechanism behind this improved anticancer activity appeared quite puzzling.

It is well known that, once introduced in the organism, NPs interact with a complex biological environment and acquire a complex signature, which can significantly affect their in vivo fate. While a great deal of attention has been focused to identify the proteins adsorbed at the nanoparticle surface, herein, the lipid nature of SQ and the capacity of circulating lipoproteins (LP) to transport hydrophobic molecules led us to believe that the interaction between the SQGem NPs and lipoproteins deserved to be deeply explored.

Materials and Methods. *Formulation.* A radiolabeled bioconjugate (^{3}H-SQGem) has been synthesized and tritiated NPs were prepared by nanoprecipitation. Size and polydispersity index were determined by DLS. *Interaction with lipoproteins*: Radiolabeled NPs and free ^{3}H-Gem were (i) incubated in vitro with human blood or (ii) administered intravenously to healthy Sprague Dawley rats. 5 minutes after, blood was collected, centrifuged and obtained plasma was separated into lipoprotein and LP deficient fraction (LPDF). The radioactivity found in each fraction was measured using a β-scintillation counter.

Results and Discussion. We clearly showed that SQGem bioconjugates spontaneously interact with the plasma lipoproteins, and in particular with the cholesterol-rich ones, both in vitro in human blood and in vivo in rodents, whereas the free drug does not interact with LPs. [2] (Figure 1) To be noted that in rodents, due to their specific lipid metabolism, the cholesterol transport is mediated by the HDL, which play the same role as LDL in Humans. Thanks to this interaction, the cholesterol-rich particles behaved as endogenous carriers of the bioconjugates and allowed an “indirect” transport of the gemcitabine to cancer cells with high LP receptor expression. [3]

Conclusions. We have demonstrated that is possible to exploit the lipoproteins as indirect carriers by simply taking advantage of the spontaneous intravascular events that occur in the circulation post administration. Moreover, not only SQGem NPs but also other squalene derivatives can interact similarly with lipoproteins thus opening an entirely new perspective, which may significantly advance the application of LDL as drug delivery systems.

Acknowledgments: This work was supported by the ERC under the FP7/2007-2013 Grant Agreement No. 249835.

[1]D. Desmaele, R. Gref et al., *J. Controlled Release* 2012, **161**, 609

[2]D. Sobot, S. Mura *et al., Nature Comm.*2017, **8**, 15678

[3]D. Sobot, S. Mura. *et al., Mol.Ther.* 2017, **25**, 1596

Figure 1. Radioactivity (magenta lines) and cholesterol (blue line) distribution among the collected fractions of plasma obtained from rats treated with ^{3}H-SQGem or free ^{3}H-Gem, 5 min post administration.

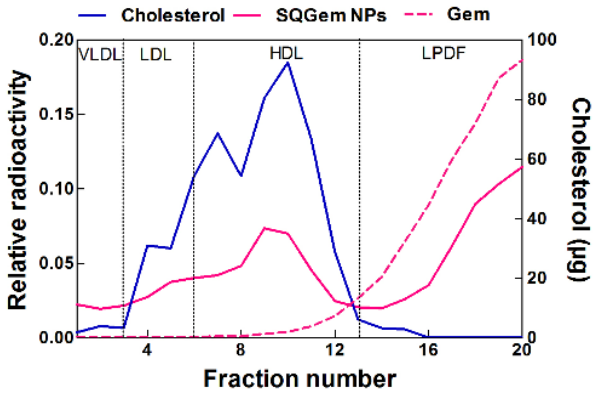


Fig 1 drug distribution in vivo.png

Heat mediated drug delivery with temperature sensitive liposomes – a synergistic approach

Wednesday, 16th October - 11:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 281

Prof. Lars Lindner [1]

1. *University Hospital of the Ludwig-Maximilians-Universitaet Munich, Germany*

Coming soon

Synthesis and Characterization of Photoresponsive Amphiphilic Star Copolymers as Drug Carriers

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 2

Mr. Fatih Genç [1], ***Dr. Binnur Aydogan Temel*** [1]

1. *Bezmialem Vakif University*

Introduction: Star polymers are the structures consisted by many linear polymers fused at a central point with a large number of chain end functionalities [1]. Through this special structure, star polymers exhibit some important properties and characteristics that cannot be reached by simple linear polymers. Compared to linear polymers, the three-dimensional spherical structures and special hydrodynamic volumes provide a very good advantage for the encapsulation of drugs [2,3]. In general, these particulate encapsulation vehicles have a core structure that allows the water-insoluble molecules to be entrapped in a hydrophobic environment and a hydrophilic shell structure that surrounds the core providing water solubility of the formed micelle structure.
Methods: Photoresponsive amphiphilic star copolymers bearing anthracene moieties at side chains were synthesized by Atom Transfer Radical Polymerization (ATRP). Final amphiphilic copolymers were used for preparation of polymeric micelles which were further loaded with DOX in order to examine their potential as drug carriers. All polymers were characterized by ^{1}H NMR, FT-IR, DSC, UV-Vis spectroscopy. Particle sizes of polymeric micelles were characterized using DLS measurement.
Results and Discussion: Anthracene bearing amphiphilic star copolymers were synthesized and their micelles were formed in water. ^{1}H NMR, FT-IR, UV-Vis data clearly revealed the successful synthesis of all structures. A hydrophobic drug DOX was used to study encapsulation of guest molecules inside the hydrophobic core of star copolymer micelle. Photodimerization characteristics of anthracene pendants in polymer micelles were investigated by UV-Vis spectroscopy. Polymer micelles were irradiated at 365 nm and obtained UV spectra showed a clear decrease with time. The micelles showed good loading capacity for DOX was clearly demonstrated.
Acknowledgements: This work was financially supported by Bezmialem Vakif University Scientific Research Projects Unit (Project No: 2.2019/19).
References:
[1] Wang, M., Wang, Y., Zhao, S., Zhang, X., and Wei, H.,*Macromolecular Chemistry and Physics*, **2018**, *219*(12), 1800061.
[2] Zhu, W., Nese, A., and Matyjaszewski, K., *Journal of Polymer Science Part A: Polymer Chemistry*, **2011**, *49*(9), 1942-1952.
[3] París, R. and De la Fuente, J. L., *Reactive and Functional Polymers*, **2008**, *68*(5), 1004-1012.

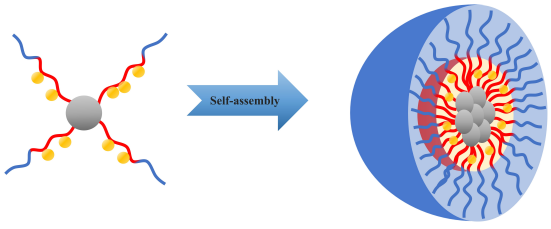


Figure 1. self-assembly of amphiphilic star copolymers bearing anthracene groups..jpg

The new types of carbosilane dendrimers as non-viral transfection vectors for siRNA cell delivery

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 32

Mrs. Regina Herma [1], ***Dr. Michaela Liegertova*** [1], ***Dr. Jan Maly*** [1], ***Dr. Dominika Wrobel*** [1], ***Dr. Marcel Stofik*** [1], ***Dr. Marek Maly*** [1], ***Dr. Dietmar Appelhans*** [2], ***Dr. Tomas Strasak*** [3]

1. *Jan Evangelista Purkyne University,* ***2.*** *The Leibniz Institute of Polymer Research Dresden (,* ***3.*** *Institute of Chemical Process Fundamentals of the CAS, v. v. i.*

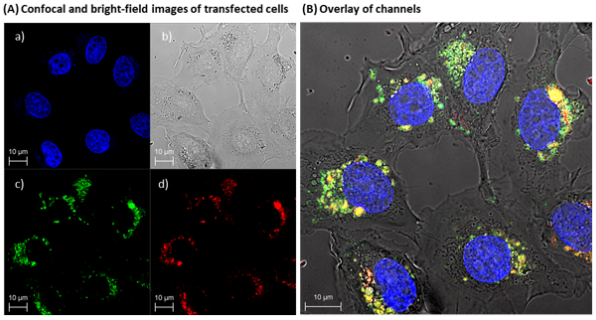
Rapidly developing concepts of gene therapy bring great expectations in potential treatment of several fatal genetic-based diseases as are cystic fibrosis, haemophilia, various types of neurodegenerative diseases, HIV infections and cancers. [1] The core of the approach lies in the specific local delivery of nucleic acids (DNA, small interfering RNA (siRNA)) in to the targeted cells to mediate the therapeutic effect on selected genes. Based on the type of nucleic acid, the genetic material must be transported either into the nucleus (DNA) or into the cytosol of the cells (siRNA). The indispensable part of the functional gene therapy concept is the availability of suitable nucleic acid carriers.

Non-viral gene delivery vectors studied in the gene therapy applications are often designed with the cationic nitrogen containing groups necessary for binding and cell release of nucleic acids. Disadvantage is a relatively high toxicity which restricts the *in vivo* use of such nanoparticles. We shown, that the 3rd generation carbosilane dendrimers possessing (trimethyl)phosphonium (PMe3) groups on their periphery were able to effectively deliver the functional siRNA into the cells (B14, *Cricetulus griseus*), release it into the cytosol and finally to achieve up to 40% gene silencing of targeted gene (glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) with the comparable or, in some cases, even better effectivity as their ammonium counterparts. Moreover, such cationic dendrimers show relatively low *in vivo* toxicity as compared to their ammonium analogues when analyzed by standard Fish Embryo Test (FET) on *Danio rerio in vivo* model, with LD50 = 6.26 μM after 48 hours of incubation. This is more than 10-fold improvement as compared to published values for various other types of cationic dendrimers. We discuss the potential of further increase of the transfection efficiency, endosomal escape and decrease of toxicity of such non-viral vectors, based on the systematic screening of different types of substituents on central phosphonium atom.

Research was supported by project [UJEP-SGS-2017-53-002-3] and partially by project 173-07-04 of Internal Grant Agency UJEP.

Reference

[1] Miele, E. et al. 2012. Nanoparticle-based delivery of small interfering RNA: challenges for cancer therapy. Int J Nanomedicine 7: 3637-3657.



Confocal fluorescence microscopy images of transfected cells.png

Using Liposomal Encapsulated Thymoquinone in Treating Cervical Cancers

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 74

Mr. Poona Matin [1], ***Dr. Lucy Ghali*** [1], ***Dr. Xuesong Wen*** [1], ***Prof. Hemda Garelick*** [1]

1. *Middlesex University*

Using Liposomal Encapsulated Thymoquinone in Treating Cervical Cancers

Poona Matin, Lucy Ghali, Hemda Garelick, Xuesong Wen*

Department of Natural Sciences, Faculty of Science & Technology, Middlesex University, London, UK

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Cervical cancer is one of the most common female malignancies worldwide of which over 99% cases are associated with a high-risk human papillomavirus (HPV) infection. Current treatments of cervical cancer involve surgery, radiotherapy and chemotherapy depending on the stage of disease. Cisplatin is the main drug of choice despite its cytotoxicity. Thymoquinone (TQ), a component of the *Nigella sativa*plant has been shown to have anti-inflammatory, antioxidant and anticancer properties. Although it has been shown to induce cell apoptosis in cervical cancer cells, its anti-HPV effect has not been investigated. Liposomes as drug delivery vesicles have been used to deliver drugs enabling reduced cellular toxicity and a controlled drug release. In this study, liposomal encapsulated TQ were prepared and used to treat HPV-16 infected cervical cancer cell line (CasKi) in order to determine whether it can reduce the HPV oncogenes level of expression of E6 and E7. C33A (HPV-negative cervical cancer cells) was used as a control cell line.

The MTT assay was used to determine the IC_{50} value of TQ and the control drug cisplatin following 48 hours drug exposure to the tested cell lines (CasKi and C33A). IC_{50} values were determined as 24μM and 15μM for TQ and 13μM and 18.5μM for cisplatin. These concentrations were used in subsequent experiments.

Immunocytochemical staining and western blotting analyses were carried out to determine the drug effect on the expression of HPV oncoproteins following the treatment. Results showed that both free and liposomal TQ decreased E6 and E7 expression levels which were similar to the results obtained when CasKi cells were treated by cisplatin. However, the expression of tumour suppressor protein p53 for CasKi cells was higher following liposomal TQ treatment than free drugs. For C33A cells, increasing levels of pRb but not p53 were observed from all three drug treated groups which might indicate a different mechanism involved in the drug action for non-HPV associated cervical cancers.

Current results have shown some promising anti-HPV effect from liposomal delivered TQ on cervical cancer cells. Further investigations are warranted to confirm the findings.

Halloysite nanotubes-mediated delivery of therapeutics in cancer therapy

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 113

Ms. Katarzyna Fidecka [1], ***Dr. Jessica Giacoboni*** [1], ***Dr. Riccardo Vago*** [2], ***Dr. Frederic Jamme*** [3], ***Prof. Emanuela Licandro*** [1]

1. *University of Milan,* ***2.*** *San Raffaele Hospital,* ***3.*** *SOLEIL Synchrotron*

I. Introduction

Traditional drug delivery presents limitations such as inability of therapeutics to reach target sites and pass cell membrane, short drugs lifetime, undesirable side effects as well as the existence of a dose threshold, which drive to develop new strategies to treat patients, overcoming the above mentioned drawbacks.

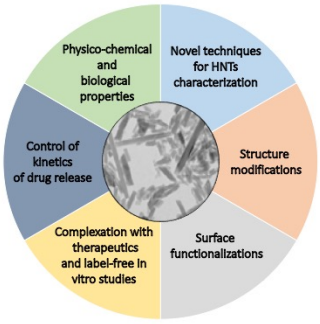
Application of nanotechnology is one of the approaches currently investigated that could bring a new insight in this field of study. On account of a wide diversity of available nanoparticles and their properties, nanomedicine offers various possibilities of engineered nanocarrier for delivering therapeutics to target organs. Herein, we present the possibility to apply the unique natural nanoparticle, namely halloysite nanotube (HNT) and the examination of its potential in the field of nanomedicine. This up to date quite unknown, nevertheless triggering attention, nanoparticle has been noticed by the scientific community as a promising high-performance nanomaterial with peculiar physico-chemical and biological properties, such as tubular hollow morphology, large aspect ratio, surface multifunctionality, ability to release incorporated molecules in a sustain way as well as biocompatibility. Moreover, halloysite is a low-cost clay mineral, available in huge quantities. Advantages of HNT make it stand out as a potential candidate for drugs immobilization by various methodologies and consequent tumor-specific drugs release. Therefore, we aim to present our research dedicated to the novel halloysite-based nanomaterials development for more efficient and less toxic anticancer therapeutic strategies.

II. Methods

Novel halloysite-based nanoconstructs were made and examined on their physico-chemical properties by standard techniques, such as FTIR, TGA, ζ – potential, XRD, nitrogen BET method and SEM/TEM microscopy. Biological in vitro characterization was also performed and consisted of toxicity assays and internalization studies using human cells. Furthermore, we have implemented novel characterization techniques for their investigation as well, such as Multiphoton microscopy.

III. Results and discussion

The primary focus of our research was to extend the knowledge in the newly developing halloysite field for the purpose of the nanoparticle application in biological and medical fields. We have discovered novel properties of halloysite nanoparticle and developed label-free characterization techniques for HNT-based nanoconstructs bioimaging. We have focused on the nanocylinder length size and inner lumen diameter modifications in order to overcome its limiting factors and create a highly competitive nanoparticle for the efficient intracellular drug delivery. We have generated nanoarchitectures with targeted affinity through specific incorporation of various therapeutics through halloysite outer surface functionalization or incorporation within its inner lumen. Finally, we wish to present strategies for drug loading improvement as well as control of kinetics of drug release through formation of the natural end-stoppers on the drug filled HNT.



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Pulmonary delivery and fate of nanomedicine in whole-murine lungs: novel insights into cellular localization and interaction

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 120

Mr. Lin Yang [1], ***Ms. Regine Gradl*** [2], ***Dr. Annette Feuchtinger*** [1], ***Dr. Kaye Susannah Morgan*** [2], ***Dr. Martin Dierolf*** [2], ***Mr. David Kutschke*** [1], ***Dr. Tobias Stoeger*** [1], ***Prof. Franz Pfeiffer*** [2], ***Prof. Daniel Razansky*** [1], ***Dr. Otmar Schmid*** [1]

1. *Helmholtz Zentrum muenchen,* ***2.*** *Technical University of Munich*

Pulmonary drug delivery is the primary route for the treatment of lung diseases. Drug nanocarriers or nanomedicines (NM) have exhibited great potential to improve diagnostic and therapeutic options by combining the merits of drug carriers (*e.g.*controlled release, enhanced tissue retention, reduced metabolic degradation, cell-specific targeting) with nanometer size properties (*e.g.* avoidance of phagocytic elimination)[1, 2]. The therapeutic effect of inhaled NM is dependent upon the delivered dose and its spatial distribution throughout the lung. Understanding the dynamic process of aerosolized drug delivery in the context of dose, distribution, biokinetics, and bioactivity of NM with cellular resolution is of central importance for therapeutic efficacy.

A novel preclinical imaging platform for whole, non-dissected lungs of mice was introduced combining two imaging modalities, *in vivo* phase-contrast X-ray imaging (PCXI) and *ex vivo* light sheet fluorescence microscopy (LSFM) on optically cleared lung tissue.

PCXI allowed for high speed (1 Hz) *in vivo* imaging of the controlled delivery of bulk NM-liquid (melamine resin fluorescent nanoparticles (NP) with iodine as surrogate for NM) via intratracheal instillation to the murine lung (*e.g.* delivering to the left (Figure A), right, or whole lung). The process of aerosol inhalation of NM is more difficult to visualize *in vivo* with PCXI due to low delivery rates and extremely uniform aerosol distribution resulting on low signal-to-noise ratio. However, certain features of the aerosol inhalation process could be observed. After *in vivo* PCXI imaging tissue optical clearance and LSFM were used for *ex vivo* complementary 3D mapping of NM spatial distribution in the entire lung. This proved that the left, right, or whole lung NM delivery was controllable with *in vivo* PCXI (Figure B, C, and D). Moreover, inhalation exhibited a more homogeneous NM distribution in conducting airways and acini as indicated by a central-to-peripheral (C/P) NM deposition ratio of unity (0.98 ± 0.13) versus a 2-fold enhanced central deposition (C/P = 1.98 ± 0.37) for instillation. Evaluation of the temporal changes in the pulmonary NP distribution has unveiled biokinetic processes on different scales ranging from lung generation/lobe to cellular level. Main outcomes include redistribution of proximally deposited particles to the periphery of the lungs most likely due to macrophage uptake and subsequent migration into the more distal regions of the lung, which becomes particularly evident 14 days after NP application (Figure E and F). LSFM imaging of whole-lobe immunostained tissue-cleared lung enable determination of the interplay between NP and vasculature system, lymphatics (Figure G), *etc.*, which is of central importance for understanding NM therapeutic efficacy and fate in vivo.

In summary, complementary utilization of PCXI and LSFM provides unprecedented insights into both pulmonary NM delivery in real time and 3D distribution, biokinetics, and cellular targeting of NM at single-cell resolution in intact mouse lungs, paving the way for the development of novel-designed NM with enhanced targeted delivery efficacy.

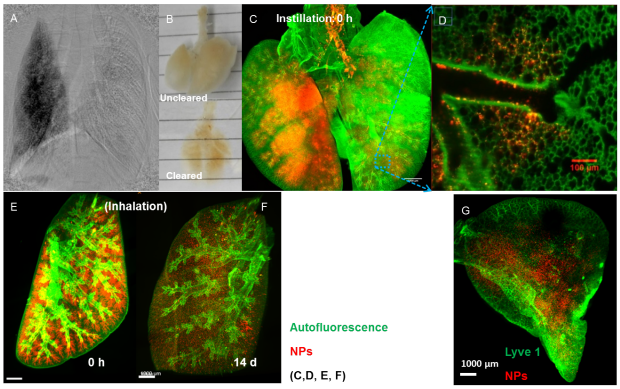


Figure: Combination of *in vivo* phase contrast X-ray imaging (PCXI) and *ex vivo* tissue-cleared light sheet fluorescence microscopy (LSFM) shows the controlled delivery and biokinetics of nanoparticles in whole-murine lungs. Targeted left half of lung delivery imaged by PCXI (B) and further confirmed by LSFM images on all scales ranging from centimeter (whole lung, C) to sub-micrometer (subcellular, D) resolution. Co-mapping the nanoparticle (re-)distribution with lung morphology at 0 h (E) and 14 d (F) after ventilator-assisted aerosol inhalation displays significant changes in term of acinar distribution uniformity. Nanoparticle distribution with respect to lyve-1 positive lymphatics/vessels in whole organ after whole-mount staining (G).

Figure 1 yang.png

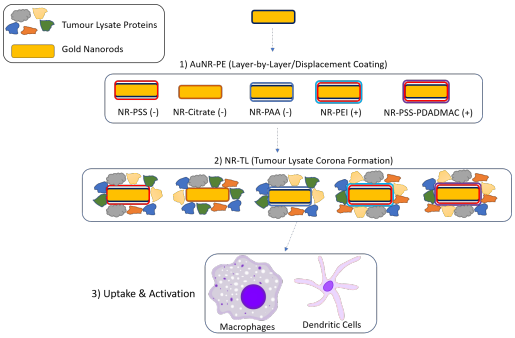
Variations in Surface Chemistry Influences Immune Response of Tumour Lysate-coated Gold Nanorods

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 155

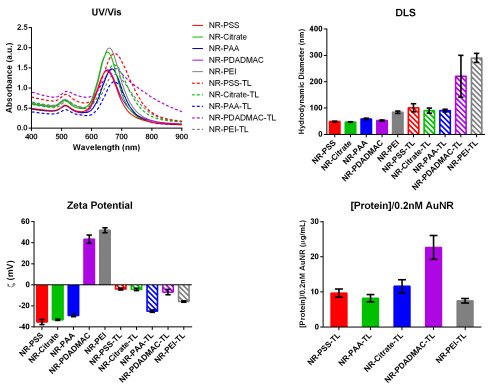
Mr. Kang Wei Cherng Malvin [1], ***Prof. Liu Haiyan*** [2], ***Dr. Kah Chen Yong James*** [2]

1. *NUS Graduate School for Integrative Sciences and Engineering,* ***2.*** *National University of Singapore*

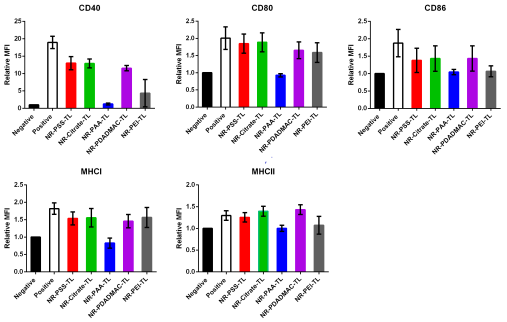
The ability for surface modification of gold nanoparticles provides a promising and versatile platform for biomedical applications. Given the ability of nanoparticles to enhance cellular uptake of drugs and biomolecules, it serves as an interesting platform for the development of vaccines, particularly cancer vaccines. Tumour lysate serves as an attractive vaccination platform given the plethora of antigens present. However, current clinical application for tumour lysate in cancer vaccination is limited to *ex vivo* dendritic cell (DC) pulsing due to rapid renal clearance upon direct vaccination, which often leads to sub-optimal clinical responses. Also, to date there has been no consensus on how variations in surface chemistry influences the immune response of vaccines. This project seeks to exploit the concept of protein corona to coat tumour lysate (TL) proteins on a variety of surface-modified gold nanorods (AuNRs) and perform *in vitro* studies using J774A.1 macrophage cell line to test for vaccine efficacy. Experimental results shows that NR-TL can generate immune activation of J774A.1 cells independent of surface charge of the original AuNR prior to coating. It was interesting to note however that hydrophobic NR-PAA when coated with TL does not generate any immune response, which contradicts existing literature on the direct correlation between hydrophobicity and immune response. When tested with controls such as mouse serum-coated NRs (NR-MS) and free TL, we observe superiority in immune response by both NR-PSS-TL and NR-Cit-TL, highlighting the efficacy of the vaccine construct due to increase antigen uptake and adjuvant effects. Future works include studies with primary immune cells such as bone marrow-derived dendritic cells to validate the efficacy of the NR-TL construct.



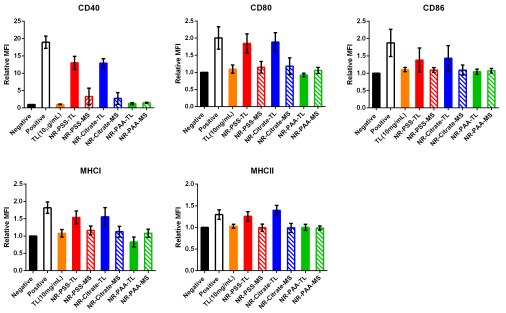
Nr-pe-tl schematic.png



Nr-tl figure 1.png



Nr-pe-tl varying surface j774a.1.png



Nr-tl vaccine with controls j774a.1.png

Evaluation of the Stability and Drug Release Behavior of mPEG5k-b-p(HPMA-Bz) Polymeric Micelles Loaded with Paclitaxel

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 172

Ms. Maryam Sheybanifard [1], ***Dr. Nataliia Beztsinna*** [2], ***Ms. Mahsa Bagheri*** [2], ***Prof. Gert Storm*** [2], ***Dr. Josbert Metselaar*** [1]

1. *University Hospital RWTH Aachen,* **2.** *Utrecht University*

The poor water solubility of paclitaxel (PTX) contributes to its suboptimal in vivo behavior and efficacy. To improve the delivery of this drug, polymeric micelles of mPEG-*b*-p(HPMA-Bz) appeared promising. Nevertheless, variations in this polymer's hydrophobicity might alter its capacity to hold, carry and deliver the drug. In the current study, we aimed to systematically evaluate the micellar formulations of PTX by changing the ratio of the drug to polymer as well as the chain length of the hydrophobic block of the polymer. To reach our aim, different hydrophobic variants of $mPEG_{5k}$-*b*-p(HPMA-Bz) were made and loaded with various quantities of PTX and assessed regarding micelle stability and drug retention. Four variants of $mPEG_{5k}$-*b*-p(HPMA-Bz) polymer were used with an identical hydrophilic part and different hydrophobic units, namely $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{18.5k}$, $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{9.6k}$, $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{4.7k}$, and $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{2.2k}$ (from the most hydrophobic to the least hydrophobic variants of the polymer respectively). Micelles were formulated using ethanol as a biocompatible and industrially feasible solvent. Our results suggest that among 5%, 10%, and 15% w/w of PTX loading, samples with 15% w/w PTX are the most unstable and that 10% and 5% w/w PTX loading is most desirable. Meanwhile, investigating the different variants of the polymer revealed that micelles of polymers $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{18.5k}$ and $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{9.6k}$ have a similar hydrodynamic diameter of around 52 nm while the polymer with the longest hydrophobic blocks showed slightly better PTX retention and release. At the same time, micelles of polymers with shorter hydrophobic blocks (i.e. $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{4.7k}$ and $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{2.2k}$) both demonstrated a smaller hydrodynamic diameter of around 39 nm and resulted in a more premature PTX leakage and release compared to the micelles of polymers with longer hydrophobic blocks. Furthermore, $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{4.7k}$ presented a slightly better PTX retention and release compared to $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{2.2k}$. In conclusion, we argue that the better stability of $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{18.5k}$ micelles and the smaller size of $mPEG_{5k}$-*b*-$p(HPMA\text{-}Bz)_{4.7k}$ micelles are both desirable features. However, each polymeric micelle fails to deliver both features simultaneously. Therefore, in vivo studies should be performed to help find the optimally performing lead candidate.

Development of Bioanalytical Assays based on Protein-stabilized Catalytic Bimetallic Nanoclusters

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 182

Dr. Laura Saa [1], ***Mrs. Verónica Mora Sanz*** [2], ***Dr. Nerea Briz*** [3], ***Dr. Valery Pavlov*** [1]

1. *CIC Biomagune,* ***2.*** *CIC biomaGUNE/ Tecnalia,* ***3.*** *Tecnalia*

Protein-stabilized metallic nanoclusters (NCs) have attracted enormous interest due to their unique electronic structures, physical and chemical properties, and their potential applications in imaging and optical biosensing (1, 2). Most of the reported protocols for the synthesis of NCs using proteins include very harsh conditions that lead to a loss in the structure and the complete denaturation of the protein. On the other hand, metallic nanoparticles (NPs) have been widely employed in bioanalysis. These NPs should be pre-synthesized and purified from the components of reaction mixtures, and afterwards, tethered to biorecognition elements. In addition, the reproducibility of the assays depends significantly on the quality of NP preparation.

In this work, we report the synthesis of bimetallic Au/Pt NCs using proteins as scaffolds under non-denaturing conditions. The resulting protein-Au/Pt NCs exhibit peroxidase-like catalytic activity and more interestingly, the proteins used for the synthesis are not denatured during the process, maintaining their native structure and functionality. These protein-stabilized Au/Pt NCs have been applied for the development of sensitive bioanalytical assays.

First, we used streptavidin, widely used in biotechnology due to their highly selective and stable interaction with biotin (3), to produce streptavidin-Au/Pt NCs that can bind biotin with high affinity. This interaction can be monitored by measuring the peroxidase activity of the streptavidin-Au/Pt NCs using the chromogenic substrate TMB which is oxidized in presence of H_2O_2. The streptavidin-Au/Pt NCs have been successfully applied for signal amplification in immunoassays employing biotinylated antibodies for the detection of anti-BSA IgG (Figure 1).

In a second approach, we employed fibrinogen to produce fibrinogen stabilized -Au/Pt bimetallic NCs with peroxidase-like activity. These molecules have been applied for colorimetric detection of thrombin activity. The assay is based on the activation of fibrinogen immobilized on the surface of a microplate and fibrinogen-Au/Pt NCs in solution in the presence of thrombin. The activated molecules can bind each other forming a catalytic fibrin fiber that remains bound on the surface. Since the generation of fibrin is correlated with the activity of thrombin, an increase in thrombin concentration is related with an increase in the readout peroxidase signal due to the polymerization of fibrin derived from fibrinogen-Au/Pt NCs. This strategy allows the development of a sensitive and simple bioassay for quantification of thrombin in the picomolar range (Figure 2).

In conclusion, we propose the synthesis of bimetallic nanoclusters by their incorporation inside of proteins, that requires mild physiological conditions that do not denature either streptavidin or fibrinogen during incorporation of the resulting atomic clusters. This strategy may address the drawbacks related with the tethering of NPs to biopolymers and result in development of new efficient strategies for biosensing.

References.

1. Chem. Soc. Rev., 2012, 41, 3594.

2. J. Nanophoton., 2012, 6, 064504.

3. Appl. Microbiol. Biotechnol., 2013, 97, 9343.

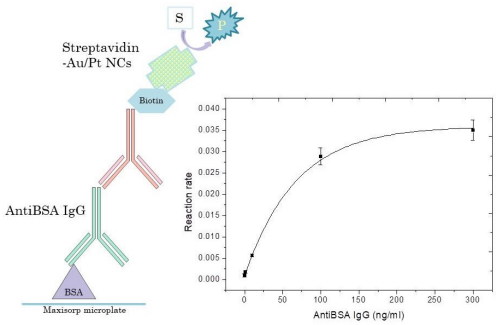


Fig1 bioanalytical assay for the detection of antibsa igg using streptavidin aupt ncs.jpg

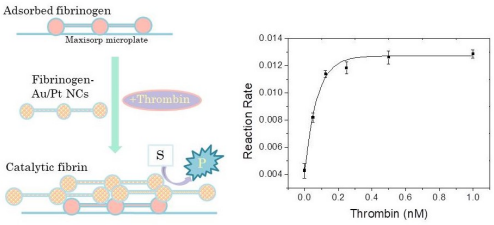


Fig 2 bioanalytical assay for the detection of thrombin using fibrinogen aupt ncs.jpg

The Uptake of Poly Lactic-co-glycolic acid (PLGA) Particles by Alveolar macrophages and epithelial cells depends on the Exposure scenario

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 192

Ms. Mehwish Ishaque [1], ***Dr. Carola Voss*** [1], ***Dr. Otmar Schmid*** [1], ***Dr. Markus Rehberg*** [1], ***Dr. Tobias Stoeger*** [1]

1. *Comprehensive Pneumology Center, Helmholtz Zentrum München, Germany; Member of the German Centre for Lung Research*

Due to its large surface area, the alveolar epithelium represents a major target for drug interaction and absorption. In this context nanoparticle-mediated drug delivery is an emerging therapeutic technique to deferent respiratory diseases, and predictive in vitro models are accordingly needed.

The aim of this study was to investigate the cell specific uptake of different sized fluorescent labelled poly lactic-co-glycolic acid (PLGA) particles as carrier surrogates in different models. To this end we exposed cocultures of alveolar epithelial and alveolar macrophage cell lines, either at conventional submerged or at the air liquid interface (ALI) setup, to different sized PLGA particles, and compared the uptake efficacy to that observed in mice. Submerged exposures are experimentally simpler and more convenient to perform, but ALI exposures are even if costlier, physiologically more realistic and hence potentially biologically more meaningful. Additionally, while the cell delivered nanocarrier dose is usually rather obscure for submerged conditions, then on the cell surface deposited dose can be determined using the ALICE Cloud setup featuring a quartz crystal microbalance. 0.1µm, 0.5µm and 1µm PLGA particles were studied. For *in-vitro* experiments, the murine cell lines LA-4 (epithelial, type II-like mouse lung adenoma cells) and MH-S (immortalized mouse alveolar macrophages) as monocultures, were exposed to PLGA particles for 24 hours in submerged and ALI conditions. For *in-vivo* experiments, C57BL/6J mice were exposed to PLGA particles (0.1µm & 1µm) by intratracheal instillation and analysed after 24 hours. The uptake was monitored and quantified by confocal microscopy and flow cytometry.

Under submerged conditions, flow cytometry revealed for epithelial cells the highest uptake for the largest particles, whereas macrophages showed more effective uptake of 0.5µm followed by 1.0µm particles. Uptake of 0.1µm particles was barely detectable at all. Under ALI conditions, significant particle uptake was only detected in macrophage and independent of particle size. Flow cytometry of particle exposed mouse lungs showed a significant uptake only for 0.1µm particles by alveolar macrophages, compared to little uptake by epithelial, type II cells, independent of size.

Finally, our current results support the idea that the more elaborate ALI exposure setup represents a more realistic model when studying particle uptake at the air tissue interface. Currently performed histological investigations shall confirm the flow cytometry based findings.

Bio-nanocarrier system : Self assembling peptide hydrogel for dual delivery of Virus Like Particles and drug to treat Brain cancer

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 221

Ms. Mrunal Wanjale [1], ***Dr. Amy Barker*** [2], ***Prof. Peter Stockley*** [2], ***Dr. GS Vinod Kumar*** [1]

1. *Rajiv Gandhi Centre for Biotechnology and University of Kerala,* ***2.*** *University of Leeds*

Among the central nervous system neoplasms, gliomas are the most common anaplastic tumors that are observed in children, adults as well as elderly people. Even after intensive therapy many of Glioma patients suffer from aggressive recurrence of these tumors. To address this problem, we have designed a biodegradable peptide hydrogel implant systems for sustained release of therapeutic agents . This system can bypass the BBB and make the drug available in appropriate amount to decrease the non specific cytotoxicity and systemic myelodysplasia . We have designed a 24 amino acid peptide which is an amalgamation of self assembling unit (KFEFKFEF) and Matrix metalloproteinase sensitive motif : VPLSLYSG selectively cleavable by MMP-7, MMP2 and MMP9 which are reported to be over expressed in glioma. Via this self assembling hydrogel we propose to deliver Temozlomide, an oral prodrug that acts by methylating DNA. But glioma cells develop resistance to this drug by over expressing an enzyme MGMT (O6-methylguanine methyl transferases). To tackle this issue in treatment of glioma chemotherapy we propose use of Virus Like Particles(VLP) the bio nano carriers of RNA(in this system-siRNA), to silence the gene expression of MGMT and sensitise glioma cells to Temozolomide.

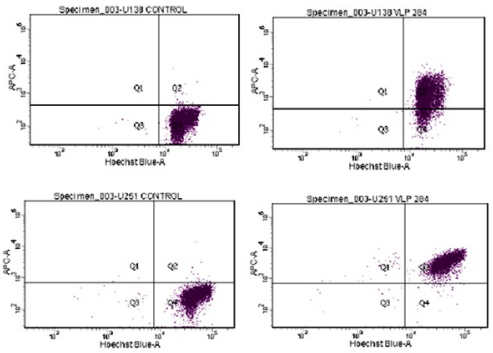
Methods:

Peptide KFEFKEEF-VPLSLYSG-KFEFKEEF was *in-silico* modelled to check aggregation using the program GROMACSv5.1.4. It was docked with the known structure of MMP7 enzyme using PATCHDOCK 3.1 software. Modelled peptide was synthesised by solid phase peptide synthesis and mass confirmed by MALDI-Toff analysis. A complex of siRNA targeting the MGMT gene and TR sequence required for VLP assembly with fluorescent tag was designed. VLP derived from the MS2 bacteriophage coat protein were assembled using this siRNA as genomic material. VLP were purified by sucrose density gradient (15-45%) ultracentrifugation. 1% agarose gel run was done along with a wild type MS2 bacteriophage to ensure similar assembly.

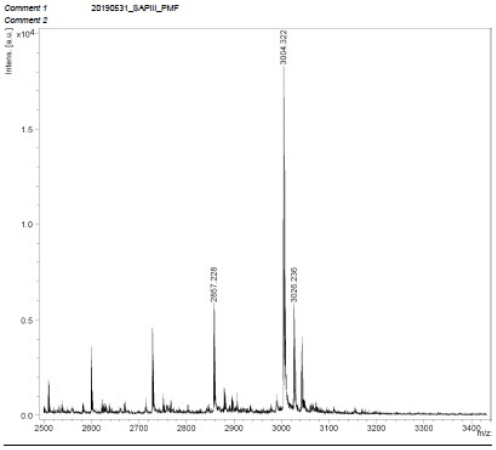
Results and discussion:

MD simulation for 20ns gave a steady conformations of peptide. It was observed to be stabilized by pi-pi-stacking, H bonds and Hydrophobic interactions. The Root Mean Square Fluctuation graph analysis shows that loop (VPLSLY) region is packed well inside the inhibitor binding pocket of MATRILYSIN. Peptide synthesis yielded correct mass of peptide upon cleavage and MALDI analysis. Transmission electron microscopy confirmed size ~28 nm and structure of the reassembled VLP which was similar to the wild type MS2. The nanocarrier VLP were tested for cell internalisation in U251 and U138 cells and showed positive uptake with 2 hours incubation. MTT assay has shown increase in cytotoxicity when Temozolomide was supplemented with VLP. Thus glioma cells are sensitized to Temozolomide treatment effectively

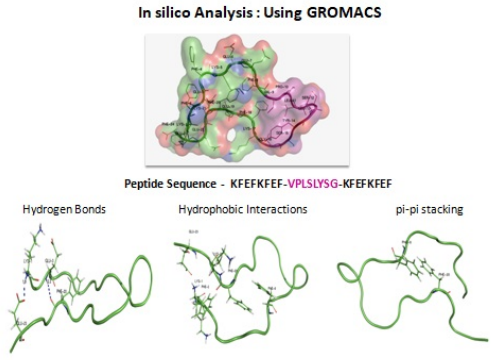
We are currently evaluating and quantitating the silencing potential of these VLP based siRNA nanocarriers and targeting of the VLP to glioma cells.The interaction of coat protein to the TR region of the RNA is very specific, ensuring specific and higher entrapment efficiency. These nanocarriers have better prospects on account of biocompatibility, cost of production. Together, this system can deliver drug and tackle resistance problem in tumor environment sparing systemic circulation of chemotherapy drugs.



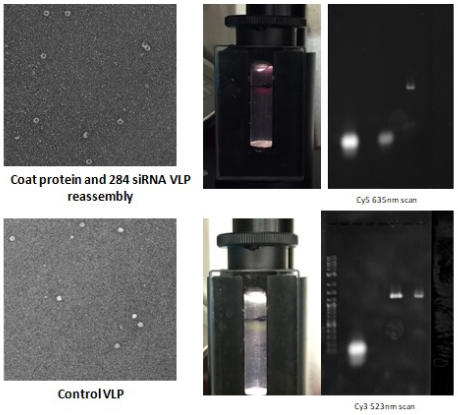
Cell uptake vlp by facs.jpg



Peptide maldi.jpg



Sap in silico.jpg



Vlp assembly.jpg

Synthesis of multivalent glyconanogels and evaluation of their interaction with lectins

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 244

Dr. Ana Sousa [1], Mrs. Noelia de la Cruz [1], Dr. Javier Rojo [1]

1. *Spanish National Research Council*

Introduction

Carbohydrate-protein interactions mediate many biological processes including tumour progression, inflammation, and viral infection. These interactions are typically characterized by a high selectivity and a low affinity, which is compensated in Nature by multivalency.

In this context, a variety of multivalent nanosized materials (dendrimers, polymers, nanoparticles, etc.) modified with several copies of carbohydrates have been designed to competitively interfere with those recognition processes. In particular, our research group is interested in the interaction of carbohydrates with C-type lectins, because these lectins play a key role in the initial stages of many viral infections, including human immunodeficiency virus (HIV) and Ebola virus (EBOV), thus becoming an interesting therapeutic target.

Methods

With the aim of obtaining a multivalent carbohydrate presentation, we have developed a simple and straightforward approach to prepare polymeric glyconanogels designed to interact with cellular receptors.

Mannosylated nanogels were synthesized from biocompatible and FDA-approved Poly(ethylene glycol) (PEG) by free radical polymerization in miniemulsion. This strategy allowed the preparation of NGs modified with simple monosacharides and with more complex structures such as glycodendrons of mannose (Figure 1).

After purification and characterization by different techniques, the ability of these nanogels to interact with the model lectin Concanavalin A (Con A) has been studied in solution.

Results and Discussion

In a first step, different PEGylated building blocks containing simple mannose or mannosylated dendrimers were synthesized. To this aim, an acrylate group was introduced on one end of the PEG chain, while the other was functionalized with terminal azides that allow the straightforward introduction of carbohydrates or glycodendrons by Click Chemistry. Afterwards, a series of mannosylated NGs were synthesized by free radical polymerization in miniemulsion. The NGs were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Interaction studies of the different NGs with the model lectin ConA by UV and DLS showed that the NGs could effectively interact with the lectin forming aggregates, and that the aggregation ability increased with the multivalency of the mannosylated ligand.

Importantly, control nanogels (displaying galactose groups on their surface) showed no activity at all.

In conclusion, biocompatible, PEG-based, glyconanogels modified with several mannose copies have been prepared and their multivalent ability to interact with a model lectin has been demonstrated.

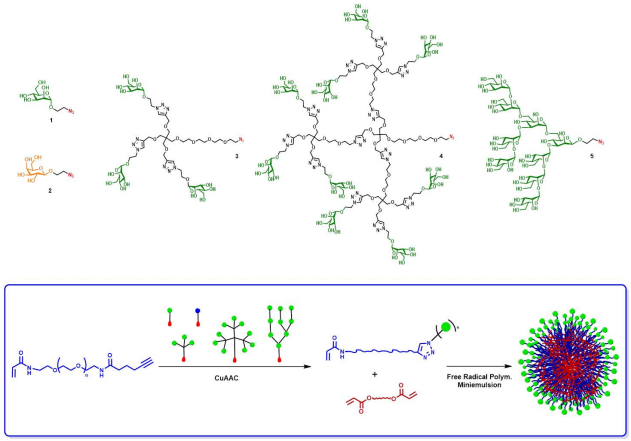


Figure 1. glyconanogels.jpg

Carbon based 0D and 2D nanomaterials for biomedical application

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 258

Dr. Katerina Polakova [1], ***Mr. Tomáš Malina*** [1], ***Mr. Jan Belza*** [1], ***Dr. Sergii Kalytchuk*** [1], ***Dr. Aristides Bakandritsos*** [1], ***Prof. Radek Zboril*** [1]

1. *Palacký University in Olomouc*

Carbon dots (0D) and graphene (2D) have attracted significant attention in last decades due to their unique mechanical, optical, electronic, thermal and chemical properties. The properties stated above implicate broad range of applications in physics, chemistry, biology and medicine.

Here, the new application of carbon dots as nanothermometers of cells based on photoluminiscence lifetime thermal sensing together with optical imaging of carbon dots labeled stem cell migration will be presented as a first.

Concerning 2D graphene, it is well known that limited reactivity of graphene has so far hampered many applications due to its low density of functionalization with limited number of carboxyl groups. We employed the pronounced and controllable reactivity of fluorographene *where* a densely functionalized and hydrophilic nitrile-functionalized graphene derivative was obtained (cyanographene, GCN). Promising biomedical application of this new graphene derivate will be introduced as well.

Nanomedicine Based in Digital Molecular Communications for Delivery of Ions Through the Beta Cell for Artificial Segregation of Insulin Granules

Wednesday, 16th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 251

Dr. Huber Nieto-Chaupis [1]

1. *Universidad Privada del Norte*

The so-called Nanomedicine **[1]** will use all compatible nano technologies encompassing biological systems in order to restore the homeostasis or delay the apparition of diseases such as type-2 diabetes for example **[2]**. In this paper we focus in the theory of Digital Molecular Communications by using the path integral theory that allows us to make predicions about the performance of nano devices working as cargo of ions. Because the prospective nano devices can among varios roles, work as antena **[3]**, fact that allows us to regulate the releasing of ions that might be required to restore funcionalities of cells and tissues. Once the theory is developed, we pass to estimate the different paths and their respective probabilities by which ions are targeted to arrive inside the beta cell. Thus, advanced nano devices would be part of an advanced network such as the Internet of Bio-Nano Things. The proposed theory that emphasizes the transportation from a nano-particles generator is resummed as follows (FIG-1):

P<A|D> =| <A|B> +<B|C> +<C | D >|^2

Where P<A|D>= Probability to accomplish the path between the nano particles generator (A) and (D) inside the beta cell.

<A|B>=Probability between the surface of the nano device.

<B|C>=Probability between the Surface of nano device and the voltaje-dependent gate located on the beta cell.

<C | D >=Probability between the voltaje-dependent gate and the insulin granules inside the beta cell.

For each path its correspondent mathematical structure for example <A|B> is given by:

$$<A|B> = \int \Phi(x\_A)\, Exp[\, -i\,(P+qA)^2/2M + eU\,]\, \Phi(x\_A)$$

where the electromagnetic wave denoted by**A** depends on the frequency that modules the digital information that the nano device should receive to release a certain amount of ions. We asume that an external bio-cyber interface is the responsable of the emission of the waves so that it is connected to the nano device pemanently.Concretely, this paper focuses on the scenario of artificial releasing of granules of insulin in beta cells (FIG-2) to avoid hyperglycemia in those type-2 diabetes patients. In order to have an efficient emission-reception system and the releasing of insulin granules is successful, we estimate the full efficiency: $\mathbf{E} = (N\_CD)/(N\_AB + N\_BC)$ with

N_CD=ions that have arrived inside the beta cell

N_AB=released ions fom the nano particle generator inside the nano device

N_BC=ions travelling in the spatial área between the cell and nano device.

Through Monte Carlo simulations based on the proposed theoretical model we have estimate that the average efficiency for a suitable improving on the glucose values of a type-2 diabetes patients would be of order of 79% with an systematic error of order of 9%.

[1] Anders H, The utility of DNA nanostructures for drug delivery *in vivo*Expert Opinion on Drug Delivery, Volume 14, 2017 - Issue 2.

[2] Nieto-Chaupis, Huber, Cheating the Beta Cells to Delay the Beginning of Type-2 Diabetes Through Artificial Segregation of Insulin, BICT 2019, Pittsburgh, PA, USA, March 13–14, 2019.

[3] Nieto-Chaupis, Huber, Modified Friis Equation for Biological Internet, 2019 IEEE iWAT Miami.

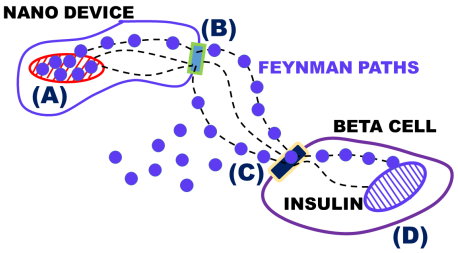


Fig-1.png

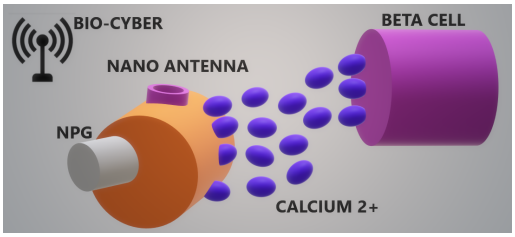


Fig-2.png

Natural Killer Cell Derived Biomimetic Nanoconstructs for Tumor Targeted Bioimaging

Wednesday, 16th October - 14:15: Nano-Imaging for diagnosis, therapy and delivery in preclinical and clinical fields (Amphitheatre N02.040) - Oral - Abstract ID: 52

Dr. Arunkumar Pitchaimani [1], ***Dr. Tuyen Nguyen*** [2], ***Mr. Ramesh Marasini*** [2], ***Ms. Achini Eliyapura*** [2], ***Ms. Tahmineh Azizi*** [2], ***Prof. Majid Jaberi-Douraki*** [2], ***Prof. Paolo Decuzzi*** [1], ***Prof. Santosh Aryal*** [2]

1. *Nanotechnology for Precision Medicine, Italian Institute of Technology, Genova, Italy.,* ***2.*** *Nanotechnology Innovation Center of Kansas State (NICKS), Kansas State University, USA*

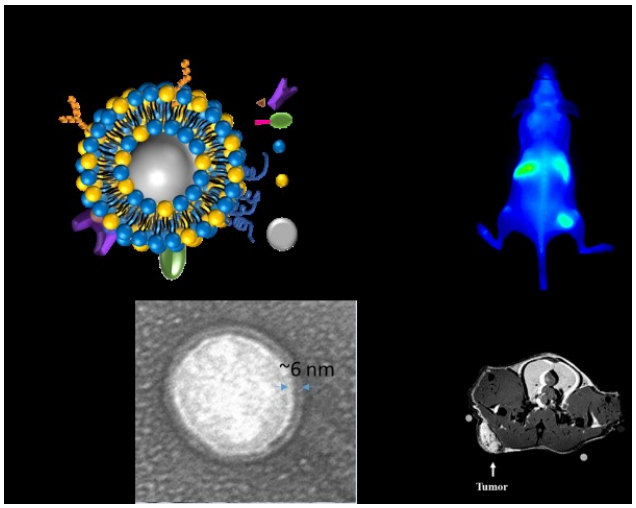
Introduction: Targeted magnetic resonance imaging (MRI) of deep solid tumors using clinical contrast agents is highly challenging due to its short-half life and rapid systemic clearance from the blood. The recent advent of Biomimetic Nanoconstructs (BNc) system shows tremendous progress in the field of nanomedicine. The advantages of incorporating biological materials with synthetic materials include biocompatibility, resistivity, cellular interaction, enhanced circulation half-life and cellular retention. Among various blood pool agents, Natural killer (NK) cells are a predominant member of the innate immune system. The major function of the NK cell includes host defense against microbial infections and tumor cells by immunosurveillance of cell surface abnormalities in major histocompatibility class I markers and cell stress markers. In the present study, biomimetic nanoconstructs was designed using tumor homing Natural Killer cell membrane, and MR imaging responsive Gd-lipid camouflaged onto the surface of polymeric nanoparticles for targeted bioimaging.

Methods: Natural Killer cell membrane was isolated using sucrose gradient ultracentrifugation method from NK-92 cells. Further, Biomimetic Nanoconstructs with Magnetic Resonance Imaging (MRI) contrast agent (DSPE-DOTA-Gd) and NIR fluorescent (DiR) molecules were fabricated using simple nanoprecipitation method. The magnetic properties of the BNc with different Gd-lipid was tested in vitro and investigated its ex-vivo tumor imaging under MR imaging, along with its pharmacokinetics and bio-distribution pattern in human breast cancer cell line MCF-7 induced solid tumor model in Nu/Nu mice. For in vivo NIR bio-imaging studies, DiR labeled BNc (10mg/kg) were injected intravenously into the MCF-7 tumor bearing mice along with bare control PLGA nanoparticles and analyzed using Pearl® Trilogy Small animal imaging system (LI-COR®).

Results: The prepared BNc are nanometer in size and shows successful retention of NK cell membrane proteins. The maximum magnetic relaxivity of BNc was found to be $7.2 mM^{-1}S^{-1}$ and was tunable by adjusting the concentration of Gd-lipid. The cellular uptake efficiency of BNc in MCF-7 cells was found to be significantly higher than the bare PLGA NPs. Confocal analysis demonstrated the uniform intracellular distribution of BNc in MCF-7 cells and found be highly biocompatible. BNc also exhibits longer circulation half-life (~9.5h) and higher biodistribution in tumor tissues (~10% injected dose) suggesting its tumor targeting potential. The ex-vivo MR images of tumor mice show clear T1-contrast in tumors tissues under 14.1 T.

Conclusion: Overall, the results demonstrated the bio imaging ability of natural Killer cell membrane camouflaged BNc for targeted Magnetic Resonance and NIR Fluorescent bioimaging of tumor. Owing to the proven immunosurveillance potential of NK□cell in the field of immunotherapy, the BNc engineered herein would hold promises in the design consideration of targeted nanomedicine.

b



Biomimetic nanoconstructs-bioimaging.jpg

The impact of multidrug resistance on tumor microenvironment remodeling and nanodrug distribution in murine breast cancer models

Wednesday, 16th October - 14:30: Nano-Imaging for diagnosis, therapy and delivery in preclinical and clinical fields (Amphitheatre N02.040) - Oral - Abstract ID: 94

Mr. Okan Tezcan [1], ***Ms. Elena Rama*** [1], ***Prof. Fabian Kiessling*** [1], ***Prof. Twan Lammers*** [2]

1. *RWTH Aachen University Clinic,* ***2.*** *ExMI – Experimental Molecular Imaging*

Introduction: Multidrug resistance (MDR) is a major limiting event in chemotherapy. Development of MDR results from changes in signaling networks and several cellular mechanisms, such as altered gene expression profiles. The cellular MDR events were further evaluated in the direction of tumor microenvironment (TME) remodeling aspects driven by the differences in regulatory mechanisms. To examine this, we developed multidrug sensitive and resistant breast tumor-bearing mouse models. To evaluate differences in TME, some of the main components of the extracellular matrix (ECM) were monitored. Additionally, differences in angiogenic vessel density and the vessel functionality as well as the vessel maturity were analysed. Lastly, to evaluate nanodrug delivery, liposome distribution was investigated in both tumor models.

Methods: Multidrug resistant 4T1 cells (4T1DOXR) were developed by stepwise selection using doxorubicin dose increments. Two groups of mice (n = 5 per group): one group was orthotopically injected with 4T1DOXR cells and the other one with sensitive 4T1 cells (4T1S). After two weeks, both groups of mice were rhodamine lectin injected and tumors were taken. Thereafter, *ex vivo* analyses, for ECM materials and vessels, were performed by immunofluorescence staining and fluorescence microscopy. As a final step, the intravenously injected liposomes were monitored by fluorescence microscopy and further penetration/distribution analysis was applied to both sensitive and resistant tumor models.

Results: Histological analysis revealed that P glycoprotein (Pgp) was overexpressed in MDR tumor (Fig.1A). Additionally, the over-deposition of ECM components, such as collagen type-I (COL-I) (Fig.1B), hyaluronic acid (HA) (Fig.1C), fibronectin (FN) (Fig.1D) and the basal lamina component, collagen type-IV (COL-IV) (Fig.1E) was also significantly higher in the microenvironment of the MDR tumor. Moreover, the angiogenic vessel density was higher in MDR tumor (Fig.1F). As for functionality and maturity of the vessels, no significant difference was noted. Nanodrug penetration analysis showed a poor liposome distribution in MDR tumor (Fig.2).

Conclusion: Our findings show an increased ECM deposition in multidrug resistant 4T1 tumors. We also supply evidence that the amount of angiogenic vessels in MDR tumor is higher. No significant difference, however, was observed in functionality and maturity of the angiogenic vessels in sensitive vs. resistant 4T1 breast tumors. The over-deposition of ECM materials as well as more COL-IV, locating at the outside of microvessels, negatively affected the liposome distribution. Together, these results provide a basis for further investigations, especially for patients with a poor response to chemotherapy as well as nanodrug delivery, such as liposome.

Acknowledgements: This work is supported by DAAD (57048249).

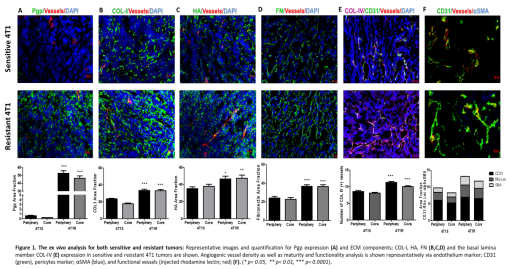


Figure. 1 okan tezcan.png

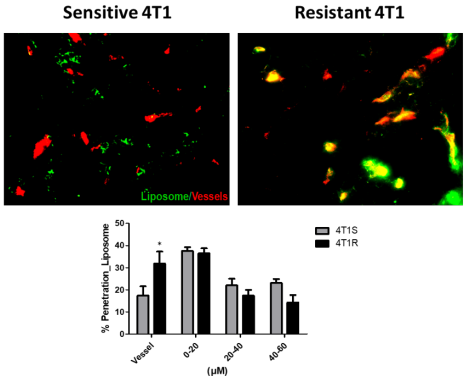


Figure 2. Liposome intratumoral distribution analysis: Representative images of liposome distribution in both sensitive and resistant 4T1 tumors and quantification. (** p< 0.05*).

Figure. 2 okan tezcan.png

Lipid-conjugated compounds dissociate from drug delivery liposomes in biological environments

Wednesday, 16th October - 14:45: Nano-Imaging for diagnosis, therapy and delivery in preclinical and clinical fields (Amphitheatre N02.040) - Oral - Abstract ID: 143

Mr. Rasmus Münter [1], ***Dr. Kasper Kristensen*** [1], ***Mr. Dennis Pedersbæk*** [1], ***Dr. Jannik Bruun Larsen*** [1], ***Dr. Jens Bæk Simonsen*** [1], ***Prof. Thomas Lars Andresen*** [1]

1. *Technical University of Denmark (DTU)*

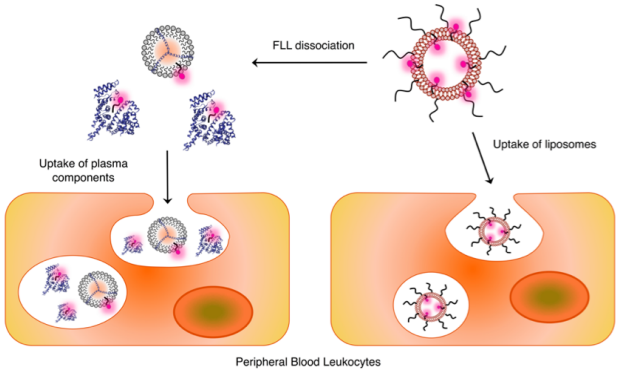
Liposomes are the most widespread used nanoparticles for medical applications such as drug delivery, diagnostics and imaging, and some liposomal formulations have even entered the clinic. Liposomes are often investigated for their ability to enter tissues and deliver drugs into subcellular compartments of the target cells. Studies on cellular uptake of liposomes are usually based on microscopy or flow cytometry, where researchers track the liposomes using fluorescently labeled lipids (FLLs) incorporated into the liposomal membrane. Such studies rely on the assumption that the FLL stays associated with the liposome for the entire duration of the experiment. However, this fundamental assumption remains largely unverified. We have applied size exclusion chromatography and a single liposome imaging assay to investigate the validity of the assumption.[1] We quantified the propensity of a range of commercially available FLLs to dissociate from PEGylated liposomes when incubated in human plasma. The FLLs displayed large variations in their dissociation propensities, varying from 10% to 75% over a 24 hour incubation period. The dissociation did not occur in buffer, demonstrating that incubation in a true biological environment is critical in order to mimic the liposomal stability in a drug delivery context.

The dissociation of the FLLs could have severe implications on studies where FLLs are used to track the cellular uptake of liposomes. To address this, we prepared liposomes with different FLLs with varying dissociation propensities, incubated them in whole human blood and investigated their uptake in peripheral blood leukocytes using flow cytometry. We observed significant differences in the apparent cell uptake, dependent on which FLL we used to track the liposomes. This uptake correlated with the dissociation propensities of the FLLs, which strongly implies that the dissociated FLLs are taken up by the cells independent on the liposomes (see figure). Consequently, the results and conclusions drawn from an uptake study can be dramatically influenced by the choice of FLL.

The dissociation of lipid-conjugated compounds from drug delivery liposomes is presumably not limited to FLLs, but could also be relevant for lipid-anchored drugs, targeting ligands, polymer coatings etc. It is thus crucial for researchers working with self-assembled nanoparticles such as liposomes, micelles and vesicles, to consider the dynamics of their systems in biological settings. We here present a simple assay that researchers can apply to study stability and dynamics of their lipid-based nanomedicine platforms in a biological environment.[2] The assay is based on fluorescence detection of FLLs, but can easily be extended to analyze other compounds. Based on our results, we present guidelines on how a stable anchoring of lipid-conjugated compounds might be achieved.

1. Münter, R. *et al.* Dissociation of fluorescently labeled lipids from liposomes in biological environments challenges the interpretation of uptake studies. *Nanoscale* (2018). doi:10.1039/c8nr07755j

2. Münter, R. *et al.* Quantitative Methods for Investigating Dissociation of Fluorescently Labeled Lipid Fluorophores from Drug Delivery Liposomes. in *Characterization Tools for Nanotechnology-based Tissue Engineering and Medical Therapy (Vol 9)* in Press



Graphical abstract - lipids dissociate from liposomes in plasma and are taken up independently by peripheral blood leukocytes.png

Drug-loaded PBCA-based polymeric microbubbles for ultrasound-mediated drug delivery across biological barriers

Wednesday, 16th October - 15:00: Nano-Imaging for diagnosis, therapy and delivery in preclinical and clinical fields (Amphitheatre N02.040) - Oral - Abstract ID: 152

Mr. Anshuman Dasgupta [1], ***Ms. Mengjiao Liu*** [1], ***Prof. Fabian Kiessling*** [1], ***Prof. Twan Lammers*** [1]

1. *RWTH Aachen University Clinic*

Introduction: Drug delivery across vascular barriers is often impaired largely due to inefficient vessel permeability and poor fenestrations between endothelial cells. Sonoporation, which refers to temporary permeabilization of cell membranes or blood vessels induced by oscillating microbubbles in the presence of ultrasound, is an emerging strategy for enhancing drug delivery across vascular barriers. In this context, most of the studies have focused on ultrasound-induced drug delivery via indirect approach, i.e., enhancing the delivery of drugs and drug delivery systems upon co-administration with microbubbles and very few studies have focused on direct approach, i.e., enhancing the delivery of drugs by encapsulating them into the shell of microbubble. In this study, we aim to encapsulate drugs (corticosteroids and dyes) into the polymeric shell of microbubble and perform in-depth characterization for applications in drug delivery across biological barriers such as tumors and brain.

Methods:PBCA-based polymeric microbubbles were synthesized by homogenizing a 300 mL aqueous solution of pH 2.5, containing 1% Triton and 3 mL butyl cyanoacrylate, at 10000 rpm for 1 h. After multiple washing steps, the drugs (corticosteroids and dyes) were incubated with the microbubbles for 19 h at room temperature. Subsequently, the drug loaded polymeric microbubbles were characterized by HPLC, plate reader and ultrasound imaging to determine the loading capacity, encapsulation efficiency, drug release and acoustic characteristics (figure 1).

Results and discussions: Corticosteroids used in this study had similar chemical structure and only varied in few functional groups depending on hydrophobicity and molecular weight. Upon loading of corticosteroids into the microbubbles, loading capacity, encapsulation efficiency and release characteristics were evaluated and these characteristics showed a linear trend with the degree of hydrophobicity (log P) as well as the molecular weight of the corticosteroids (figure 2 and 3). On the other hand, all the dyes had very different chemical structures with large variations in functional groups, and quite obviously the dye loaded microbubbles failed to show any dependency on the physicochemical characteristics of the dyes (figure 2 and 3). Moreover, in the context of ultrasound, both corticosteroid and dye loaded microbubbles responded quite similarly to acoustics, without showing any dependency on the characteristics of the loaded drug molecules (figure 4).

Conclusion: This is the first systematic analysis and characterization of PBCA-based polymeric microbubbles upon loading of corticosteroids and dyes. These data highlight the significance of physicochemical characteristics of the drug such as chemical structure, functional group, hydrophobicity and molecular weight on loading capacity, release and acoustic properties of drug loaded microbubbles. These principles and notions may provide useful guidelines while optimizing treatment protocols in ultrasound-mediated drug delivery to tumors and brain.

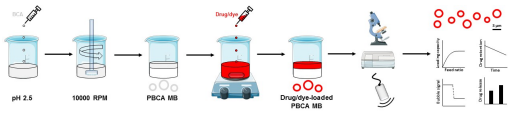


Figure 1. schematic representation for synthesizing and characterizing drug and dye-loaded pbca mb..jpg

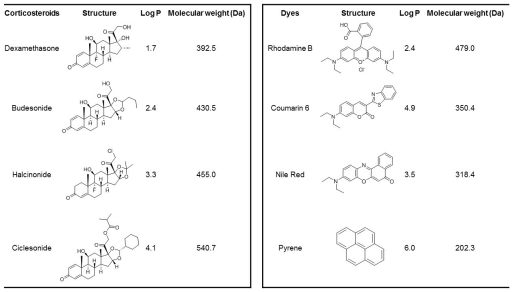


Figure 2. properties of drugs and dyes used in the study..jpg

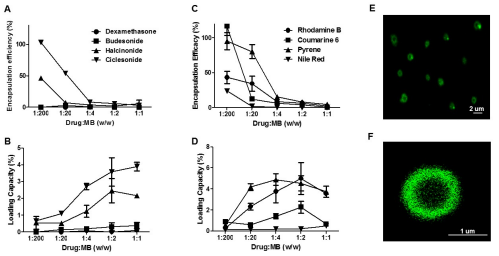


Figure 3. quantitative and qualitative analysis of corticosteroids and dye loaded pbca mb. encapsulation efficiency and loading capacity of a-b corticosteroids and c-d dyes..jpg

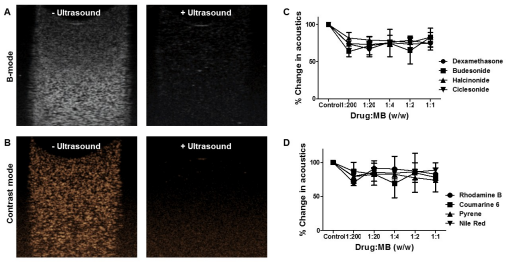


Figure 4. acoustic analysis of corticosteroid and dye loaded pbca mb. a b-mode and b contrast mode image of corticosteroiddye-loaded pbca mb before and after us treatment. .jpg

Assemblies of highly efficient iron oxide nanocubes for magnetic (fluid) hyperthermia to treat tumors

Wednesday, 16th October - 15:15: Nano-Imaging for diagnosis, therapy and delivery in preclinical and clinical fields (Amphitheatre N02.040) - Oral - Abstract ID: 234

Mr. sahitya kumar avugadda [1], ***Dr. DINA Niculaes*** [2], ***Dr. Maria Elena Materia*** [2], ***Dr. Aidin Lak*** [3], ***Dr. Rinat Nigmatullin*** [4], ***Dr. Pooja Basnett*** [4], ***Ms. Elena Marcello*** [4], ***Dr. Francisco Jose Teran*** [5], ***Dr. Ipsita Roy*** [6], ***Dr. Teresa Pellegrino*** [7]

1. *Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova. Dipartimento di Chimica e Chimica Industriale, Università di Genova, Via Dodecaneso 31, 16146 Genova,* ***2.*** *Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova,* ***3.*** *Istituto Italiano di Tecnologia,* ***4.*** *School of Life Sciences, College of Liberal Arts and Sciences, University of Westminster, New Cavendish Street, London,* ***5.*** *iMdea Nanociencia, Campus Universitario de Cantoblanco, C/ Faraday 9, 28049, Madrid, Spain. "Nanomedicina (iMdea Nanociencia), Unidad Asociada al Centro Nacional de Biotecnologia (CSIC), 28049 Madrid, Spain,* ***6.*** *Department of Material Science and Engineering, Faculty of Engineering, University of Sheffield,* ***7.*** *1Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova*

Magnetic fluid hyperthermia (MFH) is a non-invasive method, now in clinical practice, to treat solid tumors, by exposing cancerous tissues to an abnormal temperature of 40-45°C generated by magnetic nanoparticles when excited under an alternative magnetic field. In our group we have developed, cubic shaped iron oxide nanocubes (IONC) with optimized magnetic properties, that have 10 to 20 times better heating performance than commercially available spherical iron oxide nanoparticles (SPIONs), as typically measured in terms of specific absorption rates (SAR) (Guardia, P. et al, J. Mater. Chem. B 2014) . On the other hand, it is known that chains of magnetic nanoparticles produced by magnetosome bacteria have enhanced SAR properties compared to isolated nanoparticles (Alphandéry ACS Nano,2011). Since then, much attention has been dedicated to the assembly of nanoparticles from current available magnetic nanoparticles with better heat performance, in different geometrical configurations and to the study of their SAR performance. Recently, we have developed colloidally stable, water soluble short chains of core-shell IONC arranged in dimeric (two nanoparticles) and trimeric (three nanoparticles) clusters, using an amphiphilic polymer, poly(styrene-co-maleic anhydride) cumene terminated.3 By increasing the amount of polymer to nanoparticles surface ratio, we were able to fabricate the morphology of cluster from an isolated to centrosymmetric form (fig.1a-c). Among the three developed assemblies, dimer/trimeric forms possessed enhanced SAR properties (fig.1d) due to dipolar interactions between particles in chains and improved magnetic anisotropy (Niculaes, D. et al,ACS Nano,2017)
.The assembling strategy of using the same building blocks arranged in different configurations is crucial in MFH in order to reduce the doses of magnetic materials, thus avoiding toxicity concerns associated to injection of higher doses. We have also developed nanoparticle assemblies arranged in well-defined geometries that can be easily disassembled upon exposure to intra-tumoral stimuli. This assembly/disassembly process is accompanied by a significant increase in SAR that favors a more effective heat development under the alternating magnetic field (Avugadda, S. K. et al, Chem. Mater,2019). At the same time, the assembled particles, such as bi-dimensional assemblies of nanoparticles demonstrated a rapid response to an external magnet (0.3 T) compared to isolated particles, which is a crucial feature for external magnetic guided physical accumulation of particles at the target site. This indeed would favor the accumulation of nanoparticles at the tumor site.
Figure 1. a-c) TEM images of water-soluble structures produced from core-shell iron oxide nanocubes by tuning the amount of amphiphilic polymer (poly(styrene-co-maleic anhydride) cumene terminated). d) Scheme of nanoclusters production, with respective SAR properties.3

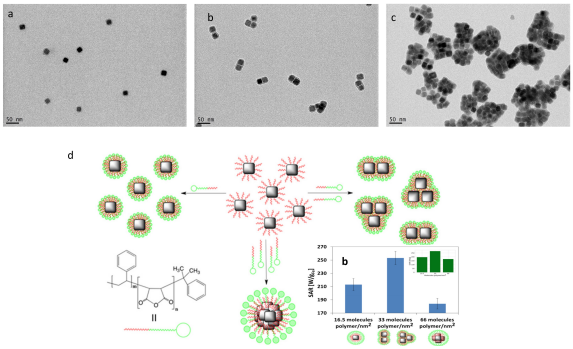


Fig 1.jpg

Nanoparticles in Phototherapies

Wednesday, 16th October - 15:30: Nano-Imaging for diagnosis, therapy and delivery in preclinical and clinical fields (Amphitheatre N02.040) - Oral - Abstract ID: 278

Prof. Havva Yagci Acar [1], ***Dr. Kubra Bilici*** [1], ***Mr. ABDULLAH MUTI*** [1], ***Prof. Alphan Sennaroğlu*** [1], ***Ms. NAZLI ATAC*** [1], ***Prof. FUSUN CAN*** [1]

1. *Koc university*

Superpramagnetic iron oxide has been around for decades for magnetic resonance imaging, magnetic hyperthermia, magnetic separation, and drug/gene delivery. It is now being investigated for photothermal therapy (PTT). Small SPIONs with 3-aminopropyltrimethoxysilane (APTMS) coating are popular due to their surface amine groups allowing conjugation of drugs, ligands etc for delivery of therapeutic cargo, yet, they have never been investigated for PTT. We will discuss the nanoparticle dose and laser intensity dependent temperature increase in the aqueous NP solutions upon irradiation at 795 nm. Indocyanine green (ICG), an FDA approved fluorescent dye, has been recently used for photodynamic therapy (PDT). We investigated the PTT and PDT (dual therapy) potential of ICG loaded to APTMS@SPIONs. Therapeutic efficiency of PTT, PDT and dual therapy was tested on colorectal and breast cancer cells at safe nanoparticle doses and laser irradiation power. Dual therapy was able to kill tumor cells almost totally on both cell lines showing higher efficiency compared to the application of either therapy scheme alone.

This strategy is also utilized for the eradication of biofilms of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus epidermidis. The* efficiency of PTT, PDT and dual therapy on growth inhibition in either planktonic cells or biofilms will be discussed.

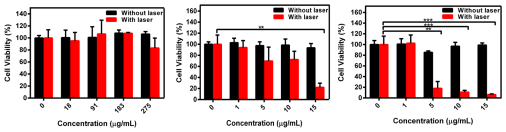


Figure 1. Viability of MCF7 cells treated with (a) NP, (b) ICG, (c) NP/ICG with and without laser irradiation (10 min irradiation at 1.8 W/cm^2)

Fig 1 iconan19.png

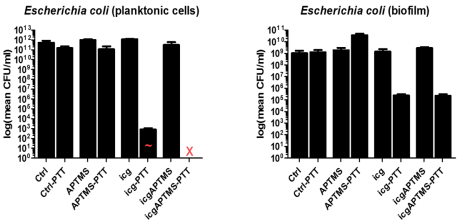


Figure 2. Colony count of E. Coli planktonic cells and biofilm treated with laser and NPs, ICG, NP/ICG with and without laser treatment (10 min 795 nm 1150 mW laser otput power).

Fig 2 iconan 2019.png

Supramolecular dynamic non-viral vectors: synergistic effect of low molecular weight polyethylenimine and polyethylene glycol on transfection efficiency and cytotoxicity

Wednesday, 16th October - 14:15: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 21

Dr. Lilia Clima [1], ***Mr. Bogdan Florin Craciun*** [2], ***Dr. Mariana Pinteala*** [3]

1. *"Petru Poni" Institute of Macromolecular Chemistry,* ***2.*** *"P. Poni" Institute of Macromolecular Chemistry,* ***3.*** *Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, Iasi*

Using polyethylenimine (PEI) as a component of non-viral vectors for delivery of nucleic acids, often rise issues related with high toxicity, low transfection efficiency and nucleic acid polyplex formation. The shielding of polyplexes charge by hydrophilic polymers, in particular polyethylene glycols, human serum albumin and dextran also known as "stealth technology", has been intensively applied. In some cases, however, they are also responsible for the non-specific ionic interactions between the complex and target cells, thus decreasing the transfection efficiency. The molecular weight of PEI, PEGylation, biocompatibility and supramolecular structure of potential carrier can each influence the nucleic acid condensation behavior, polyplex size, and transfection efficiency and cytotoxicity. Therefore, it is important to find a correlation between the constituent components, as well as the possible synergy between them, to efficiently transport and to release in specific manner different molecules of interest. Our main interest was to synthesize new compounds from commercially available or "easy to prepare" units, which will further self-assemble in a complex, tuneable and multifunctional non-viral vectors, suitable for specific targeted gene delivery.

In this communication, the preparation of libraries of polymeric micellar supramolecular assemblies based on Pegylated squalene derivative, linked with linear polyethylene glycol and low molecular weight branched PEI through a multifunctional core connector, and testing as non-viral vectors for plasmid DNA delivery were reported. Dynamic Combinatorial Chemistry approach was used to produce libraries of compounds by modifying the components and their ratios in the composition of vectors. PEGylated squalene, poly-(ethyleneglycol)-bis(3-aminopropyl) (dPEG, 1500 Da or 2000 Da or 3000 Da) and branched PEI (800 Da or 2000 Da), were reversibly connected in a hyperbranched structure to a carbonyl functionalized core *via* reversible imine bond. Driven by self-assembly properties of squalene derivatives, obtained amphiphilic architectures form hydrophobic-hydrophilic particles spontaneously in water. The prepared compounds were capable of interaction with nucleic acids, forming stable polyplexes and to deliver plasmid DNA to cells. It has been shown that studied vectors exhibited distinctly higher efficiency in transfection of pDNA to HeLa cells as compared with starting PEIs and transfection efficiency was closely related to ratio and size of PEI and dPEG.

This research was funded by H2020 WIDESPREAD 2-2014: ERA Chairs Project no 667387: SupraChem Lab Laboratory of Supramolecular Chemistry for Adaptive Delivery Systems ERA Chair initiative and from a grant of Ministry of Research and Innovation, CNCS-UEFISCDI, project number PN-III-P1-1.1-TE-2016-1180, within PNCDI III.

DNA mediates the release of nanoparticles from a hydrogel environment in a cascade-like fashion

Wednesday, 16th October - 14:30: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 27

Ms. Ceren Kimna [1], ***Prof. Oliver Lieleg*** [1]

1. *Department of Mechanical Engineering and Munich School of Bioengineering, Technical University of Munich, Boltzmannstraße 11, 85748 Garching, Germany*

Introduction

Current medication treatments require the patient to be in charge of ensuring correct dosing, timing and order of the drugs to be taken. This becomes an important problem in the case of patient non-compliance, e.g., among elderly people who are prone to forgetting individual doses. Therefore, a smart delivery mechanism providing a programmable release order of agents is needed to achieve medication at distinct, pre-defined time points for delivery of multiple molecules at correct dosages.

Methods

Self-complementary DNA sequences were designed to achieve a cascade-style release of three different nanoparticles (Figure 1). For initiating the cascade, a displacement DNA (dDNA) was used to generate electrostatically stable silver clusters. Two DNA sequences (pDNA) linked to Fe_2O_3 NPs were combined with a symmetric bridge-like DNA (brDNA) sequence to create a second cluster type, which could be separated again in the presence of the displacement DNA (dDNA). Later in the release cascade, this bridge-like DNA was used to break down gold-NP clusters that were crosslinked via partially self-complementary DNA sequences (crDNA). All three NP cluster variants were placed separately in agarose hydrogel layers. The release was monitored spectrophotometrically after addition of 150 mM NaCl solution, which triggered the release cascade.

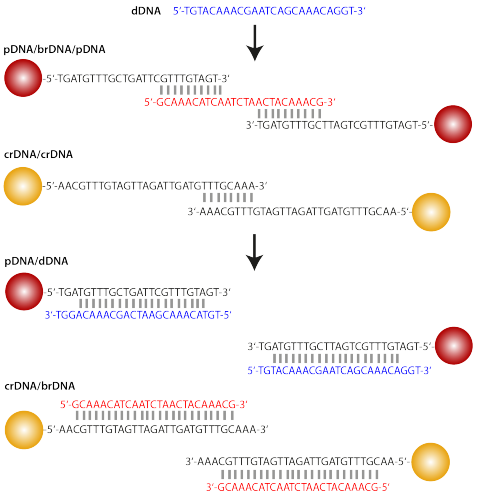
Results

Here, we showed that the release of three different nanoparticles can be initiated by subjecting the hydrogel matrix to a physiological salt solution. Electrostatically stable silver/DNA clusters placed in the bottom layer of the hydrogel were disaggregated after exposure to 150 mM NaCl – which gave rise to Debye screening effects. This triggered the release avalanche by liberating the displacement DNA that can fully bind to pDNA by forming complementary, thermodynamically favorable DNA-base pairs (Figure 2). Therefore, the Fe_2O_3clusters were disaggregated and brDNA strands were liberated. Finally, these liberated brDNA sequences induced the disaggregation of gold NP clusters and caused the release of gold nanoparticles.

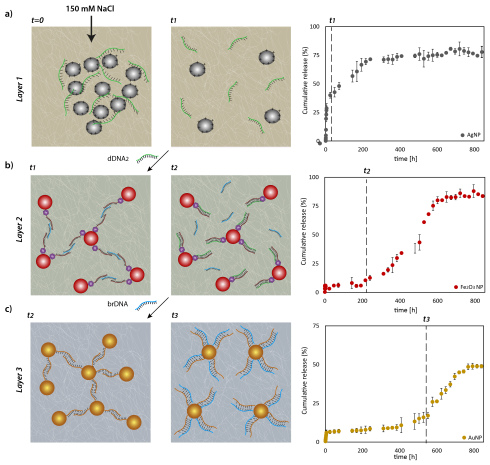
Depending on whether the NP clusters were deposited into three different layers of the hydrogel matrix or mixed together into a single layer, the release cascade takes ~4 weeks or ~6 days to complete. In any case, we achieved a high cumulative efficiency of NPs between 50% and 75% and very high precision and reproducibility.

Discussion

Our results show that the DNA-mediated release system can provide a precise control over the release of three different NPs that can be used as drug carriers. In addition, the release time can be altered to achieve different treatment durations without affecting the efficiency of the release mechanism. Thus, the DNA-based mechanism developed here can be useful for developing smart delivery systems to minimize a patient's medication management duty by providing an automated, multicomponent release cascade.



Molecular design of the dna sequences for three-stage np-release.png



Schematic illustration and cumulative release profiles of np in 3-layer agarose gel.png

Carbon nanotube active-by-design nanocarriers for cancer therapy

Wednesday, 16th October - 14:45: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 35

Prof. Mónica L. Fanarraga [1], ***Dr. Eloisa González-Lavado*** [1], ***Ms. Esperanza Padín*** [1], ***Ms. Nerea Iturroz*** [1], ***Ms. Lourdes Valdivia*** [1], ***Dr. Lorena García-Hevia*** [2], ***Prof. Rafael Valiente*** [1], ***Dr. Jesús González*** [1]

1. *Universidad Cantabria-IDIVAL,* ***2.*** *International Iberian Laboratory*

In the treatment of human cancer, multiple-drug resistance is a major problem. To circumvent this issue, clinicians combine several drugs. However, this strategy could backfire resulting in more toxic or ineffective treatments in themed-term.

Carbon nanotubes*(CNTs) are nanomaterials with a high interest in the industry that recently have drawn special attention in the field of nanobiotechnology. As other carbon allotropes, CNTs display an extraordinary capacity to capture biomolecules from the environment, acquiring different biological identities. Their unidimensional nature endows CNTs with unique properties in vivo. These nanofilaments can cross many different types of membranes and penetrate inside cells or tissues. More interestingly, their unique morphology and surface properties prompts their biomimetic interaction with the intracellular biological nanofilaments, namely microtubules,*[1] *actin*[2] *and DNA*[3]*, triggering interesting effects at the cellular level that deserve special attention when developing nano-vectors for anticancer therapies. The multi-walled nanotube (MWCNT) biomimetic properties result in intrinsic anti-proliferative,*[4,5] *anti-migratory*[6] *and cytotoxic*[7] *properties in vitro, resulting in anti-tumoral properties in vivo*[8–10] *that are maintained (and enhanced) in tumours produced cell resistant to traditional microtubule-binding chemotherapies such as Taxol®.*[8]

Here, we propose the use of MWCNTs, in the design of ***active-by-design nanocarriers****, attempting to enhance the effect of broadly used chemotherapies. Our results demonstrate how the total effect of the drug 5-Fluorouracil (5-FU) is remarkably improved (50% more effective) when delivered intratumorally coupled to MWCNTs both, in vitro and in vivo in solid tumoral models.*[10] *Our results demonstrate how using MWCNTs as anti-cancer drug delivery platforms is a promising approach to boost the efficacy of traditional chemotherapies, while considerably reducing the chances of resistance in cancer cells.*

Acknowledgements

This work was founded by Spanish MINECO, Instituto de Salud Carlos III-European Regional Development Funds (ERDF) under Projects ref. PI16/00496, NanoBioApp Network (MINECO-17-MAT2016-81955-REDT), IDIVAL and the University of Cantabria.

References:

1. Rodríguez-Fernández, L., *et al.* Multiwalled carbon nanotubes display microtubule biomimetic properties in vivo, enhancing microtubule assembly and stabilization. *ACS Nano* **6**, 6614 (2012).

2. Holt, B. D. *et al.* Carbon nanotubes reorganize actin structures in cells and ex Vivo. *ACS Nano* **4**, 4872 (2010).

3. Li, X., Peng, Y. & Qu, X. Carbon nanotubes selective destabilization of duplex and triplex DNA and inducing B-A transition in solution. *Nucleic Acids Res.* **34**, 3670 (2006).

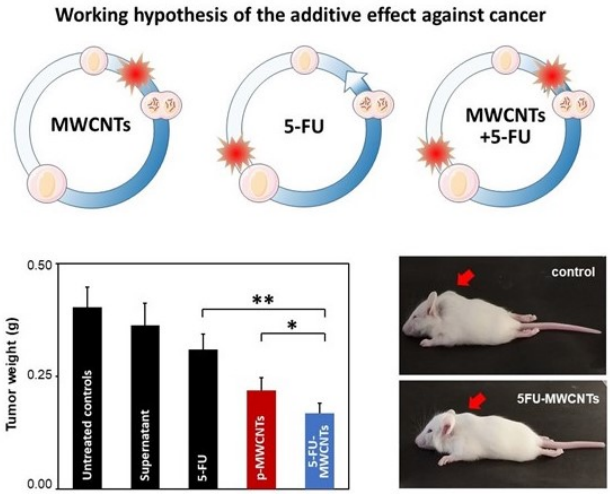
4. Sargent, L. M. *et al.* Single-walled carbon nanotube-induced mitotic disruption. *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.* **745**, 28 (2012).

5. García-Hevia, L. *et al.* Nanotube interactions with microtubules: implications for cancer medicine. *Nanomedicine* **9**, 1581 (2014).

6. Garcia-Hevia, L. *et al.* Inhibition of Cancer Cell Migration by Multiwalled Carbon Nanotubes. *Adv. Healthc. Mater.* **4**, 1640 (2015).

7. García-Hevia, L. *et al.* Anti-cancer cytotoxic effects of multiwalled carbon nanotubes. *Curr. Pharm. Des.* **21**,

(2015).
8. García-Hevia, L. *et al.* Multiwalled Carbon Nanotubes Inhibit Tumor Progression in a Mouse Model. *Adv. Healthc. Mater.* **5**, 1 (2016).
9. González-Lavado, E. *et al.* Biodegradable multi-walled carbon nanotubes trigger anti-tumoral effects. *Nanoscale* **10**, 11013 (2018).
10. González-Lavado, E. *et al.* MWCNTs complement the anti-tumoral effect of 5-Fluorouracil. *Oncotarget* **10**, 2022 (2019).



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Fullerene, siloxane, cyclodextrin and polyrotaxane : a non-viral approach in gene delivery

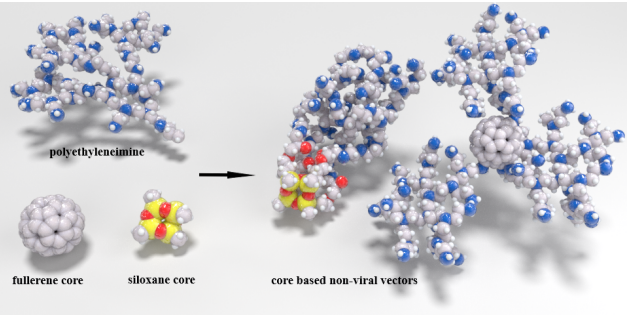
Wednesday, 16th October - 15:00: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 38

Dr. Dragos Peptanariu [1], ***Dr. Mariana Pinteala*** [1]

1. *Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, Iasi*

The development of scientific fields such as biochemistry and molecular biology have led to a better understanding of disease mechanisms and their genetic basis. This has increased the desire to find new and more effective treatments and gene therapy has begun to claim an increasing importance.
Gene therapy promises the possibility of curative treatment of genetic diseases by introducing genes into patients' cells instead of using common drugs that only provide symptomatic treatment.
Gene therapy might use viruses or non-viral agents like liposomes and cationic polymers. Viral vectors are much more common, but they raise many issues such as the potential for rejection by the patient's body, the possibility of unwanted genome integration and the inability to repeat their administration.
In the design of non-viral vectors, our group has applied various strategies based on molecules such as fullerene, siloxane, beta-cyclodextrin, which functions as the core to which several polyethyleneimine molecules are attached. This combination is versatile because it offers the possibility of attaching other groups such as peptides for targeted delivery. Also, using several cyclodextrin molecules with polyethyleneimine and polyethylene glycol, a polyrotaxane has been constructed that has proven to be very effective as a transfectant.

The synthesis of the vectors proceeded in two steps: in the first step the functionalization of the core molecule took place, and in the second step the addition of the polyethylenimine arms was done.
After chemical characterization, the synthesized vectors were tested as transfection agents on cell culture. These vectors have demonstrated good plasmid binding and transport capacity as well as efficient *in vitro* transfection for reporter genes.
This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 667387 WIDESPREAD 2-2014 SupraChem Lab and from a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number PN-III-P3-3.6-H2020- 2016-0011, within PNCDI III.



Vector synthesis.png

Forward genetic screening as a tool to study endocytosis of nanocarriers

Wednesday, 16th October - 15:15: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 51

Ms. Daphne Montizaan [1], ***Dr. Diana Spierings*** [2], ***Dr. Victor Guryev*** [2], ***Dr. Anna Salvati*** [1]

1. *University of Groningen,* ***2.*** *European Research Institute for the Biology of Ageing*

Nanocarriers offer great potential for targeted drug delivery. For efficient delivery, the nanocarriers do not only need to reach the target tissue, but they also need to cross the cell membrane and locate the drug to the appropriate intracellular location. To be able to achieve this, it is crucial to understand the nanocarrier-cell interactions. Most research studying these nanocarrier-cell interactions have used methods like RNA interference, overexpression of proteins, or chemical inhibitors, in which only known targets are usually studied. However, the mechanism by which nanocarriers enter cells is still ambiguous and it might be that novel mechanisms are involved.

In this work, to prevent exclusion of novel targets which have no clear relation to known endocytic pathways as yet, a forward genetic screening method originally developed by Carette *et al.* (2009) was used to study the internalization of nanocarriers. A hundred million human haploid HAP1 cells were mutagenized with a retrovirus, and the library of mutagenized cells was subsequently exposed to fluorescently labelled nanoparticles. Silica nanoparticles (50 nm) were used as a model to test this forward genetic screening method. Cells with a reduced uptake were enriched by several selections using fluorescence-activated cell sorting. The enriched population was sequenced to determine the integration site of the retrovirus causing the mutation. Different from previous studies, multiple sorting procedures were necessary to enrich for cells with a reduced nanoparticle uptake. Nonetheless, enrichment was achieved and sequencing allowed to identify a high number of targets involved in the uptake of nanoparticles. Among them, one of the identified targets was the low-density lipoprotein receptor, which is known to be involved in the uptake of the silica nanoparticles under the particular conditions used. Multiple other novel targets involved in nanoparticle internalization were discovered and are currently being verified. Overall, these results show that forward genetic screening can be used to study the internalization of nanocarriers and discover novel targets not yet associated the uptake of nano-sized objects.

Aqueous stable gold nanostar/ZIF-8 nanocomposites for light triggered release of active cargo inside living cells

Wednesday, 16th October - 15:30: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 175

Dr. Carolina Carrillo-Carrion [1]

1. *Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Universidade de Santiago de Compostela*

Introduction

In recent years, the interest in nanoscale materials for theranostic applications, for instance in diagnosis and treatment of cancer, has increased. In this direction, multifunctional hybrid nanostructures with improved performance in physiological scenarios are required. The combination of plasmonic nanoparticles such as gold nanoparticles with metal–organic frameworks (MOFs) offers unique opportunities for the development of nanoplatforms with theranostic potential thanks to the versatile drug encapsulation properties of MOFs [1].

Methods

Here, a plasmonic core-shell nanostar@ZIF-8 nanocomposite (NC) was developed for thermoplasmonic-driven release of encapsulated molecules inside living cells [2]. The NCs were loaded with Hoechst molecules as functional cargo (DNA staining), and further functionalized with an amphiphilic polymer that prevents ZIF-8 degradation and Hoechst leaking in aqueous media and inside living cells. Confocal fluorescence microscopy and surface enhanced Raman spectroscopy (SERS) were used to evaluate the performance of the system.

Results and discussion

The newly developed NC has a similar capacity for drug loading than conventional ZIF-8 based nanosystems, but importantly it is stable in aqueous solution, and it can be activated to release the drug upon illumination with near–IR (NIR) light. This concept is demonstrated both in aqueous solution and inside living cells (HeLa cells). The proposed drug-release mechanism relies on the use of NIR light coupled to the plasmonic absorption of the core nanostars, which creates local temperature gradients, thereby leading to thermodiffusion of cargo molecules.

The intracellular monitoring of cargo delivery upon illumination with a NIR light (785 nm) was first carried out by using SERS imaging in cells. A clear signal of Hoechst was recorded in certain regions of the cell (very likely the lysosomes) previously to the light illumination. The release kinetics showed that around the 75% of the Hoechst was released during the first 2 h of illumination. These results contrast with those obtained without illumination, where SERS spectra showed clear Hoechst signals along the time (during 8 h) indicating that the integrity of the drug carrier was not compromised. SERS data fully agree with equivalent studies by confocal microscopy (Figure 1). Accumulation of the NCs (blue fluorescent dots) was found in the vicinity (very likely in lysosomes) of the nuclei before illumination. Then, cells containing the NCs were irradiated with the NIR illumination setup (5 min, 7 W/cm^2) leading to the Hoechst release as can be concluded from the nuclei staining observed in the confocal images.

Conclusion

An aqueous stable (even when stored in cells) ZIF-8 based nanomaterial with combined thermoplasmonic and high drug-loading capabilities has been developed. This approach is an important step forward in the development of MOF-based intracellular vehicles for the controlled release of drugs.

References

[1] Zheng, G. et al., Small 2016, 12(29), 3935.

[2] Carrillo–Carrión, et al., Angew. Chem. Int. Ed. 2018, 58(21), 7078.

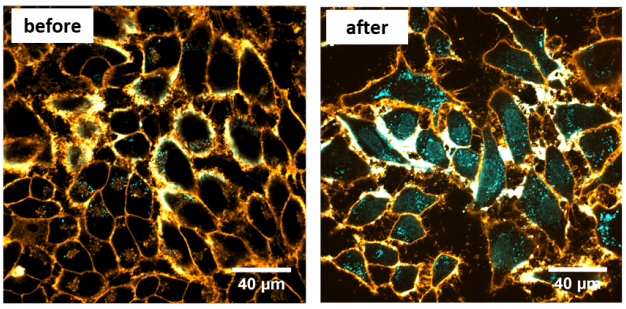


Figure 1. confocal microscopy images before and after nir irradiation.png

Reactive oxygen species (ROS)-responsive polymersomes with site-specific chemotherapeutic delivery into ROS-rich tumor in vivo

Wednesday, 16th October - 15:45: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 245

Mr. Vladimir Sincari [1], ***Dr. Eliezer Jager*** [1], ***Dr. Martin Hruby*** [1], ***Dr. Petr Stepanek*** [1]

1. *Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic*

Introduction: Polymersomes (PS) made from block copolymers are spherical self-assembled structures which resemble cell membranes of living organisms. In comparison to liposomes the PS have improved stability and higher loading capacity for drug encapsulation. The release of loaded drugs from PS can be triggered by external or internal physical or chemical stimuli such as pH, redox potential, light, magnetic field, temperature or the presence of enzymes. Some of these proposed "smart" PS were able to release their payloads, or show reduced sizes or converted charges under conditions (e.g., reduced pH, hypoxia, tumor-specific enzymes) within the tumor microenvironment (TME). Among the aforementioned stimuli, one straightforward targeting approach is exploiting the presence of reactive oxygen species (ROS) in cancer cells. ROS such as, hydrogen peroxide (H_2O_2), is a component of cell signalling pathways that are necessary for the growth, development, and fitness of living organisms. However, imbalances in H_2O_2 production lead to oxidative stress and inflammation events, which damage tissue and organ systems and are correlated with the onset and advancement of various diseases, including cancer. Hence, the design of PS able to be responsive to these inherent feature of the TME has been proposed as a promising approach for the cancer treatment.

Methods: A new oxidative responsive amphiphilic diblock copolymer based on a novel methacrylamide monomer bearing pinacol-type boronic ester functional moieties and *N*-(2-hydroxypropyl)methacrylamide (HPMA) was synthesized by a reversible addition-fragmentation chain transfer (RAFT) polymerization and characterized by standard techniques (^{1}H NMR and SEC). Monodisperse polymersomes (PS1 and PS2) ~ 100 nm in diameter loaded with the chemotherapeutic drug doxorubicin (Dox) were produced by microfluidic flow-focusing method. Characterization of the PS and their degradation under presence of $H_2O_2$was probed by ^{1}H NMR, SEC, dynamic and static light scattering techniques. The therapeutic effect of PS was tested on in vitro and in in vivo biological models and was compared with the marketed free chemotherapeutic Dox.

Results and discussion: The developed PS platform allows the specific delivery of Dox at the inherent ROS levels typically found in the TME (Figure 1, top). The in vivo results clearly show efficient suppression of tumor cell growth in mice bearing EL4 T cell lymphoma (Figure 1, bottom right). The survival time of the animals was also extended if compared with DOX-free (Figure 1, bottom left). Along, the reduced side-effects of the chemotherapy were substantially improved with the PS such as balance of the body weight, less cardiotoxicity of DOX, one of the main drawbacks of this current chemotherapeutic drug in the clinic, as observed after the decreasing of serum creatine kinase levels monitored in blood. The results from this pioneer work strongly suggest that ROS-responsive PS has potential for tumor-targeting DOX delivery based on the ROS-triggered release mechanism in vivo.

ACKNOWLEDGEMENTS:

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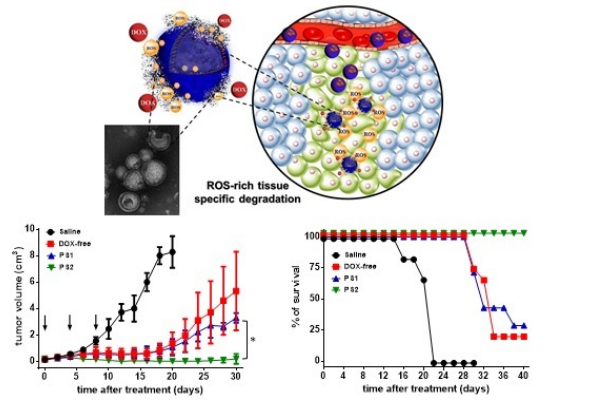

Figure 1.: Mechanism of ROS-triggered degradation (top), In vivo effect of PS vs DOX-free and Saline on the growth of T cell lymphoma EL4 (bottom right) and Kaplan-Meier survival plot of mice after 3 x 5 mg DOX (equivalent)/kg administration and untreated control (n=4-6; black arrows indicate injections) (bottom left).

Figure1.jpg

CriPec® nanomedicines: academic to industry and preclinical to clinical translation

Wednesday, 16th October - 16:45: Start-up session: How to successfully start a bio-nano-pharmaceutical company? (Amphitheatre N02.040) - Workshop - Abstract ID: 259

Dr. Rachel Hu [1], ***Dr. Rob Hanssen*** [1], ***Dr. cristianne rijcken*** [1]

1. *Cristal Therapeutics*

Introduction

Cristal Therapeutics is a clinical stage pharmaceutical company developing next generation nanomedicines based on its proprietary CriPec® platform to treat various diseases, including cancer. CriPec® is perfectly suited for the rational design of (targeted) nanomedicines with superior efficacy and safety profiles. The most advanced product in development is CPC634 (CriPec® docetaxel) for treatment of solid tumours, while other CriPec® products are in preclinical development.

Methods

CriPec® is a pioneering approach to transform a broad range of therapeutic compounds into rationally designed nanomedicines to assure optimal treatment of various diseases.

Results

CriPec® has been successfully combined with small molecules, peptides or oligonucleotides, thereby generating monodisperse CriPec® nanoparticles with tuneable sizes between 30 and 100 nm with high drug entrapment efficiencies and predetermined drug release kinetics. The surface can be modified by targeting ligands. CriPec® products are customisable and biocompatible, with a robust manufacturability at clinical scale.

CPC634 is a 65 nm sized nanoparticle entrapping docetaxel designed which improves tumour accumulation and tolerability compared to conventional docetaxel by taking advantage of the presumed enhanced permeability and retention (EPR) effect. In preclinical models, an enhanced therapeutic index is observed due to an improved pharmacokinetic profile, increased tumour uptake and superior tolerability.

The first-in-human study demonstrated a favourable plasma PK profile of CPC634. Safety evaluation specifically demonstrated less neutropenia. One partial response and sixteen cases of stable disease (RECIST 1.1) were confirmed. The RP2D was set at 60 mg/m2 with dexamethasone premedication.

In a randomized cross-over study, tumour biopsies were taken after intravenous administration of CPC634 and conventional docetaxel (or vice versa). CPC634 generated higher intratumoural total docetaxel (323%, $p < 0.001$) and comparable released docetaxel levels relative to conventional docetaxel.

Next, CPC634 was labelled with zirconium-89 to facilitate non-invasive PET imaging of its biological fate. Tumour retention showed intra- and interpatient heterogeneity with the highest intensity at 96h post injection and with a mean %ID/kg of 3.43 [1.14-9.32], confirming the EPR effect in patients.

Conclusion

The clinical results of CPC634 illustrate the improved safety profile and increased tumour uptake. A phase II efficacy study of CPC634 in patients with platinum resistant ovarian cancer (NCT03742713) is currently ongoing. The innovative CriPec® platform allows for the rational design of nanomedicines of a variety of drug molecules with an anticipated superior therapeutic performance.

Acknowledgements: H2020-SMEInst-2016-2017 – INTACT #784127

Translating pre-clinical drug delivery research into products for clinical use by starting a company

Wednesday, 16th October - 17:10: Start-up session: How to successfully start a bio-nano-pharmaceutical company? (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 275

Dr. Josbert Metselaar [1]

1. *Department of Nanomedicine and Theranostics Institute for Experimental Molecular Imaging RWTH Aachen University Clinic; Enceladus Pharmaceuticals BV*

In my talk I will present practical experience as to the trajectory of commercializing breakthrough scientific results into products for clinical use. The following items are highlighted: setting out a clinical development strategy, building a patent portfolio, entering into collaborations, and last but not least: how to acquire financing. A personal view is taken with my drug development company Enceladus Pharmaceuticals as an 'example case'.

Enceladus started at the time as a spin-off from the Utrecht University. Enceladus focused on the development of Nanocort and Oncocort, two liposomal pharmaceuticals that contain corticosteroids and selectively target actual sites of pathology in inflammation and cancer respectively. After a range of preclinical studies that showed that these i.v. liposomal targeted corticosteroid products can achieve a dramatic increase of the efficacy and safety of corticosteroids, clinical trials were undertaken in several diseases including a recently concluded successful phase 3 trial.

Long-acting drug delivery: are we witnessing a paradigm shift in treatment of chronic diseases?

Thursday, 17th October - 09:00: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 263

Prof. Andrew Owen [1]

1. *University of Liverpool*

Long-acting drug delivery has emerged as an effective option for indications such as contraception, schizophrenia, androgen ablation and opioid substitution therapy. The approach encompasses implant and injectable technologies with a growing interest also evident in microarray patches and devices for extending half-life from oral administration. Many opportunities exist for therapy and prevention of disease, and long-acting injectables for HIV drugs have spurred recent interest across infectious diseases. such as tuberculosis, malaria and viral hepatitis. Despite success, considerable knowledge gaps exist in terms of the physiological and molecular processes that govern drug absorption for some technologies. Preclinical studies have highlighted potential roles for macrophage infiltration, granuloma formation and/or angiogenesis at the depot site for long-acting injectables, but the quantitative importance of these mechanisms in humans is uncertain. Moreover, the clinical application of long-acting medicines requires consideration of several factors relating to posology and clinical pharmacology of the administered drug. For example, a long sub-therapeutic pharmacokinetic tail may be a more important consideration for infectious diseases with a resistance liability than for non-communicable indications. The implications for drug-drug interactions with the long-acting agent as a victim or perpetrator is also not well characterised and may be different for some drugs when the route of administration is changed, avoiding the gastrointestinal tract and first pass metabolism. Other questions relate to whether there will be new DDIs mediated through different absorption mechanisms and/or whether an oral lead-in to mitigate toxicity will be necessary for all long-acting medicines. This presentation will summarise existing data for long-acting medicine, with a focus upon long-acting injectable formulations.

Phenotypic targeting via multiplexed and chemotactic polymersomes

Thursday, 17th October - 09:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 273

Prof. Giuseppe Battaglia [1]

1. *University College London*

We have advanced our ability to deliver drugs combining the high selectivity of active molecules with molecularly engineered carriers equipped with the necessary attributes to navigate biological environments. A critical element of such a nanomedicinal effort is the introduction of ligands that enable targeting and selectivity to guide carriers across biological barriers. Such an approach is now allowing to extend drug discovery to target biological macromolecules that are not accessible via simple diffusion such as the inside of cells or the central nervous system. However, tight control on the selectivity of drugs and nanomedicines' interaction with biological systems is paramount for the development of targeted therapies. A large number of synthetically tuneable parameters makes it difficult to identify optimal design "sweet spots" without rational guiding principles. Here I address this problem combining super-selectivity theory (SST) with analytical models from soft matter and polymer physics into a unified theoretical framework. Starting from an archetypal system, a polymer-stabilized nanoparticle functionalized with targeting ligands, we use our model to identify the most selective combina- tion of parameters in terms of particle size, brush polymerization degree and grafting density, as well as tether length, binding affinity and ligands number. I further discuss how to combine multivalent interactions into mul- tiplexed systems which act holistically as a function of the density of more than one receptor type, to achieve binding only when multiple receptors are expressed above a threshold density. I christen this as "phenotypic" targeting, and I propose its use for drugging different cell populations enabling personalized therapies. I will show few examples of phenotypic targeting in both blood-brain barrier crossing, cancer targeting and immune cell recognition.

Finally, I will combine phenotypic targeting with chemotaxis in all effect amplifying molecular interaction to create organotropic diffusion profile. I will show this with a fully synthetic, organic, nanoscopic system that exhibits attractive chemotaxis driven by enzymatic conversion of glucose. This is achieved by encapsulating glucose oxidase alone or in combination with catalase into nanoscopic and biocompatible asymmetric polymer vesicles (known as polymersomes). I will show that these vesicles self-propel in response to an external gradient of glucose by inducing a slip velocity on their surface, which makes them move in an extremely sensitive way toward higher-concentration regions. I will finally demonstrate that the chemotactic behaviour of these nanoswimmers, in combination with LRP-1 (low-density lipoprotein receptor-related protein 1) targeting, enables a fourfold increase in penetration to the brain compared to non-chemotactic systems

Prodrugs and associated opportunities in localized drug synthesis

Thursday, 17th October - 10:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 261

Dr. Alexander Zelikin [1]

1. *Aarhus University*

Biocatalytic impants and nanozymes, antiviral macromolecular prodrugs, remote activation of biomaterials these areas of research are in the focus of my lab and during this presentation, I will discuss our recent findings on these avenues. I will present the development of biocatalytic implants as novel tools for site specific drug delivery. We will also discuss the design of nanozymes as fundamentally important materials for biomedicine. Finally, I will outline our recent successes in the design of macromolecular prodrugs as long-acting tools of drug delivery, as broad spectrum antiviral agents, and latency reversing agents against HIV. Presentation aims to be of interest for diverse audience, specifically scientists in the fields of medicinal chemistry and virology, polymer science and engineering, and biomaterials.

Drug Delivery with Multifunctional Mesoporous Nanoparticles

Thursday, 17th October - 11:00: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 269

Prof. Thomas Bein [1]

1. *Ludwig-Maximilians-Universität München*

Over the years, numerous intriguing types of multifunctional mesoporous nanoparticles have been developed as carriers for targeted drug delivery, for example in the context of cancer therapy. In our recent work, we have addressed several key challenges related to the development of multifunctional mesoporous silica and other mesoporous nanoparticles, including stimuli-responsive release systems, targeting ligands for specific cellular receptors, biodistribution and biocompatibility.

For example, folate and epidermal growth factor (EGF) have been used for successful cell targeting with mesoporous silica nanoparticles (MSNs). Considering triggered release in the endosome, a novel pH-responsive system has been created based on genetically modified carbonic anhydrase (CA) gatekeepers. A pH-dependent CA inhibitor was covalently attached to the surface of the MSNs, resulting in the desired opening mechanism caused by the endosomal pH change. Addressing endosomal escape, we have covalently attached a red-light sensitive phthalocyanine photosensitizer to the MSN, surrounded by a lipid bilayer, which releases cargo upon illumination. Moreover, we have exploited the proton sponge effect during acidification of the endosome with polymer-functionalized MSNs to achieve endosomal release. Taking advantage of overexpressed matrix metalloproteinases in cancer tissue, we have also achieved spatially selective extracellular release of bioactive molecules.

Organically functionalized MSNs were developed to reversibly adsorb oligonucleotides such as siRNA at high loading and with high cellular activity upon release. Moreover, cellular uptake of proteins such as small antibody fragments was successfully achieved using a novel release mechanism from MSNs.

An important feature of the MSNs regards their biodistribution and their fate after successful drug delivery. Our recent quantitative analysis of the dissolution kinetics of different types of multifunctional MSNs demonstrates that their dissolution rate can be tuned over a wide range, leading to lifetimes of under one hour to several weeks in aqueous media. In contrast, these MSNs are stable in ethanol for long periods exceeding two years.

Recently, we have expanded the scope of mesoporous materials to mesoporous metal phosphates with very high surface area and the ability to dissolve in the acidic cytosol. Their intracellular behavior opens new vistas in targeted drug release with porous nanoparticles. Specifically, we will discuss a novel synthesis method for spherical, amorphous mesoporous calcium phosphate-citrate nanoparticles (CPCs) with an average size of 50 nm. Internalized CPCs dissolve once the endosomal pH turns acidic. This leads to a rapid release of Ca^{2+} ions into the cytosol. Remarkably, mesenchymal cancer cell lines are much more strongly affected by the Ca^{2+} shock and induce apoptosis at lower IC_{50} values than epithelial cancer cells, while healthy cells are not affected. These nanoparticles show promising therapeutic activity in a malignant pleural effusion (MPE) mouse model. Our results strongly suggest the possibility to efficiently treat certain lung tumors while at the same time dramatically reducing toxic side effects.

Optimizing Nucleic Acid Nanomedicines by Chemical Evolution of Carrier Sequences

Thursday, 17th October - 11:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 225

Prof. Ernst Wagner [1]

1. *Pharmaceutical Biotechnology, Department of Pharmacy, Ludwig-Maximilians-Universität, Munich*

It took five decades from first gene transfections to approval of gene therapies as medical drugs. Up to date, >2930 clinical gene therapy trials have been initiated, nine gene therapy products, one siRNA drug, and eight oligonucleotide drugs received market authorization. Intracellular delivery has been critical for the success of these therapeutic macromolecules. A further refinement of delivery carriers will have a tremendous impact for efficacy of future nanomedicines. Different chemical evolution approaches are pursued toward synthetic nucleic acid or protein nanocarriers. Our strategy focuses on a bioinspired sequence-defined process including (i) artificial amino acids active in specific delivery steps, (ii) precise assembly into defined sequences by solid phase-assisted synthesis, (iii) screening for a pre-defined delivery task and selection of top candidates, followed by random or educated variation for a next round of selection. The selected type of cargo (pDNA, siRNA, miRNA, PMOs, mRNA, or Cas9/sgRNA) directs the optimal motifs and sequences of nanocarriers.

Increased in vivo biocompatibility of PASylated nanocarriers in targeted therapy of breast carcinoma

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 12

Ms. Barbora Tesarova [1], ***Dr. Simona Dostalova*** [1], ***Mrs. Veronika Smidova*** [1], ***Ms. Zita Goliasova*** [1], ***Mrs. Hana Michalkova*** [1], ***Dr. Petr Michalek*** [1], ***Dr. Hana Polanska*** [2], ***Prof. Marie Stiborova*** [3], ***Prof. Tomas Eckschlager*** [4], ***Dr. Zbynek Heger*** [1]

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Introduction

There is an increasing trend of studying the biological identity of nanoparticles in order to improve the ability to predict their biological outcomes. Surface modifications of nanoparticles could be used to potentially speed up of their translation to the clinic. PASylation represents one of the most promising modifications of nanocarrier surface. Modification is based on PAS sequences, which are abundant on small residues of amino acids Proline, Alanine and Serine (PAS). PASylation is overcoming drawbacks of PEGylation, *e.q.* decreased biological activity of a drug after PEGylation, lack of biodegradability or inherent polydispersity, even though PEGylation is the only one clinically approved surface modification of drug by FDA for human oral, intravenous and dermal pharmaceutical use. PEGylation can be generally defined as modification of proteins, peptides or small organic molecules by covalent binding with one or more poly-ethylene glycol (PEG) chains. As a nanocarrier was chosen ubiquitous protein 22*L/2H*-ferritin (FRT), which is naturally found in human body. For the purpose of this study was used experimental drug ellipticine (Elli).

Methods

Encapsulation of Elli into FRT was realized *via* changing the pH of FRT solution, because the structure of FRT is dependent on the pH values (Fig. 1). Formed FRTElli nanoparticles were modified with PAS-10 sequences leading to formation of PAS-10-FRTElli. For *in vivo* testing was used MDA-MB-231 cell line, which represents triple negative breast carcinoma

derived from metastatic site. Female Nu/Nu mice were treated with 250 μg of Elli in the form of FRTElli, PAS-10-FRTElli and nonencapsulated Elli. Extracted organs (tumor, liver, kidney, heart and spleen) were evaluated in terms of Elli uptake, morphology (hematoxylin and eosin staining), Fe detection (Prussian blue staining), complement C3b activation and protein corona formation.

Results

Due to the prolonged circulation of PAS-10-FRTElli, higher transport efficiency in the tissue of an ectopic triple negative breast carcinoma (73% higher than in FRTElli) with a significant reduction in Elli transport to non-target tissues was achieved (Fig. 2). No morphological changes were detected after treatment with PAS-10-FRTElli nanoparticles, while unmodified FRTElli caused significant changes in tissue morphology. The use of FRT as a nanocarrier did not lead to iron loss in tissues. PASylated FRT also reduced protein corona formation and did not activate complement C3b in mice.

Discussion

Overall, *in vivo* experiments of PAS-10-FRTElli showed promising results in the anticancer therapy. Modification of FRT with PAS-10 sequences led to higher internalization of Elli into tumor while protecting non-target tissue.

The authors gratefully acknowledge financial support from the Grant Agency of the Czech Republic (GACR 17-

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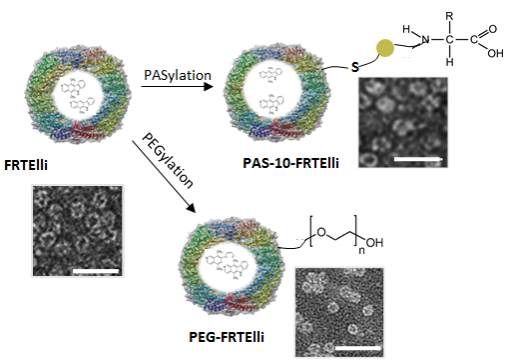

Fig 1 schematic representation of preparation and layout of modified frtelli nanoparticles.png

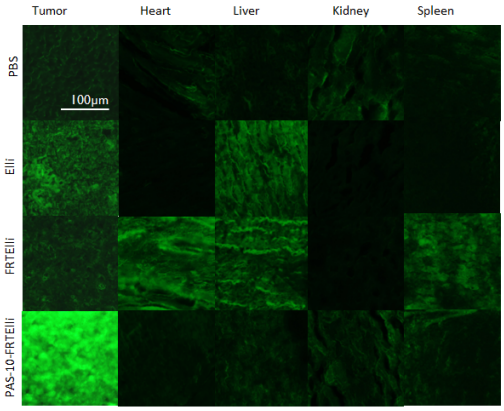

Fig 2 internalization of elli into target and non-target tissue.png

Positively charged human recombinant ferritin as a potent tool for gene silencing

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 39

Ms. Markéta Charousová [1], ***Dr. Simona Dostalova*** [2], ***Mr. Michal Mokry*** [3], ***Dr. Zbynek Splichal*** [1], ***Dr. Vladimir Pekarik*** [4], ***Dr. Zbynek Heger*** [2]

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Introduction

Use of horse spleen ferritin (EcaFtLH) as a suitable nanocarrier is an often studied approach in nanomedicine. Among many benefits belongs small size, ability to disassemble and reassemble itself in pH dependent manner, and ability to encapsulate various molecules. However, it has a low encapsulation efficiency for negatively charged molecules, such as siRNA. Therefore, we produced recombinant human H-chain ferritin (HsaFtH), and its derivative HsaFtH-RK, which has a positively charged sequence on E-helix at the *C*-end formed by arginine and lysine. The experimental results confirmed its ability to encapsulate negatively charged molecules.

Methods

Structure of ferritins was analysed by transmission electron microscopy (TEM) and by dynamic light scattering (DLS). The amount of encapsulated non-coding fluorescently labelled siRNA was detected by agarose electrophoresis. Cell experiments were performed using HEK293 cell line. Internalization of ferritins with encapsulated siRNA was detected by flow cytometry and fluorescence microscopy. Finally, silencing of Bcl-2 using encapsulated anti-*Bcl-2* siRNA was evaluated using western blotting.

Results

Spherical structures with size of approximately 12 nm were detected by TEM after encapsulation of non-coding siRNA. The encapsulation efficiency was in both ferritins above 85 %. However, agarose electrophoresis revealed a different mode of association between non-coding fluorescently labelled siRNA and ferritins. Whereas siRNA was encapsulated inside HsaFtH-RK, it is mostly linked to the external surface of EcaFtLH. Long-term stability test was performed in two solutions – in water and in solution that simulates human plasma. The size was constant for at least 24 hours (measured by DLS) and percentage of lost siRNA was under 20 % for both ferritins. The main difference in ferritins was in their ability to internalize encapsulated siRNA inside HEK293 cells. Both ferritins were able to internalize inside cells (HsaFtH-RK with higher efficiency), but only Hsa-FtH-RK was able to deliver siRNA inside cells. Functional analysis with anti-*Bcl-2* siRNA revealed a successful, 54 % silencing of *Bcl-2* using HsaFtH-RK.

Discussion

We were able to create and produce recombinant human ferritin with a suitable size and ability to encapsulate negatively charged siRNA inside its cavity. The encapsulation efficiency and further stability are more than sufficient for gene silencing. The intensity of internalization of HsaFtH-RK inside cells, as well as the *Bcl-2* silencing efficiency confirm our expectation that this ferritin could be a potent nanocarrier for negatively charged molecules.

The authors gratefully acknowledge financial support from the GAČR project 17-12816S and AF-IGA2019-IP044 and League Against Cancer Prague. M. Ch. is a Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.

Protein nanoparticle-based inhibition of DNA methylation for the treatment of atherosclerosis

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 115

Mrs. Ana C. Marquez-Sanchez [1], ***Mr. Alejandro Manzanares-Guzman*** [2], ***Dr. Dannia Colin-Castelan*** [1], ***Ms. Dalia Rodriguez-Rios*** [2], ***Mr. Enrique Ramirez-Chavez*** [2], ***Dr. Jorge Molina Torres*** [2], ***Dr. Gloria Barbosa-Sabanero*** [1], ***Dr. Agustino Martinez-Antonio*** [2], ***Dr. Yolanda Alvarado-Caudillo*** [1], ***Dr. Ramon Carriles-Jaimes*** [3], ***Dr. Gertrud Lund*** [2], ***Dr. Lino Sanchez-Segura*** [2], ***Dr. Silvio Zaina*** [1]

1. *University of Guanajuato,* ***2.*** *CINVESTAV-IPN Irapuato,* ***3.*** *Center for Research in Optics*

Introduction. We omit technical details in order to protect a patent application. Epigenetic mechanisms participate in the etiology of atherosclerosis. Particularly, the DNA of the atherosclerotic lesion is hypermethylated in the initial and intermediate phases of the disease in comparison with the histologically normal vascular tissue of the same patient. Therefore, we aim to design nanoparticles to deliver a DNA methylation inhibitor (DMI) selectively to macrophages, a critic cell type in atherogenesis. For this purpose, the DMI is encapsulated in human protein nanoparticles (HP-NPs) functionalized with an exposed peptide that specifically binds to a macrophage internalization receptor (PP1). HP-NPs were tested in human THP-1 macrophages and in APOE-null mice, a model of hyperlipidemia-induced atherosclerosis.

Methodology. HP-NPs were synthesized by the desolvation method. Morphometric characteristics were evaluated by transmission electronic microscopy, and the Z potential and PDI by Dynamic Light Scattering. The efficiency of drug encapsulation (EE) was determined by HPLC. Functionalization with PP1 was verified by dot blot and immunogold.

The internalization kinetics were analyzed by multiphotonic microscopy and flow cytometry in THP-1 macrophages, stimulated with HP-NPs with and without DMI and the peptide for 15 min, 30 min and 4h. HP-NPs were detected by eosin-Y staining, and SynaptoRed C2 and DAPI were used for the detection of cellular membranes and nuclei, respectively.

Five-month-old APOE-null mice fed with high fat diet were treated with HP-NPs corresponding to 0.19µg/g DMI, or vector (deionized water, pH 7.4) by subdermal injection for 8 weeks. Whole mount aortas were stained with oil red O to identify lipid-rich lesions. The lesion relative area (% of the luminal surface occupied by lesions) was measured with the image J software. Groups were compared with a Mann-Whitney U test. Glucose, cholesterol and triglycerides were measured at the end of the treatment.

Results. HP-NPs had an average diameter of 320.6+149.61 nm, a PDI of 0.9+0.1 and a Z potential of -44.6+0.8 mV. The EE was 22.6%. We observed a fastest HP-NPs uptake functionalized with PP1, relative to not functionalized counterparts. The relative lesion area in HP-NPs-treated mice (n=10) was 8.7+0.16%, corresponding to a ~44% reduction in comparison with controls (15.5+0.4%; n=10; $p<0.001$). HP-NPs did not exert any effect on glucose and blood lipids, nor caused any macroscopic adverse effects.

Discussion. Functionalization with the PP1 peptide accelerated HP-NP uptake by THP-1 macrophages, already after 15 a minute-stimulation. The data suggest an interaction between the DMI and PP1 that further promotes internalization. In vivo, the effectiveness of HP-NPs in reducing atherosclerotic lesion area, confirms that the DNA methylome is a promising target in cardiovascular prevention and therapy.

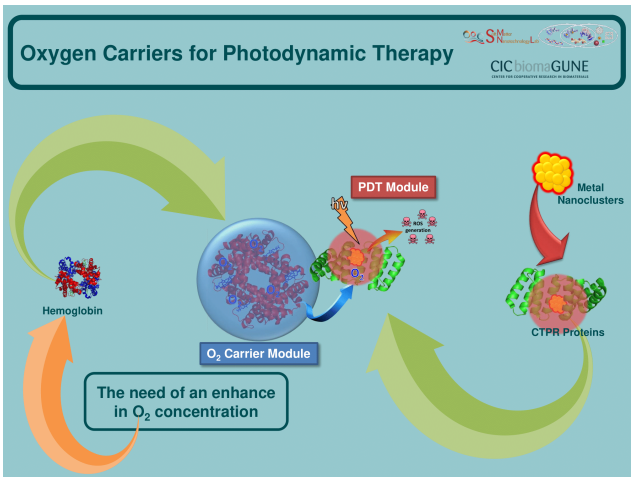
Protein-based nanocarriers for photodynamic therapy

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 127

Dr. Eduardo Guisasola [1], ***Prof. Aitziber L. Cortajarena*** [1], ***Dr. Sergio Moya*** [1]

1. *CIC Biomagune*

Introduction:
The ability of nanocarriers to target specific tissues offers a great potential in the development of advance therapies in medicine, helping to solve drawbacks from the current standard medical approaches. Nanocarries combine cargo ability with targeting capacities that are certainly helpful to find efficient and selective therapies. Photodynamic therapy (PDT) has been showed to be an effective treatment for earlier stages cancer and can selectively destroy tumor tissues with a minimal invasive procedure. Clinical studies demonstrate that PDT can be applied in cycles and as cotreatment of surgery, radio and chemotherapies. Besides, no mechanisms of resistance to PDT have been described. The fundamental elements involved in PDT are a light source, a photosensitizer moiety, and the oxygen availability.
The mechanism of action of PDT is driven by the light irradiation of the photosensitizer that generates an oxidative stress by reactive oxygen species (ROS) generation that provokes a significant damage to cell structures. Unfortunately, the lack of oxygen within hypoxic tumor regions can decrease the efficacy of this therapy. In addition, the perfusion of small molecules into necrotic tumors is hindered by the internal pressure of these tissues. Taking into account these issues, we have designed protein-based hybrid nanomaterial based on the stabilization of photoactivatable nanoclusters (NCs) on engineered proteins as photosensitizers, in combination with hemoglobin as oxygen transporter.
Methods:
Different approaches are applied for the protein encapsulation. Layer by layer technique with biocompatible polyelectrolites ensures the hemoglobin encapsulation free of covalent bonding and therefore unrestricted movement for oxygen release. Also a radical polymerization was explored to keep the protein free of covalent bonding by encapsulation within nanogel particles. Recombinant protein expression technology is used to produce and isolate engineered tetratricopeptide repeat proteins (TPR) with specific mutations to create a preferential metal coordination sites. NCs are grown on the protein scaffold by reduction different metal salts.
Results:
Polymer-hemoglobin capsules with different sizes were generated and their protein entrapment efficiency and oxygen release performance were evaluated. The recombinant proteins were designed via incorporation of a cluster coordination site. The results support the stabilization of metal NCs within the protein scaffold, which showed tunable fluorescence and biocompatible properties besides that promoted the internalization in cells. Moreover, the NCs have photocatalytic properties which make them able to generate ROS as common photosensitizers.
Discussion:
In the oxygen carrier module a polymeric capsule protects the hemoglobin structure from release in bloodstream, to ensure the delivery of the oxygen captured by the protein once the nanostructure targets the tumor tissue. The photosensitizer module is based on repeat protein modules as scaffolds for the synthesis of metallic nanoclusters. The main role of this module is to generate ROS species inside the cancer cells through its metallic NCs catalytic activity. The fluorescent properties of metallic nanoclusters allow the protein tracking. Furthermore, these recombinant proteins allow the expression of homing peptides to address specific targets.



General scheme-1.png

pH driven enhancement of drug loading on iron oxide nanoparticles for drug delivery in macrophages.

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 163

Ms. Karishma Cotta [1], ***Prof. Rajdip Bandyopadhyaya*** [1], ***Prof. Sarika Mehra*** [1]

1. *Indian Institute of Technology Bombay (IITB)*

Introduction

Nanoparticles play an important role in modern pharmaceutics, wherein their small size and large specific surface area have been found to greatly improve drug delivery. Understanding the drug-nanoparticle interaction can help increase drug coating, in turn overcoming limitations of toxicity, while facilitating drug delivery. In our work, we explore the interaction of norfloxacin (NOR) and iron oxide nanoparticles (IONPs) at different pH and also the effect of norfloxacin coated iron oxide nanoparticle (NOR@IONP) systems on drug uptake in macrophages.

Methods

Synthesis and Characterization:

IONPs were synthesized by co-precipitation of $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$, in presence of ammonia, followed by magnetic separation and washing. Norfloxacin, a class of anti-tubercular drug, was coated onto the nanoparticles at either pH 5 ($NOR@IONP_{pH5}$) or at pH 10 ($NOR@IONP_{pH10}$) with subsequent centrifugation. Finally, pellets were dried and stored as powders. Nanoparticles were characterized for their physical properties and drug coating.

Drug uptake:

Differentiated THP 1 cells were treated with either free drug or drug coated nanoparticle, at same drug concentrations, and incubated for 48 hrs. Post treatment, cells were washed and lysed. Internalized NOR concentration was estimated through fluorescence spectroscopy (excitation/emission wavelengths: 280/420 nm).

Results

$NOR@IONP_{pH5}$ and $NOR@IONP_{pH10}$consisted of 50 to 100 nm aggregates of 8 to 12 nm sized individual nanoparticle (Figure 1a, 1b). These exhibited a zeta potential of + 29 and – 16.5 mV, respectively.

Absence of COO^- and OH vibrational spectra in FTIR data indicates that drug-nanoparticle interaction could be via COO^- of NOR (Figure 1c, 1d). A shift in the fluorescence spectra of NOR on NOR@IONPs, further confirms this observation. A pH of 5 facilitated this interaction; we observed a higher drug coating of 50.2 ± 7.3 µg/mg of the nanoparticle, as opposed to a coating of only 6.5 ± 2.1 µg/mg of the nanoparticle, achieved at pH 10. As desired, a slow drug release profile was achieved in $NOR@IONP_{pH5}$, which was not affected by external pH (Figure 2a, 2b).

The uptake of NOR in differentiated THP1 cells is a low amount of 0.62 ± 0.19 pg/cell, on treatment with 32 µg/mL of extracellular NOR. Coating of NOR onto IONPs enhances uptake by 20 or 28 fold when coated at pH 5 or pH 10, respectively (Figure 2c). However, due to very low drug loading at pH 10, the Fe concentrations required to achieve such a drug concentration is itself high (Figure 2d).

Discussion

Alteration of pH during drug coating is a possible route, whereby zwitterionic drug loading can be increased on IONPs; e. g. norfloxacin coating being facilitated at lower pH. Interactions occur between the positive charge on the nanoparticle surface and the negative COO^- group present in the drug. Such drug-nanoparticle interaction slows down drug release in time, which is independent of the release medium pH. This system in turn enhances the delivery of antibiotics to macrophages, while also allowing us to overcome the limitation due to nanoparticle toxicity. Application of such a system can be realized in the treatment of intracellular pathogens as in phagocytized Mtb.

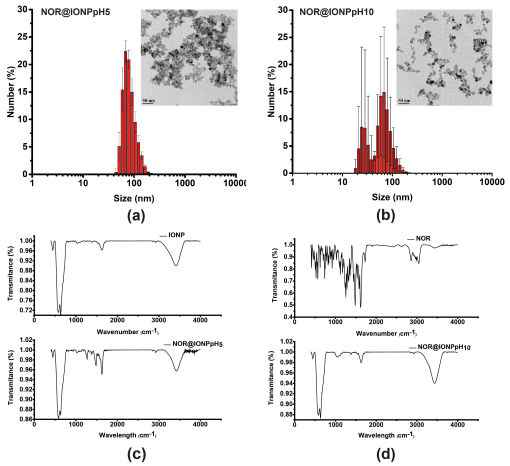


Figure 1. characterization of drug loaded ionps synthesized at ph 5 and ph 10.jpg

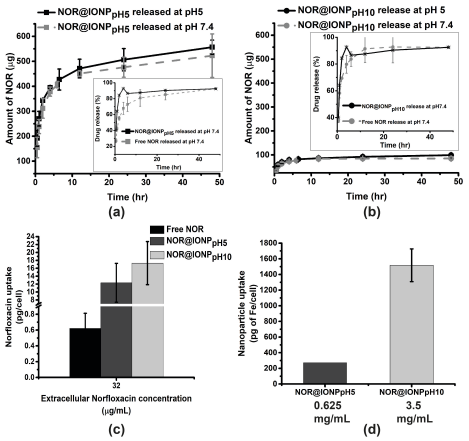


Figure 2. drug release and drug or nanoparticle uptake.jpg

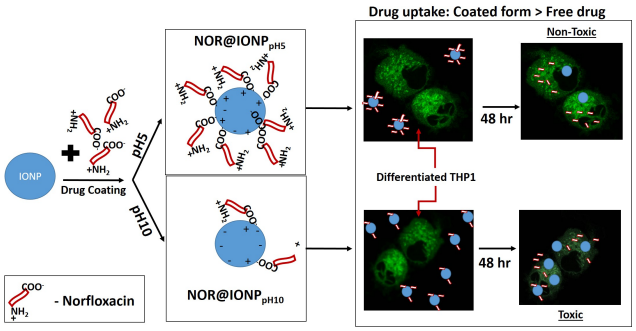


Figure 3. overall process schematic.jpg

Use of antibodies modified with catalytic Au/Pt nanoclusters in immunoassays

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 177

Mrs. Verónica Mora Sanz [1], ***Dr. Laura Saa*** [2], ***Dr. Nerea Briz*** [3], ***Dr. Valery Pavlov*** [2]

1. *CIC biomaGUNE/ Tecnalia,* ***2.*** *CIC Biomagune,* ***3.*** *Tecnalia*

Nanoclusters (NCs) have attained great attention in the last few years due to their size dependent optical and chemical properties[1]. Nowadays a large number of methods using proteins as scaffold have been developed for NCs synthesis. Up to now any synthetic method has been described using an antibody as scaffold. Its structure, highly like that of proteins, makes antibodies suitable biomolecules to act as templates for the incorporation of NCs. Usually the synthetic conditions for the synthesis of NCs stabilized with proteins require extreme conditions of pH or temperature. These conditions cause the denaturalization of the proteins.

In this work we present the first method for the synthesis of catalytic NCs using antibodies as scaffold and the first immunoassay carried out using antibodies modified with NCs. The synthesis of antibodies modified with NCs is performed under physiological conditions, which do not affect the antibody structure. The resulting antibodies still maintain the affinity for target antigens.

Bimetallic nanoclusters composed by Au and Pt were chosen as candidates for the modification of an antibody due to its catalytic properties[2]. The peroxidase-like activity of the NCs can be measured by monitoring the colour change of the chromogenic substrate TMB in presence of H_2O_2(Figure 1.).

The NCs have been characterized by TEM, MALDI-TOF and circular dichroism. The results indicate that the NCs have a mean diameter less than 2 nm (Figure 2.), the NCs are bound to the antibody and the antibody secondary structure stays unalterable after the synthesis.

The Au/Pt NCs-IgG can be used as detection antibody in a direct sandwich ELISA, the concentration of the target analyte can be related with the reaction rate of TMB oxidation (Figure 3.A.). This immunoassay was compared with a conventional method used in sandwich ELISA using the primary antibody labeled with HRP (Figure 3.B.). In Figure 4. the calibration curve for both methods is shown. The limit of detection (LOD) for the ELISA that uses Au/Pt NCs IgG is 1.84 ng/mL and for the conventional method 52.03 ng/mL.

In conclusion, this study provides the first method for the synthesis of bimetallic NCs using antibodies as scaffolds. The physiological conditions using during the synthesis make that the antibody structure remains unalterable after the modification, thus antibody still have affinity for its target analyte. The Au/Pt NCs have catalytic properties with the chromogenic substrate TMB. The Au/Pt NCs-IgG can be used as detection antibody in a direct sandwich ELISA and the concentration of the target analyte can be related with the reaction rate of TMB oxidation. In comparison with the conventional method using an IgG labelled with HRP the LOD is improved 28 times.

1. Li-Yi Chen, Chia-Wei Wang, Zhiqin Yuan, and H.-T. C. Fluorescent Gold Nanoclusters: Recent advances in Sensing and Imaging. *Anal. Chem.* **87**, 216–229 (2015).

2. Feng, J., Huang, P. & Wu, F. Gold–platinum bimetallic nanoclusters with enhanced peroxidase-like activity and their integrated agarose hydrogel-based sensing platform for the colorimetric analysis of glucose levels in serum. **142**, 4106–4115 (2017).

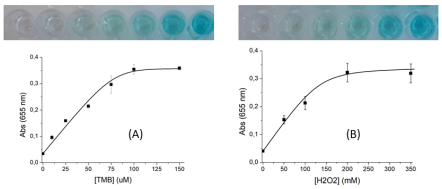


Figure 1. intensity of absorbance peak 655 nm at different a tmb and b h2o2 concentration..png

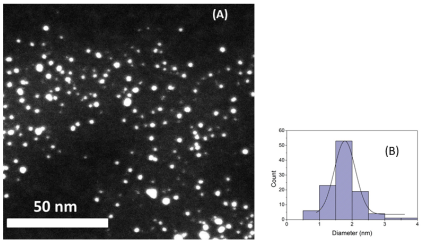


Figure 2. a typical stem image and b size distribution of aupt ncs igg..png

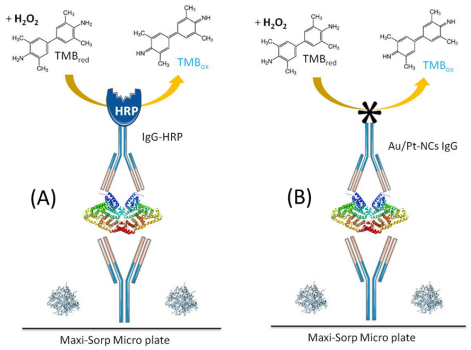


Figure 3. scheme of elisa sandwich performed with detection antibodies labelled with different catalytic elements. a igg labelled with hrp. b igg labelled with aupt ncs..png

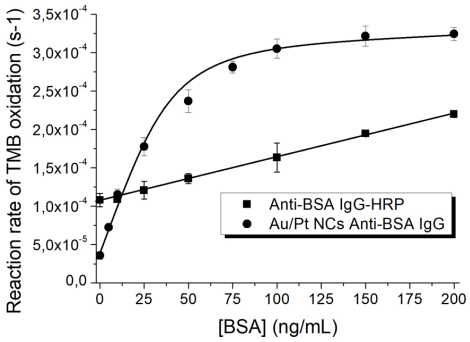


Figure 4. calibration curve of the direct sandwich elisa system based on igg-hrp and aupt ncs igg..png

Characterization and evaluation of graphene in cellular interactions and infection prevention as future application

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 188

Ms. Lucila Menacho [1], ***Mrs. Jacqueline Zarria*** [2], ***Dr. Angel Bustamante*** [2]

1. *Universidad Nacional de Ingenieria,* ***2.*** *Universidad Nacional Mayor de San Marcos*

In response to the wide spread of microbial contamination induced by bacterial pathogens, the development of novel material as graphene with remarkable bactericidal activity on a wide range of bacteria activity is of great interest [1,2]. In addition to this, graphene has been already reported as a preventive of infections in open wounds as new proposals to treat patients immediately, avoiding bleeding and septicaemia conditions [3]. In this study, E. coli (ATCC 13706) bacteria were used to check the growth inhibiting activity of Graphene particles in DMF. Graphene particles concentrations of $0{,}2x10^{-2}$ mg/ml and $0{,}1x10^{-2}$ mg/ml was tested in a bacterial culture medium. DMF has shown inhibiting activity of 33% whilst for two concentrations of Graphene particles diluted in DMF was obtained bacterial growth inhibiting of 65%. It shows that at low concentrations, graphene manages to inhibit bacterial growth by itself, demonstrating its potential use in gauze sponges or surgical tapes to protect open wounds from infections. We have already investigated the graphene by X-Ray diffraction (XRD), Fourier transform infrared (FTIR), field emission scanning electron microscopy (FE-SEM), thermogravimetric analysis (TGA) and Raman spectroscopy (for more details see Figure 1).

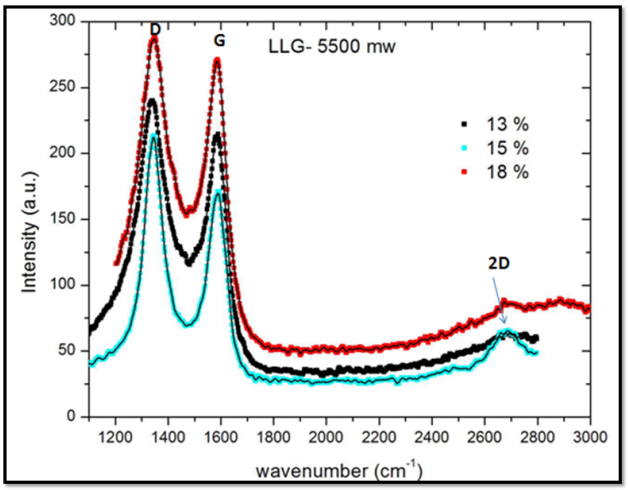


Figure 1.png

Supramolecular Amphiphiles of Beta-Cyclodextrin and Oleyl Amine for enhancement of Vancomycin delivery

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 205

Mr. Mohammed Salih [1], ***Dr. Calvin Omolo*** [2], ***Dr. Nikhil Agrawal*** [1], ***Dr. Pavan Walvekar*** [1], ***Dr. Ayman Waddad*** [1], ***Dr. chunderika Mocktar*** [1], ***Dr. Charlotte Ramdhin*** [1], ***Prof. Thirumala Govender*** [1]

1. *University of Kwazulu Natal,* ***2.*** *United States International University*

The global threat of antimicrobial resistant strains calls for innovative strategies to utilize nano drug delivery systems to enhance the delivery of antibiotics, thus reducing the development of resistance. Supramolecular amphiphiles that can self-assemble into nanostructures are one such nano delivery system, that are showing potential for effective drug delivery. The aim of this study was to synthesize and formulate a novel sugar-based cationic amphiphile (BCD-OLA) derivative from a Beta-cyclodextrin (BCD) head and long C18 carbon chain with a terminal amine; oleyl amine (OLA), using inclusion complexation for application in antibiotic delivery. A suspension method was used for preparation of the BCD-OLA amphiphile, which was then utilized for the formulation of nanovesicles. The complexation of BCD-OLA was confirmed by FTIR, NMR and molecular dynamic (MD) simulations. Thereafter, biosafety was evaluated using the *in vitro* MTT cytotoxicity assay. Size, zeta potential (ZP), polydispersity index (PDI), entrapment efficiency, *in vitro* drug release and antimicrobial activity of BCD-OLA-loaded nanovesicles was also evaluated. MD of the BCD-OLA simulation showed that the mechanism responsible for amphiphile formation was through hydrophobic inclusion of OLA in BCD. MTT results showed cell viability of 75-100%, thus affirming biosafety of BCD-OLA complex. TEM images showed the self-assembled structures to be vesicles. The formulated nanovesicles size was shown to be 119.8±1.12 nm with a PDI of 0.220±3.98, and ZP of 25.8±6.96 mv. The encapsulation efficiency of vancomycin was 40.2±4.5%. Vancomycin release from the nanovesicles was found to be sustained, with an 80% release over a 48h period. The *in vitro* antibacterial test showed that the BCD-OLA had a 2- and 4-fold lower minimum inhibitory concentration (MIC) against *Staphylococcus aureus* (SA) and Methicillin-resistant *Staphylococcus aureus* (MRSA), compared to bare vancomycin. Further, intracellular and macrophage studies showed that the system had a 459-fold reduction of intracellular bacteria using infected human embryotic kidney cells (HEK), and an 8-fold reduction in infected macrophages, in contrast with the bare vancomycin. These discoveries affirmed the potential of the BCD-OLA complex as a promising biosafe effective nanocarrier for antibiotic delivery

Transcriptomic response of primary human hematopoietic stem cells to graphene quantum dots

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 235

Mr. Stefan Fasbender [1], ***Ms. Lisa Zimmermann*** [1], ***Dr. Ron-Patrick Cadeddu*** [2], ***Dr. Martina Luysberg*** [3], ***Mr. Bastian Moll*** [1], ***Prof. Christoph Janiak*** [1], ***Prof. Thomas Heinzel*** [1], ***Prof. Rainer Haas*** [2]

1. *Heinrich-Heine-Universität Düsseldorf,* ***2.*** *Universitätsklinikum Düsseldorf,* ***3.*** *Forschungszentrum Jülich*

Introduction: Graphene quantum dots (GQDs) consist of one or a few layers of graphene. They are used in biomedical research not only for drug delivery applications, but also for long term and deep tissue imaging, cancer diagnostics and intracellular sensing [1,2]. All of these applications require profound knowledge about the response of the cells to GQDs. Here we report the cellular gene expression of primary human CD34+ hematopoietic stem cells after exposure to GQDs [3].

Methods: Fluorescent GQDs were synthesized using the recipe of Qu et al. [4] with slight modifications. They were characterized with fluorescence spectroscopy, UV-vis, XPS, AFM, TEM and Raman spectroscopy. Primary human hematopoietic stem cells were collected from leukapheresis products of in total seven healthy individuals. The cells were exposed in vitro to a high concentration (500 µg/ml) of GQDs for 36 hours. The uptake of GQDs into the cells was measured by flow cytometry and validated by confocal fluorescence microscopy. Total RNA was extracted using the Qiagen RNeasy Mini Kit and gene expression analysis was performed using Affymetrix Clariom S assays.

Results: According to XPS, the GQDs consist of 62 % C, 19 % O and 19 % N atoms. They have an average diameter of 3.3 nm and present with a graphene-like, hexagonal crystal structure. At 405 nm excitation, they show a broad fluorescence emission around 460 nm. Confocal fluorescence microscopy shows an accumulation of GQDs near the nucleus of hematopoietic stem cells after an incubation period of 36 hours. Gene expression analysis reveals that only one, namely the selenoprotein W, 1 of 20 800 recorded gene expressions is significantly changed by the GQDs and intracellular selenoprotein W measurements show a decrease of intracellular selenoprein W content. A meta-analysis, which takes into account the more prominent effects by cell culture, reveals that the expression of 1171 genes with 8 corresponding signaling pathways is weakly affected. [3]

Discussion: We conclude that our GQDs show only marginal effects on the transcriptome as well as low toxicity. These results are in line with recent observations that the GQD are encapsulated in the endosomal and lysosomal system of the cells [1], which might protect the cells from possible toxic effects of the GQDs. This situation may be useful for some diagnostic or therapeutic applications, while for others, endosomal release, and the corresponding toxicity studies thereafter, would be required.

[1] Kersting et al., *Nanotechnology*, **2019**, 30, 395101 https://doi.org/10.1088/1361-6528/ab2cb4

[2] Shen et al., *Chem.Commun.*, **2012**, 48, 3686-3699 https://doi.org/10.1039%2FC2CC00110A

[3] Fasbender et al., *Scientific Reports*, **2019**, 9, 12028 https://doi.org/10.1038/s41598-019-48567-6

[4] Qu et al., *Light: Science and Applications*, **2015**, 4, e364 https://doi.org/10.1038/lsa.2015.137

Liposomes Decorated with G-Quadruplex Decoy Oligonucleotides: Their Nanoparticle Delivery and Efficient Bioactivity in Pancreatic Cancer Cells

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 249

Prof. Erik Pedersen [1], ***Prof. Stefan Vogel*** [1], ***Prof. Luigi E. Xodo*** [2], ***Dr. Ulla Jacobsen*** [1]

1. *University of Southern Denmark,* ***2.*** *University of Udine*

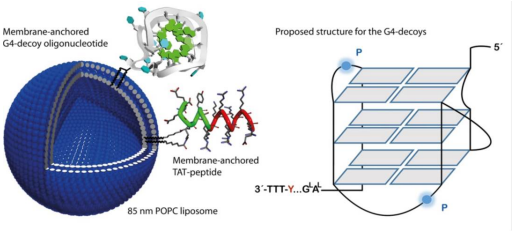
KRAS is mutated in >90% of pancreatic ductal adenocarcinomas (PDAC). As its inactivation leads to tumour regression, mutant KRAS is considered an attractive target for anticancer drugs. In this study we report a delivery strategy for a G4-decoy oligonucleotide (ON) that sequesters MAZ (myc-associated zinc-finger), a transcription factor essential for KRAS transcription. However, the challenge is delivery of G4-decoy oligonucleotides across cell membranes. We present a liposomal delivery platform and demonstrate the potency of the strategy in pancreatic cancer cells by clonogenic assays.

POPC liposomes were formulated with lipid-modified G4-ONs and TAT/R8-peptides that spontaneously anchor to the liposome surface. The molecules can move freely on the lipid surface and the G4-decoy can efficiently interact with the target proteins.[1-2]The membrane anchor of the G4-decoy consists of two palmityl chains (membrane anchor **Y**).[3]ODN-1 and ODN-2 are designed with: (i) the sequence of truncated G4-proximal comprising G-runs 2-3-4-5, (ii) two para-TINA (**P**) units to stabilize the unimolecular folding of the G4-ON and a membrane anchor **Y** followed by thymidines at the 3' end to prevent ON self-aggregation of the lipid-modified G4-Ons (Figure 1).

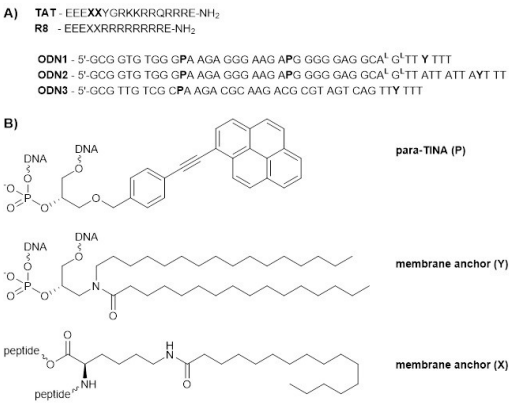
G4-decoy oligonucleotides inhibit KRAS transcription by a decoy mechanism (MAZ scavenger) and consequently the proliferation of cancer cells. Panc-1 cells were treated with the liposomes functionalized with cell penetrating peptides and ODN-1 and the G4-decoys ODN-1 and ODN-2 strongly reduced colony formation to ~10 % of the control.

The POCP liposome strategy has been extended to downregulation of miR-216b in pancreatic ductal adenocarcinoma (PDAC) using a palmitoylated amino-LNA as the lipid anchor of the oligonucleotide.[4] Together with the results from the previous work[1] this demonstrates the potential of using many different types of oligonucleotides in this delivery strategy. Also, the two very different anchors presented in these two publications shows that a large freedom is possible when selecting the lipid anchors.

1. S. Cogoi, U. Jakobsen, E. B. Pedersen, S. Vogel, and L. E. Xodo, *Sci. Rep*. **2016**, *6*, 1-13.
2. S. Cogoi, M. Paramasivam, B. Spolaore, L.E. Xodo. *Nucleic Acids Res*. **2008**, 36, 3765.
3. O. Ries, P. M. G. Löffler, S. Vogel, *Org.Biomol. Chem.* **2015**, *13*, 9673.
4. A. Ferino, G. Miglietta, R. Picco, S. Vogel, J. Wengel, L. E. Xodo, *RNA Biology* **2018**, DOI: 10.1080/15476286.2018.1526536.



Oligonucleotides and peptides anchored to liposomes.jpg



Sequences and structures of oligos with lipid anchors.jpg

Prospective Procedure for detaining Angiogenesis by using Quantum-Mechanics-theory-based electrically charged Nanodevices

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 255

Dr. Huber Nieto-Chaupis [1]

1. *Universidad Privada del Norte*

From the fact that tumorization is achieved by the so-called angiogenesis, we present a hybrid physics-based scheme by which a nano device can perform the tasks of (i) ions anomalous concentration identification, and (ii) exerting electric forces in order to create instability to the electric mechanisms that would lead to tumor formation (FIG-1). In this manner, we employ the conjunction of the concept of probability as defined in Quantum Mechanics as well as those equations derived from Classical Electrodynamics that emerges as an suitable theory that would be used to identify events where attraction and repulsión take place as previous to angiogenesis that would end in the formation ofanomalous tissue or tumor [1].

Essentially, we simulate the action of an engineered nano device [2] that through electric forces either repulsion or attraction might draw asymmetriesin contrast in clean zones, thus it would reveal the presence of apossible spatial region that is perceived as an early phase of anomalous vascularity formation. Particularly we have used the Jackson's electric potential that iswell adjusted to the morphology and spatial distribution of new vessels. We propose a physical scenario where nano particles plays the role of***in-situ*** sensor that can be able to recognize the presence of an anomalous formationof tissue.

In this paper we paid attention to the blocking probability that measures the capability of nano device to avoid ions accumulation along the sprouting process that depends on the surface ionic charge density. By knowing the blocking probability and its corresponding spatial location, we turn to achieve an approximate reconstructionof the spatial cavity of sprouting.

Essentially we use Quantum Mechanics to estimate probabilities as to block the so callled Vascular Endhotelial Growth into a theoretical approach that allows us to determine from the obtained probabilities a spatial reconstruction of the sprouting (FIG-2 and FIG-3). For this end we focus on the propagator that is derived form the usage of the evolution operator as commonly done in in Quantum Mechanic. Thus, the choice of the Hamiltonian becomes crucial to calculate the different forms of probabilities from the fact that

$\mathbf{P} = |U|x> |\mathbf{2}>$

$\mathbf{P} = | Exp-i\mathbf{H}(t-t') |x> |\hat{}\mathbf{2}$

with **H** the hamiltonian in its elementary versión and written as $\mathbf{H} = \mathbf{P}^2/2M + \mathbf{\Phi}(r,\theta,z)$ being the electric potential the source of the electric field and force so that

$E = -\nabla\mathbf{U}(r,\theta,z)$,

$F = -Q\nabla\mathbf{\Phi}(r,\theta,z)$.

Once the probability of the event is established, we pass toinsert inside of a Metropolis algorithm in order to estimate the best values of the free parameters of model.

In addition for a certain tissue and its bio-physical intrinsic parameters, the probability to detain angiogénesis reads

$\mathbf{P} = \Sigma_N$ **Sqrt[** $2M\, h/\Delta T$ **]** **Exp**[$M\, \Delta z^2 /\Delta T$] $J_N(\Delta T)$

with J_N the integer-order Bessel function.

[1] Domenico Ribatti and Silvia Baiguera, Phase II angiogenesis stimulators, Expert Opinion on Investigational Drugs, Volume 22, 2013 - Issue 9.

[2] Huber Nieto-Chaupis, Macrophage-Like Nanorobots To Anticipate Bacterial Dynamics, 2019 IEEE 9th Annual Computing and Communication Workshop and Conference (CCWC).

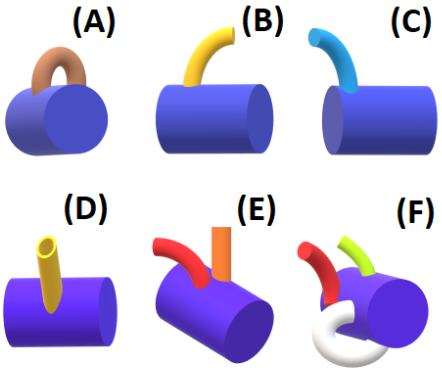


Figure-1.png

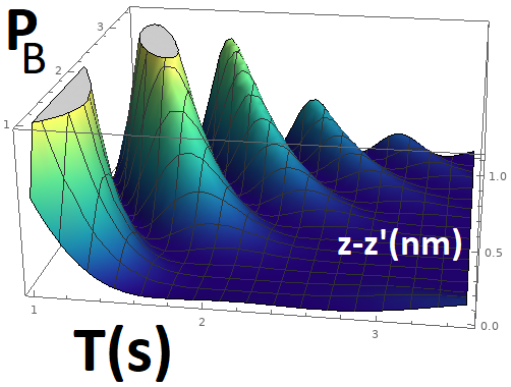


Figure-2.png

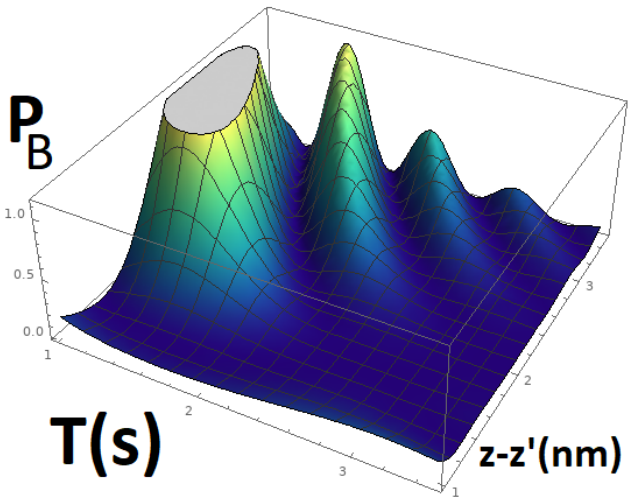


Figure-3.png

Biosynthesis of gold nanoparticles using extract of Xylopia aethiopica and evaluation of its antioxidant and anticancer properties

Thursday, 17th October - 13:30: Poster Presentations (Foyer N02.083) - Poster - Abstract ID: 189

Ms. Scholastica O. Anadozie [1], ***Prof. Saartjie Roux*** [1], ***Dr. Hajierah Davids*** [1]

1. *Nelson Mandela University*

Biosynthesis of gold nanoparticles using extract of Xylopia aethiopica and evaluation of its antioxidant and anticancer properties.

Scholastica O. Anadozie[1,2], Hajierah Davids[1], Saartjie Roux[1]

[1]Department of Biochemistry, Microbiology and Physiology, Nelson Mandela University, Port Elizabeth, South Africa.

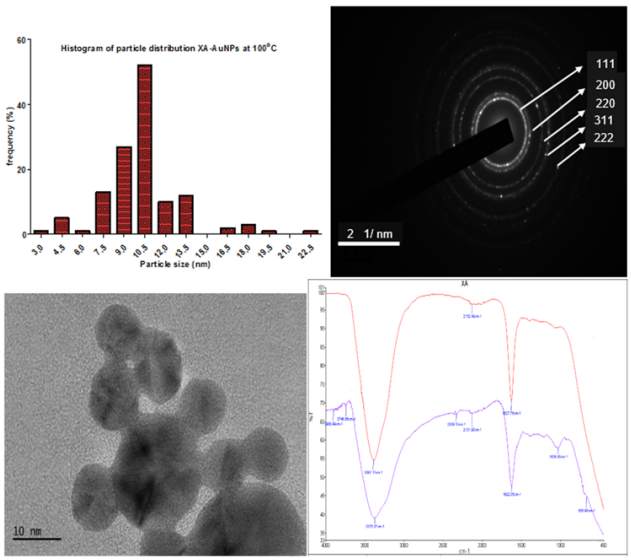
[2]Department of Biochemistry, Afe Babalola University, P.M.B 5454, Ado-Ekiti, Nigeria.

Background: Over the last few decades, the development of gold nanoparticles (AuNPs) using green approach has drawn a considerable interest in the field of nanomedicine. Its wide application in clinical diagnosis, imaging and as therapeutics portrays its importance for human existence. Antioxidant and anticancer pontential of aqueous fruit extract of *Xylopia aethiopica* (XA) biosynthesised AuNPs was investigated in this study.

Methods: The biosynthesised AuNPs were characterised using ultraviolet (UV-Vis) visible spectroscopy, dynamic light scattering (DLS), high-resolution transmission electron microscopy (HRTEM) and fourier transform-infrared spectroscopy (FTIR). *In vitro* antioxidant activities of the extract and biosynthesised AuNPs were evaluated by phosphomolybdeum, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability and ferric reducing antioxidant power (FRAP) assays. The 3,−4, 5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) viability test was used to evaluate the effect of the AuNPs on a human breast carcinoma cells (MCF-7), human colorectal adenocarcinoma cells (HT-29), human epithelial colorectal adenocarcinoma cells (Caco-2) and normal human fibroblast cells (KMST-6).

Results: The synthesised AuNPs were mostly spherical in shape with an average size of 10.61± 3.33 nm, exhibiting surface plasmon absorption maximum at 517 nm. The *in vitro* antioxidant studies showed that the biosynthesised AuNPs exhibited DPPH radical scavenging activity and ferric reducing antioxidant power potential in a concentration-dependent manner. The cytotoxicity result except for the KMST-6 cells showed a viability less than 70% at the maximum treatment concentration of 200 µg/ml.

Conclusion: The biosynthesised AuNPs showed good antioxidant and anticancer activities, thus, can be suggested as a potential therapeutic agent for breast and colorectal cancers.



So anadozie xylopia aethiopica.png

ShRNA-mediated knock-down of CD200 using the self-assembled nanoparticle-forming derivative of polyethylenimine

Thursday, 17th October - 14:15: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 48

Dr. Ali Dehshahri [1], ***Dr. Bahman Khalvati*** [2]

1. *Shiraz University of Medical Sciences,* ***2.*** *Yasuj University of Medical Sciences*

Introduction: ShRNA-mediated silencing strategy is considered to be a potent therapeutic approach. The present study aimed to assess the ability of the cross-linked LMW polyethylenimine (LMW PEI) derivative for the shRNA knock-down of the *CD200* gene on the cells obtained from the patients with chronic lymphocytic leukemia (CLL).

Methods: Since there are several investigations regarding the role of *CD200* over-expression in the progression of several malignancies (e.g., CLL), polyplexes were prepared using succinylated cross-linked PEI and the plasmid encoding anti-*CD200* shRNA. The ability of the nanoparticles for *CD200* silencing at the levels of protein and mRNA, as well as the apoptotic effects induced by unmodified PEI and its derivative, were evaluated.

Results: The results of apoptosis assay demonstrated that 92.1% of the cells remained alive after treatment with the nanoparticles based on modified PEI. In addition, *CD200* knock-down evaluations demonstrated a 50% reduction in the expression of the gene in the samples obtained from patients with CLL, while using the same formulation on the cells obtained from healthy donors decreased the $CD200^{+}$ cells up to 10%. The results of *CD200* silencing at the mRNA level revealed that the shRNA formulation could reduce the *CD200* level in the cells of the patients by 3.2-6.06-fold relative to the cells transfected with non-effective, scrambled shRNA.

Discussion: Our findings supported the application of succinylated cross-linked PEI for the down-regulation of the *CD200* gene in the upcoming attempts to develop nano-carriers for gene therapy.

Green synthesized gold nanoparticles antibacterial and anti-inflammatory agents

Thursday, 17th October - 14:30: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 50

Dr. Abdulrahman Elbagory [1], ***Prof. Ahmed Hussein*** [2], ***Prof. Mervin Meyer*** [1]

1. *University of the Western Cape,* ***2.*** *Cape Peninsula University of Technology*

Introduction: Gold nanoparticles (AuNPs) have been showing great potentials in many biomedical applications. The antibacterial activity of the AuNPs presents a therapeutic option against bacterial infections.

Macrophages and Natural Killer (NK) cells are integral part of the innate immune system. These cells produce pro-inflammatory cytokines to defend against bacterial infections. However, prolonged inflammation can be an etiology of several conditions such as rheumatoid arthritis and inflammatory bowel disease. Therefore, reducing the secretion of pro-inflammatory cytokines is an effective treatment strategy for these conditions. The immunomodulatory activity of AuNPs can make them useful in the management of prolonged inflammation caused by bacterial infections.

The synthesis of AuNPs can be achieved by a variety of physical and chemical methods. However, the drawbacks of these "conventional" methods in terms of high cost, adverse health side effects and incompatibility with the ecosystem cannot be undermined. Thus, safer and more cost-effective protocols have been reported for the synthesis of AuNPs. Plants have provided alternative synthesis methods to chemically synthesize AuNPs. Hypoxis hemerocallidea is an important medicinal plant that is indigenous to South Africa and is used in traditional medicine as an immunophytotherapy.

Methods: The biosynthesis and characterization of AuNPs from H. hemerocallidea and its major metabolite, hypoxoside, were investigated in this study. The study investigated the antibacterial activity of the biosynthesized AuNPs against several Gram-positive and Gram-negative bacterial strains using Alamar blue assay. Additionally, the immunomodulatory effect of the biosynthesized AuNPs on the pro-inflammatory cytokines production from the lipopolysaccharides (LPS) stimulated macrophages (Differentiated from THP1 cells) and NK cells (NK92 cell line) was examined using solid phase sandwich ELISA assay. Also, the study investigated the toxicity of the biosynthesized AuNPs to non-cancerous human fibroblast cells (KMST-6) using MTT assay.

Results: The study was successful to produce AuNPs from H. hemerocallidea (Hypoxis-AuNPs) and hypoxoside (Hy-AuNPs) with similar particle size of 25 nm. The Hypoxis-AuNPs exhibited antibacterial activity. The AuNPs were also found to have anti-inflammatory responses as shown by the reduction of pro-inflammatory cytokines (Fig 1). All the biosynthesized AuNPs showed no toxicity against KMST-6.

Conclusion: The data suggest that H. hemerocallidea and hypoxoside produce biologically safe AuNPs with antibacterial and anti-inflammatory activities that can be exploited in the treatment of bacterial infections and in the management of chronic inflammation.

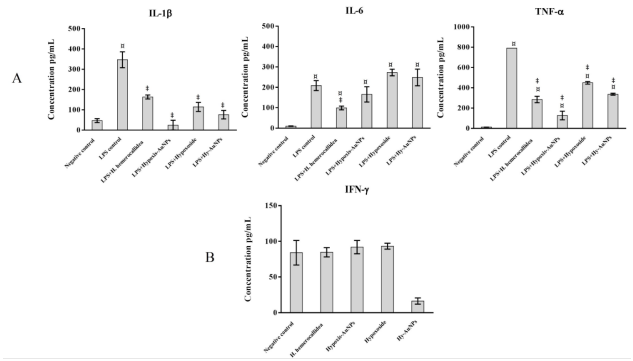


Figure 1 Quantification of cytokines release from THP1 and NK92 cells following treatment with *H. hemerocallidea* extract, Hypoxoside, Hypoxis-AuNPs and Hy-AuNPs. (A) THP1 cells were stimulated with LPS for 6 h. The LPS containing medium was then replaced by the respective treatments and the cells were incubated for another 18 h, after which the cytokine production (IL-1β, IL-6 and TNF-α) were quantified by ELISA. (B) NK92 cells were exposed to the respective treatments for 24 h, after which IFN-γ production was quantified by ELISA.
¤ Statistical significance ($p < 0.05$) compared to the negative control.
‡ Statistical significance ($p < 0.05$) compared to the 6 h treatment with LPS (LPS control).

Figure 1 abstract.png

Successful preparation of siRNA polyplexes into inhalable dry powder

Thursday, 17th October - 14:45: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 78

Mr. Tobias Keil [1], ***Prof. Olivia Merkel*** [1]

1. *Ludwig-Maximilians University Munich*

Introduction:

Enormous potential and high versatility are major characteristics of siRNA based therapies. Local administration e.g. into the lung, is ideal to minimize drug dose and side effects. To achieve lung deposition, siRNA containing nanoparticles can be embedded via spray drying into inhalable microparticles. Here, we investigate the effect of different excipients and inlet-temperatures (T-In) on polyplex size distribution, siRNA and polymer content as well as integrity and biological activity.

Methods:

For this proof-of-concept study, we used a model system for siRNA therapeutics made of siGFP and a PEG-PCL-PEI (PPP) block copolymer. Polyplexes are prepared at nitrogen to phosphate ratio of 10 in either 5 or 10% wt/wt mannitol or trehalose solutions. After dilution to 5 ml, the suspensions were spray dried at 120° or 145°C T-In. Size distribution is assessed before and after spray drying by DLS. Results are reported as peak size, particle size distribution (PSD) (distribution width) and intensity. For nucleic acid and polymer quantification, a heparin competition assay followed by SYBRgold™ dye exclusion assay and a TNBS assay were used, respectively. To confirm siRNA integrity, a 4-20% TBE gel was loaded with redispersed polyplexes upon siGFP release. Cell culture experiments were performed with H1299 expressing eGFP. For 72 h under standard conditions, cells were transfected with 100 nM siGFP and analyzed via flow cytometry.

Results & Discussion:

Comparisons of size peak, distribution width and intensity of fresh and redispersed polyplexes showed no significant difference. Due to mannitol monomer peaks appearing at 1 nm, Z average and PDI results for these experiments do not represent meaningful parameters. Polyplexes prepared with trehalose showed no significant losses in siGFP and PPP content. However, polyplexes prepared with mannitol underwent significant losses of ~20% and 53-65% for siGFP and PPP, respectively. The different T-In had no effect on trehalose formulations. Also, the siGFP losses of mannitol formulations did not differ with increased T-In. However, PPP losses were greater when mannitol formulated polyplexes were spray dried at higher temperatures. These findings suggest that trehalose through its amorphous structure is able to stabilize polyplexes to a greater extent than the recrystallized mannitol. This is underlined by DSC measurements, showing a glass transition of formulations spray-dried in trehalose whereas the mannitol formulations showed the typical crystalline melting point. Further on, gel integrity testing revealed unaffected base pair length of siRNA released from spray dried polyplexes in both formulations at both temperatures. First preliminary *in vitro* data suggest that the ability of redispersed polyplexes applied in cell culture to downregulate eGFP expression is not affected by spray drying in comparison to freshly prepared polyplexes confirming their biological activity.

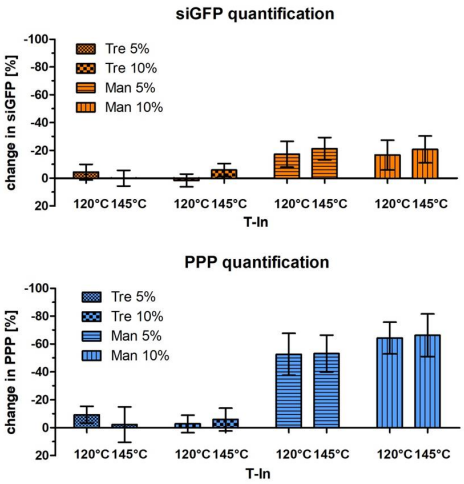


Fig-2 quantification.jpg

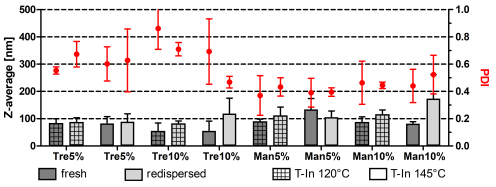


Fig-1 polyplex size analysis.jpg

Nanoparticles for in vivo genome editing mediated by crispr-cas9 delivery for undruggable KRAS driven lung cancers

Thursday, 17th October - 15:00: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 107

Dr. Aditi Mehta [1], ***Prof. Olivia M. Merkel*** [2]

1. *Faculty for Chemistry and Pharmacy, LMU Munich,* ***2.*** *Faculty for Chemistry and Pharmacy LMU*

Introduction:*KRAS* is the most frequently mutated gene in human cancers, with most mutations occurring in codon 12 or 13. However, due to its important role in cell signaling, picomolar affinity between KRAS and GTP, and the relatively smooth protein surface, KRAS has proved to be an impossible target for novel small molecule drugs, and KRAS tumors are often regarded untreatable, making it a promising candidate for nucleic acid based therapies, especially genome editing. The greatest hurdle facing nucleic acid-based therapy is its safe and efficient delivery. Since nucleic acids are negatively charged hydrophilic macromolecules, they are incapable of crossing the biological membrane on their own to reach target cells. Therefore, we aimed to engineer nanocarriers to specifically deliver nucleic acids to lung tumor cells.

Methods: KRAS G12S mutation specific guide RNAs (sgRNAs) were designed using crispr.mit.edu and cloned into the pCas9-2A-GFP plasmid (Addgene). ERBB3 affibody was overexpressed in *E.coli BL21*and purified using affinity chromatography. Low molecular weight Polyethylenimine (PEI) was coupled to the ERBB3 affibody to yield a lung cancer specific plasmid DNA (pDNA) delivery system. Characterization of ERBB3-Aff-PEI/pDNA polyplexes included measurements of size and zeta potential by dynamic light scattering and determination of pDNA encapsulation efficiency and stability in lung fluids via SYBR gold assays. Uptake and genome editing were tested *in vitro.* A549 cells and cancer hallmarks, such as migration, apoptosis, cell proliferation were tested.

Results: To achieve targeted delivery of lung cancer cells, we used affibodies against ERBB3, a member of the EGFR family. Affibodies are small, single domain proteins with high affinities to their targets and are internalized by the cells.We found high levels of ERBB3 at the membrane of lung cancer cells while little to no expression of ERBB3 in the healthy lung epithelial cells. Polyplexes of the ERBB3-Aff-PEI conjugate and pDNA showed favourable sizes smaller than 200 nm and slightly negative zeta potentials, displaying optimal characteristics for *in vivo* application.

A549 cells, known to carry a KRAS G12S mutation, were transfected with ERBB3-Aff-PEI/pDNA polyplexes, thereby deleting the mutant allele. Minimal off target effects were observed, as confirmed by targeted gene sequencing. Transfected cells also demonstrated decreased viability, proliferation and migration coupled with an increase in apoptosis, confirming the therapeutic benefit of KRAS G12S deletion.

Discussion: Our data demonstrate the successful conjugation of ERBB3 affibody and PEI resulting in an efficient lung cancer-targeted nucleic acid delivery system. ERBB3-Aff-PEI/pDNA polyplexes are successfully and preferentially taken up A549 cells, resulting in efficient deletion of the mutant allele. Using genome editing, we interfere with disease progression early-on, resulting in the direct and heritable deletion of the tumour driving mutation.

In comparison to systemic chemotherapeutic anti-cancer drugs, the direct localized administration of nucleic acids via the pulmonary route allows higher retention in lung tissues and reduces systemic toxicity for better treatments of lung cancer. Using the efficient and precise CRISPR-Cas9 gene editing approach, mutant *KRAS*alleles can be deleted resulting in sudden cessation of signaling and ultimate cell death, in accordance with the theory of Oncogene Addiction.

Enhancing cancer immunotherapy by a tumor targeted chemotherapeutic nanomedicine

Thursday, 17th October - 15:15: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 157

Dr. Yang Shi [1], ***Ms. Qingxue Sun*** [1], ***Ms. Diana Möckel*** [1], ***Prof. Fabian Kiessling*** [1], ***Prof. Twan Lammers*** [2]

1. *RWTH Aachen University Clinic,* ***2.*** *ExMI – Experimental Molecular Imaging*

Combining nanomedicines with immunotherapy has great potential to fully harness the capability of cancer immunity. Although cancer immunotherapy has demonstrated unprecedented therapeutic outcomes, this is only achieved in a fraction of patients. The low response rate of immunotherapy can be improved by nanomedicines which induce, e.g., immunogenic cell death (ICD). By such ICD effect, dying cancer cells release antigens and danger-associated molecular patterns, which improve antigen presentation and cytotoxic T cell generation.[1,2] The immunomodulatory treatment turns “cold tumors” with low immunogenicity and poor immune infiltration into “hot tumors”, in which immunotherapy (e.g., checkpoint blockade therapy) exhibits the most optimal effectiveness.[3] The ICD effect of small molecule chemotherapeutics (anthracyclines, cyclophosphamide, and oxaliplatin) is often compromised due to their very low tumor disposition, which can be improved by nanomedicine-based tumor targeting.[1] The immunomodulation effect of nanomedicines can be further improved by pharmacological microenvironment modulators increasing tumor penetration of the nano-drugs, as well as infiltrating immune cells and immunotherapeutics such as checkpoint blockade antibodies. In this study, polymeric micelles stabilized by π-π stacking with enhanced loading capacities for multiple drugs and tumor targeting efficacy[4] are engineered with varying size to achieve optimal tumor penetration, which deliver anthracycline ICD inducers and pharmacological modulators to prime tumors for potentiating checkpoint immunotherapy.

Methods: The polymeric micelles are prepared from amphiphilic polymers synthesized by free radical or reversible addition-fragmentation chain transfer polymerization with different molecular weight via the nanoprecipitation method. Various hydrophobic ICD-inducers and pharmacological tumor penetration enhancers are loaded in the micelles. The ICD induction and cytotoxicity are studied in vitro, and optimized formulations are injected in 4T1 tumor-bearing syngeneic mice to assess the immunomodulation effects and therapeutic efficacy in combination with anti-PD1 checkpoint blockade antibodies.

Results: Polymers with different molecular weights are synthesized, which form micelles with varying sizes between 30 and 100 nm. Hydrophobic compounds, (ICD-inducing) chemotherapeutics and pharmacological modulators, are efficiently loaded (loading capacity >20 wt%) in the micelles due to π–π stacking interactions. The PM induce significant ICD in vitro as characterized by calreticulin translocation and show high targeting efficiency in vivo. The micellar formulations induced complete tumor regression, which have the potential to synergize with immunotherapy.

Conclusions: Polymeric micelles with enhanced loading capacity, stability, and efficient tumor penetration are prepared, in which ICD inducers and pharmacological modulators are efficiently loaded. These micelles that prime the tumor microenvironment are potential candidates in the setting of combination immunotherapy.

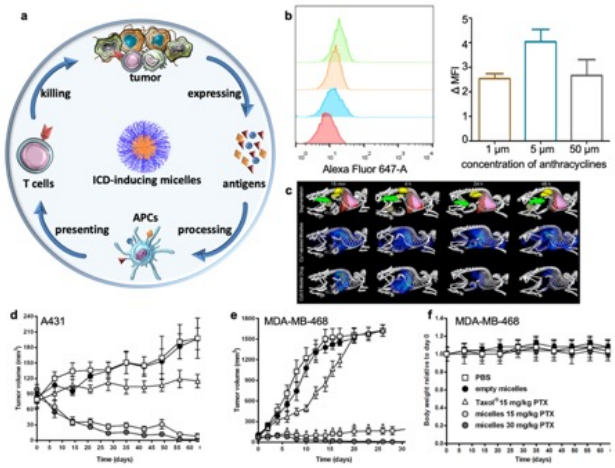
References:

[1] Shi Y, Lammers T. Combining Nanomedicine and Immunotherapy. Acc. Chem. Res. 2019, 1543-1554

[2] Sun Q, Barz M, De Geest BG, Diken M, Hennink WE, Kiessling F, Lammers T, Shi Y. Nanomedicine and macroscale materials in immuno-oncology. Chem. Soc. Rev. 2019, 351-381

[4] Shi Y, van der Meel R, Theek B, Blenke EO, Pieters EHE, Fens MHAM, Ehling J, Schiffelers RM, Storm G, van

Nostrum CF, Lammers T, Hennink WE. Complete regression of xenograft tumors upon targeted delivery of paclitaxel via π-π stacking stabilized polymeric micelles. ACS Nano. 2015, 3740–3752



Picture1.jpg

Effects of antineoplastic agents delivery by plasma membrane-derived nanoparticle on cancer cells and immunoregulatory properties on monocytes.

Thursday, 17th October - 15:30: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 161

Mr. Edson Comparetti [1], ***Prof. Valtencir Zucolotto*** [1]

1. *Physics Institute of São Carlos, USP - University of São Paulo*

A recent advance in nanoengineering uses the main components from cell membrane to camouflage the nanostructures in the organisms. Lipids and proteins are isolated from the organelles by ultracentrifugation to create nanovesicles that can be load with antitumor drugs in order to guide drug release within the tumor cells. The present study aims the development of nanocarriers to transport antineoplastic agents using the main components from plasma membrane (MNPs). Currently, studies investigate the simultaneous application of different chemotherapeutic molecules in order to improve treatments efficiency. Considering that, we synthesized lipid nanoparticles with the major components from pancreatic tumor cell membrane (PANC-1) conjugated with two first-line drugs used in clinical treatment, such as gemcitabine (GEM) and paclitaxel (PTX). First, we investigated its stability by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). Atomic force microscopy was used to study the structure and morphological changes induced by incorporated material. In cancer cell line and healthy cell, MNPs were tested *in vitro* for particle internalization, and cytotoxic activity of Gemcitabine/Paclitaxel incorporated on nanocarriers. Moreover, particles were labeled with fluorescein isothiocyanate, a fluorescent substance used to evidence internalization by target cells. In order to observe particle specificity, nanocarriers were exposed to PANC-1 cells (human pancreatic tumor), HEPA-RG cells (healthy phenotype liver cell line) and peripheral blood monocytes from healthy donors. Adhesion/internalization was investigated by flow cytometry and the cytotoxic activity of immobilized drug was analyzed by methyl tetrazolium (MTT) reduction and cell viability by Annexin V (apoptosis) and 7AAD (cell death) after 24h and 48h of incubation. The main factor that favors neoplastic cells growth and expansion is their ability to modulate tumor microenvironment and evade immune surveillance. Nanocomposites can prevent the production of immunosuppressive cytokines and increase the activity of the main mechanisms of cellular immunity. We also study the nanocarriers ability to carrier immunomodulatory agents, both inside tumor cells and immunocompetent cells. Therefore, MNPs were used to deliver antineoplastic agents to cancer cells, antigenic material to antigen presenting cells to modulate the activity of immune system, establishing a pro-inflammatory response in tumor sites. Our preliminary results indicate excellent nanovesicle stability and higher cytotoxic effects of MNPs-GEM-PTX than pure GEM-PTX and MNP-GEM, indicating that membrane nanoparticle favors the interaction with tumor cells. The most interesting finding was that in monocytes and dendritic cells membrane nanoparticle increases the expression of CD80, CD83, CD86, and HLA-DR, indicating that drug-carrier property of MNPs has a low toxic effect on normal monocytes.

Table 1. Particles size and zeta potential based on NTA and DLS data.

	pH 4.4			pH 7.4		
	Size (nm)		Zeta Potential	Size (nm)		Zeta Potential
Nanoparticles	NTA	DLS	ζ (mV)	NTA	DLS	ζ (mV)
MNPs	238.6 ± 4.0	447.17 ± 45.3	4.33 ± 0.9	187.7 ± 5.5	242.83 ± 2.1	-11.23 ± 0.7
MNPs-GEM	155.5 ± 5.4	411.17 ± 75.5	1.54 ± 0.7	130.4 ± 0.7	142.20 ± 1.9	-4.26 ± 1.6
MNPs-PTX	226.1 ± 6.8	359.70 ± 56.7	-6.92 ± 0.9	122.4 ± 1.8	139.73 ± 2.6	-5.02 ± 2.3
MNPs-GEM-PTX	268.6 ± 21.8	253.23 ± 59.2	-8.61 ± 0.7	154.2 ± 5.2	152.70 ± 3.8	-4.15 ± 1.1

Size and surface charge.jpg

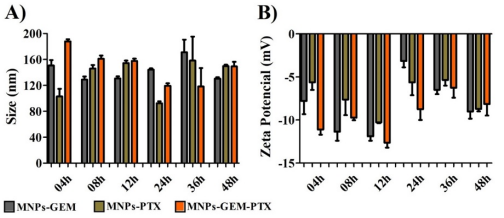


Figure 1 – Size (A) and zeta potential (B) of MNPs-GEM, MNPs-PTX and MNPs-GEM-PTX at 4, 8, 16, 24, 36 and 48 hours with DMEM 10% FBS.

Nanoparticles stability in culture media..jpg

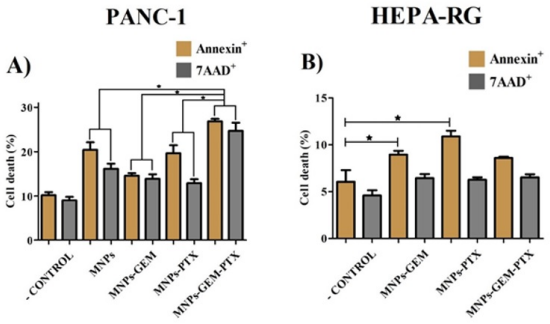


Figure 2 - Effects of modified membrane nanoparticles on viability of A) tumor (PANC-1) and B) healty cells (HEPA-RG) (Annexin V and 7AAD) after 48h exposed to MNPs, MNPs-GEM, MNPs-PTX, MNPs- GEM-PTX.

Cell death.jpg

Au@DTDTPA NPs endocytosis alters glioblastoma cell lines behavior

Thursday, 17th October - 15:45: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 176

Ms. Elodie Lelievre [1], Ms. Alicia Chateau [2], Dr. Alexandre Berquand [3], Mr. Maxime Durand [2], Dr. gautier laurent [4], Dr. Rana Bazzi [4], Prof. Stéphane Roux [4], Dr. Sophie Pinel [2], Dr. Jerome DEVY [1]

1. *URCA / UMR 7369 CNRS,* ***2.*** *UL / UMR 7039 CNRS,* ***3.*** *URCA / LRN,* ***4.*** *UTINAM / UMR 6213 CNRS*

Glioblastoma (GBM) represents the most common and aggressive primary brain tumor with a poor survival rate. Providing new therapies and new diagnostic solutions is still worldwide challenging. The standard treatment of GBM is multimodal including surgery, radiotherapy and chemotherapy. In the past twenty years, the use of nanoparticles (NPs) in cancer therapy and imaging has attracted an increased interest. In this context, we developed Au@DTDTPA(Gd) NPs. These multifunctional NPs are composed of Au core for X-ray radiotherapy coated by gadolinium chelates for MRI imaging. Considering that few data exist concerning the effects of NPs on cell behavior after endocytosis, we were interested by studying the biological effects of Au@DTDTPA(Gd) NPs on two GBM cell lines after their endocytosis. Different *in vitro* approaches such as videomicroscopy, atomic force microscopy and 3D spheroids tumor invasion assays have been applied to characterize U251 and U87 cell lines behavior as well as NPs effects. AFM experiments revealed a young's modulus (YM) of 2.23 kPa and 1.31 kPa for U87 and U251 respectively. After NPs endocytosis the YM increased in these two cell lines to reach 6.05 kPa and 4.48kPa in U87 and U251 respectively (Figure 1). Interestingly, videomicroscopy experiments demonstrated a significant effect of NPs on cell migration only on U251 cell line. In fact, NPs endocytosis induced a decrease of velocity, Euclidian distance and accumulated distance by a factor 1.8, 2.7 and 1.8 respectively. These results were confirmed by using a wound healing model as well as by a 3D spheroid tumor invasion assay (Figure 2). Indeed, forU251 cells, migration in wound healing assays was 2-fold reduced after exposition to NPs (16h post-scratching, recovery of the injury reached 16% for NPs-treated cells vs 32% for control cells) and invasive capacities were decreased of about 21%.

Collectively, our results indicate that NPs induced a stronger effect in term of the cell elasticity on U251 and U87 (an increase of YM by a factor of 3.4 and 2.7 respectively) and corroborate with a decrease of cell migration/invasion capabilities.

Above all, these results pointed out the beneficial effect of these NPs on GBM cancer cell migration independently of their potential in radiotherapy enhancement.

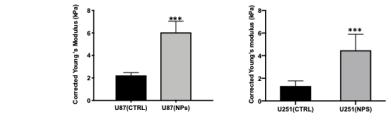


Figure 1. NPs endocytosis leads to an increase of the Young's modulus by 2.7 to 3.4-fold on U87 and U251 cells. Quantification of the average Young's modulus of U87 (left) and U251 (right) cells pretreated with NPs (gray) or not (black). *** $P < 0.001$ (t-test).

Figure1.png

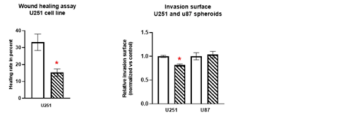


Figure 1. NPs endocytosis impairs the migration and the invasion capabilities of U251 cells. Quantification of the average healing rate of U251 cells (left) and the relative invasion surface of U251 and U87 cells (right) pretreated with NPs (striped lines) or not (white). *$P < 0.05$ (Mann Whitney-test).

Figure2.png

Quantitative analysis of nanoparticle composition using open microcavities

Thursday, 17th October - 16:00: Nanomedicine for immune system and cancer diagnosis & therapy (Amphitheatre N02.040) - Oral - Abstract ID: 203

Ms. Kiana Malmir [1], Dr. Aurelien Trichet [2], Dr. Robert Nyman [2], Dr. Benjamin Ash [2], Dr. Matteo Contino [2], Dr. Dean James [2], Prof. Claire Vallance [1], Mr. Jeremy Warren [2], Prof. Jason Smith [1]

1. *University of Oxford,* ***2.*** *Oxford HighQ Ltd*

Introduction:

Improved techniques for the characterisation, identification and analysis of nanoparticles in fluids are important in nanomedicine, with a particular challenge related to the measurement of drug load and variation of drug load on nanoparticles for targeted delivery. Here we present a new approach to the analysis of single nanoparticles using optical microcavities through which minute quantities of fluid can be flowed, allowing accurate quantitative measurements of size and composition at the single particle level.

Methods:

The microcavities are plano-concave Fabry Pérot resonators with Q factors exceeding 10,000 supporting well-characterised Gaussian modes of volume ~1 mm^3 (Figure 1). The mode is monitored via laser transmission relative to a reference cavity on the same chip to suppress noise. A nanoparticle entering the trap causes a red-shift and broadening of the mode that is dependent on the position of the nanoparticle within the confined field distribution. Monitoring these parameters at 200 microsecond intervals captures the diffusive motion of the nanoparticles revealing size information [1].

Results:

Figure 2 shows a trace of the mode displacement for a 200 nm diameter polystyrene particle diffusing in the optical trap. The magnitude of the resonance shift reveals the particle polarisability while the time autocorrelation function reveals the hydrodynamic radius, providing a useful fingerprint that is sensitive to the material composition of the nanoparticle. The technique therefore allows the differentiation of particles of similar size, as shown by the distinction of polystyrene and PMMA particle in figure 3.

Discussion:

The sensing method provides a new means for speciation of nanoparticles in fluids, or for detection of small changes in nanoparticles, such as those resulting from drug loading in nano-medicine applications. Measurement of the distribution of polarisabilities from a monodispersed reference sample of 200 nm PMMA nanoparticles in water reveals an instrument resolution of order 8% (Figure 4). Since the polarisability is proportional to the difference in density between the particle and the surrounding water, this is equivalent to a resolution of around 2% in the density of the particle.

The same device can provide enhancements to the sensing of chemicals via optical absorption, fluorescence and Raman spectroscopy. They are well-suited to integration into microfluidic systems, with the optical mode spanning the channel and thus sampling across the flow profile, offering potential for flexible chip-scale sensing at the nanoscale.

[1] A. A. P. Trichet et al, Nanoparticle trapping and characterisation using open microcavities, Nano Letters 16, 6172 (2016).

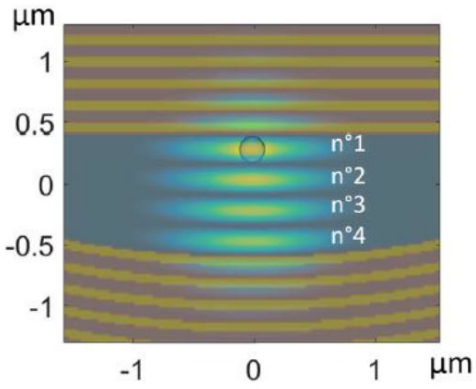


Figure 1: False colour-scale FDTD simulation of an open microcavity, showing distributed Bragg reflectors top and bottom and a confined optical field with four field antinodes.

Fig1.jpg

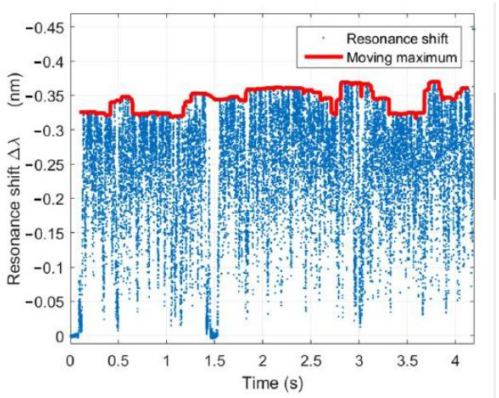


Figure 2: Mode displacement trace for a trapped 200 nm diameter particle

Fig2.jpg

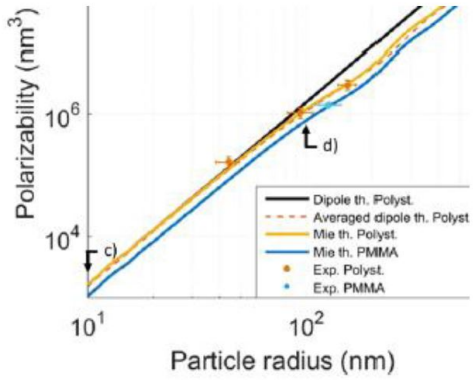


Figure 3: Differentiation of polystyrene and PMMA nanoparticles using optical microcavity analysis.

Fig3.jpg

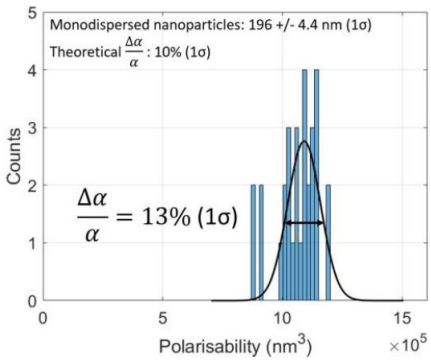


Figure 4: Measured polarisability distribution of monodisperse PMMA nanoparticles, indicating an instrument resolution of about 8%.

Fig4.jpg

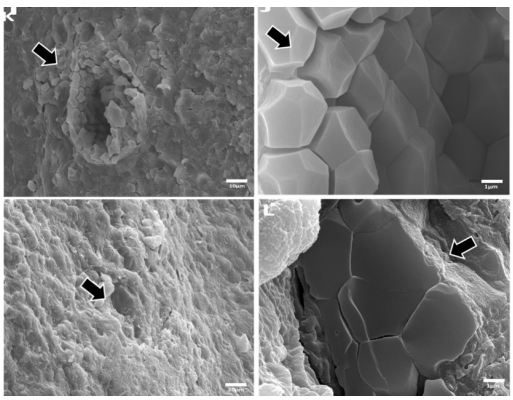
Tumor vascular heterogeneity and the impact of sub-tumoral nanoemulsion in vivo biodistribution

Thursday, 17th October - 14:15: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 123

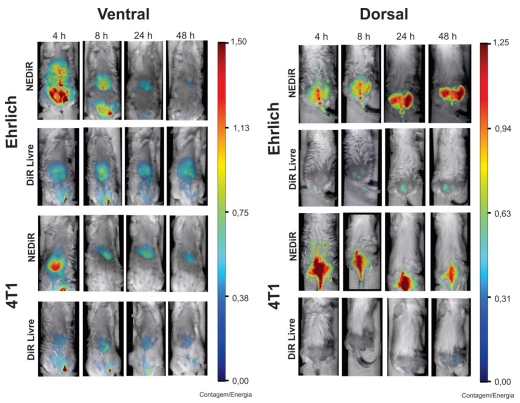
Dr. JOAO PAULO LONGO [1], ***Mrs. Jaqueline Vaz Oliveira*** [1]

1. *University of Brasília*

Tumor growth and dissemination leads to increased expression of angiogenic factors, inducing the formation of an altered vascular network, which supports its survival and expansion, as well as providing a transport pathway to metastasize tumor cells. The idea of nanomedicine is to offer effective therapies to help compounds stay in circulation longer and accumulate in tumors instead of healthy tissue. Much of these therapies are based on the idea that particles accumulate in tumors due to a phenomenon in which blood vessels present fenestrations that facilitate the preferential passage to the tumor tissue and remain in place longer due to inefficient lymphatic activity, event known as Permeability Effect and Enhanced Retention (EPR). However, many researchers have questioned this premise because of the difficulty of drug-loaded nanoparticles to demonstrate greater efficacy in clinical trials. Thus, the study of how tumor vascular structures affect the accumulation of nanoparticles in tumor tissues is a topic of interest to the scientific community. The aim of this study was to study the vascular vascular structure of two experimental carcinoma models (Ehrlich carcinoma and murine mammary adenocarcinoma) and to analyze their influence on the biodistribution of nanoemulsion containing the fluorescent marker DiR (1,1'-dioctadecyltetramethyl indotricarbocyanine Iodide). To this end, imaging and morphological analysis techniques such as scanning electron microscopy, computerized microtomography, molecular fluorescence tomography, vascular permeability (evans blue), histology and immunohistochemistry (CD31 and KI67) were used to study how blood vessels behave in the fourth week of tumor development. Using these techniques, it has been observed that even the two carcinoma lines being at a similar stage of development and with the same anatomical location, exhibit distinct vascular behavior within and between tumor types so that this affects the perfusion dynamics and vascular permeability and in the passive biodistribution of the nanoemulsion into tumor tissue. Thus, it is recommended that for a better clinical application in therapies using nanostructured materials, the biological behavior of the tumor should be evaluated individually so that the most appropriate strategy for the treatment can be selected and, consequently, the clinical success in nanomedicine is improved.



Mev.jpg



In vivo.jpg

Effect of the antimicrobial silver nano-particles on microbes at different replicating or non replicating stages.

Thursday, 17th October - 14:30: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 186

Ms. Archi Ghosh [1], ***Dr. Mahua Ghosh Chaudhuri*** [2], ***Prof. Prasanta kumar Maiti*** [1]

1. *Dept. of Microbiology,Institute of Post Graduate Medical Education and Research , Kolkata.,* ***2.*** *School of Materials Science and Nano Technology, Jadavpur University,Kolkata*

Introduction:

Conventional antibiotics have selective target of action during stages of cell replication. But in some clinical situations, the dormant & non-replicating microbes poses a serious therapeutic problem either in treatment refractoriness in case of biofilms or longer requirement of treatment during conventional antibiotic therapy. Silver nanoparticles (AgNPs) having multiple targets of action and higher permeability with efficacy irrespective of cell replication may overcome these limitations.

Methods:

I) Three different types of colloidal AgNPs had been prepared capped with tri -sodium citrate , Carboxy Methyl Cellulose (CMC), poly vinyl pyrrolidone (PVP) and characterized for physical properties along with antimicrobial properties against *E.coli, S. aureus, P.aeruginosa, C.albicans*(reference strains & clinical isolates).

II) MIC values of *E.coli and P.aeruginosa* for amikacin ; *S. aureus* for penicillin and C. albicans for fluconazole were determined. Similarly respective MIC values of all organisms for different tested AgNPs were determined.

III) Microbial suspention was stored at refrigeration temperature (0-4 °C) to arrest replication.

IV) At respective 4 MIC concentrations of AgNPs and relevant antibiotics were added to broth cultures of each organism adjusted at o.5 McFarland standard in two sets .One was incubated for 24 hours at 37 ° C and another at 0-4 ° C . After 24 hours, both the refrigerated samples and incubated samples were washed thrice with sterile phosphate buffer saline, by passing through membrane filter, then reconstituted in respective culture medium and adjusted to 0.5 McFarland standard .All tubes were incubated at 37 ° C for 24 hours. Viability of organisms was checked by subculture on blood agar medium.

V) Transmission electron microscopic analysis was also performed to note presence of AgNPs after washing.

VI) Dead and viable cell assay were done by flow cytometry both for directly incubated and washed refrigerated microbes.

Results:

Prepared Citrate, CMC and PVP capped silver nanoparticles showed average size of 10 nm , 9.5 nm, 20nm respectively with triangular shape.

Total killing of microbial cells with all antimicrobials including AgNPs were noted at 37 ° C incubation, whereas almost all organisms were intact in refrigerated cultures after washing antimicrobials. Subsequent incubation showed active growth of antibiotic washed refrigerated organisms while no growth for AgNP treated organisms. In flow cytometric analysis, viability of washed out antimicrobial cells was nearly 100% and there was total inhibition of cells incubated after exposure to AgNP at refrigeration temperature, whereas similarly treated mictobes with antibiotics at refrigeration shows about 95% viability.

Discussion:

Due to no replication of microbes at refrigeration temperature, conventional antimicrobial agents could not act whereas silver nanoparticles could attach, invade negatively charged microbial cells and on subsequent incubation completed cascade of reactions. Such microbial damage might occur either through ROS production or enzymatic inhibition resulting in microbial death. So, in treatment refractory biofilm colonizations, silver

nanoparticle may be effective. Unlike antibiotics as nanoparticles work on all active or dormant cell population, their prolong course with repetitive dosages may not be required.

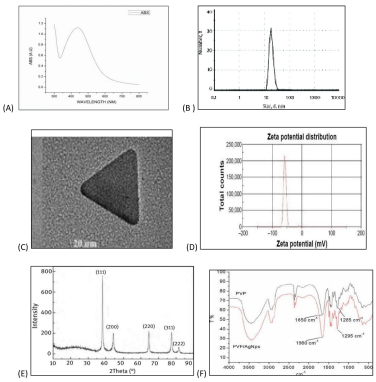


Figure 2. (A) UV-Vis absorption spectra of PVP capped silver nanoparticles at 420 nm and narrow distribution (B) TEM image of triangular shaped PVP capped silver nanoparticles, (C) Size distribution obtained from DLS measurements of PVP capped silver nanoparticles. (D) Zeta potential of PVP capped silver nanoparticles .(E) XRD of PVP capped silver nanoparticles. (F) FTIR spectrum of pure PVP and PVP capped silver nanoparticles.

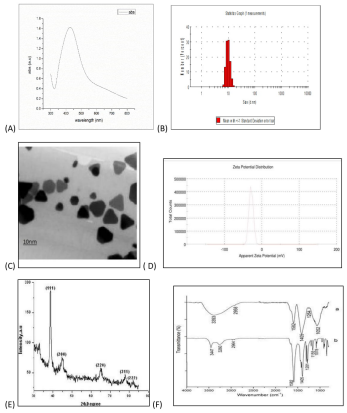


Figure 1. (A) UV-Vis absorption spectra of citrate capped silver nanoparticles at 410 nm and narrow distribution (B) TEM image of triangular shaped citrate capped silver nanoparticles, (C) Size distribution obtained from DLS measurements of Citrate capped silver nanoparticles. (D) Zeta potential of citrate capped silver nanoparticles . (E) XRD of Citrate capped silver nanoparticles. F) (a) FTIR spectrum of citrate capped silver nanoparticles. (b)FTIR spectrum of pure trisodium citrate.

Pvp figure final-1.jpg

Citrate figure-1.jpg

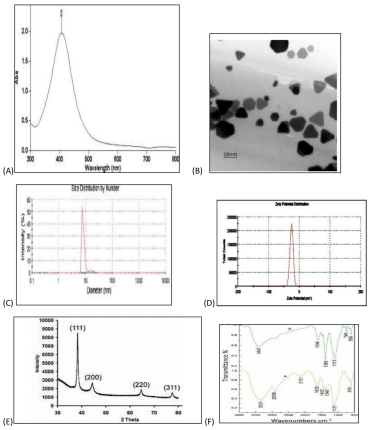


Figure 3. (A) UV-Vis absorption spectra of carboxy methyl cellulose capped silver nanoparticles at 409 nm and narrow distribution [Reference 5](B) TEM image of triangular shaped carboxy methyl cellulose capped silver nanoparticles [Reference 5] , (C) Size distribution obtained from DLS measurements of carboxy methyl cellulose capped silver nanoparticles. [Reference 5] (D) Zeta potential of carboxy methyl cellulose capped silver nanoparticles[Reference 5] .(E) XRD of carboxy methyl cellulose capped silver nanoparticles.(F)(a)FTIR spectrum of pure Carboxy methyl cellulose;(b) FTIR spectrum of carboxy methyl cellulose capped silver nanoparticles.

Table1:Anti-microbial enhancement of CMC , Citrate , PVP capped silver nanoparticles by shifting MIC following nano conversion:

AgNPs	Organism	MIC of colloidal AgNP	MIC of AgNO3 solution	"Bonus effect"
CMC Capped silver nanoparticles.	*S.aureus* ATCC 43300	1/512 dil(~ 0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 fold
	S.aureus MDR	1/512 dil(~ 0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	E. coli ATCC 25922	1/512 dil(~ 0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	E.coli MDR	1/512 dil(~ 0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	C.albicans ATCC 10231	1/1024 dil (~ 0.025 mg/ L)	1/2 dil(13.2 mg/L)	512 folds
	C.albicans MDR	1/1024 dil (~ 0.025 mg/ L)	1/2 dil(13.2 mg/L)	512 folds
	P.aeruginosa ATCC 27853	1/512 dil(~ 0.05 mg/ L Ag)	1/4 dil(6.1 mg/L)	128 folds
	P.aeruginosa MDR	1/512 dil(~ 0.05 mg/ L Ag)	1/4 dil(6.1 mg/L)	128 folds
Citrate capped silver nanoparticles.	*S. aureus ATCC 43300*	1/256 dil (~ 0.1 mg/ L Ag)	1/2 dil (13.2 mg/L)	128 folds
	S.aureus MDR	1/256 dil(~0.1 mg/ L Ag)	1/2 dil(13.2 mg/L)	128folds
	E. coli ATCC 25922	1/512 dil(~ 0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	E. coli MDR	1/512 dil(~ 0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	C.albicans ATCC 10231	1/1024 dil (~ 0.025 mg/ L)	1/2 dil (13.2 mg/L)	512 folds
	C.albicans MDR	1/1024 dil (~ 0.025 mg/ L)	1/2 dil (13.2 mg/L)	512 folds
	P.aeruginosa ATCC 27853	1/256 dil(~ 0.1 mg/ L Ag)	¼ dil(6.1 mg/L)	128 folds
	P.aeruginosa MDR	1/256 dil(~ 0.1 mg/ L Ag)	1/4 dil(6.1 mg/L)	128 folds
PVP capped silver nanoparticles.	*S. aureus ATCC 43300*	1/512 dil (~ 0.05 mg/ L Ag)	1/2 dil (13.2 mg/L)	256folds
	S. aureus MDR	1/512 dil(~0.05 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	E.coli ATCC 25922	1/256 dil(~0.1 mg/L Ag)	1/2 dil(~ 13.2 mg/L)	128 folds
	E. coli MDR	1/256 dil(.~ 0.1 mg/ L Ag)	1/2 dil(13.2 mg/L)	128 folds
	Calbicans ATCC10231	1/1024 dil (~ 0.025 mg/ L)	1/2 dil (13.2 mg/L)	512 folds
	C.albicans MDR	1/1024 dil (~ 0.025 mg/ L)	1/2 dil(13.2 mg/L)	512 folds
	P. aeruginosa ATCC 27853	1/512 dil(~ 0.1 mg/ L Ag)	1/2 dil(13.2 mg/L)	256 folds
	P.aeruginosa MDR	1/512 dil(~0.1 mg/L Ag)	1/2 dil(13.2 mg/L)	256 folds

Cmc physical data final-1.jpg

Table.jpg

Nanoplatforms for the design of engineered biopolymer nanostructures for therapy and multimodal imaging applications

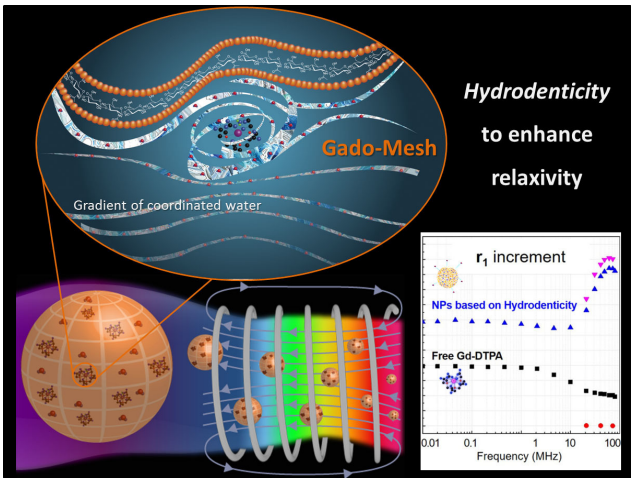
Thursday, 17th October - 14:45: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 265

Dr. Enza Torino [1]***, Prof. Paolo Antonio Netti*** [2]

1. *Fondazione Istituto Italiano di Tecnologia - Center for Advanced Biomaterials for HealthCare - Napoli, Italy,* ***2.*** *University of Napoli Federico II*

Recently, rational design of a new class of contrast agents (CAs), based on biopolymers (hydrogels), have received considerable attention in Magnetic Resonance Imaging (MRI) diagnostic field. Several strategies have been adopted to improve relaxivity without chemical modification of the commercial CAs, however, understanding the MRI enhancement mechanism remains a challenge. Furthermore, the combination of more Imaging Modalities(Multimodal Imaging)[1, 2] and its integration with the therapy (Theranostics)[2] is acquiring more attention because it allows integration of the strengths of individual modalities while overcoming their limitations and provides more accuracy in the response. A multidisciplinary approach is used to highlight the basic principles ruling biopolymer-CA interactions in the perspective of their influence on the relaxometric properties of the CA. Changes in polymer conformation and thermodynamic interactions of CAs and polymers in aqueous solutions are detected by Isothermal Titration Calorimetry (ITC) and later, these interactions are investigated at the molecular level using NMR to better understand the involved phenomena[3]. Furthermore, different strategies based on microfluidic, high-pressure homogenization and calorimetry are presented enabling the production of different complex architectures[4-6] with multimodal imaging and theranostic properties.The effect of the hydration of the hydrogel structure on the relaxometric properties and its application to the nanomedicine field, is exploited. The effect that results from the complex equilibrium established by the elastic stretches of polymer chains, water osmotic pressure and hydration degree of GdCAs has been called Hydrodenticity. A comparison in vitro and in vivo among different techniques and architectures is possible regarding impact of the interaction between the biopolymers and the tracers to design rationally further nanovectors with improved properties and able to overcome many biological barriers[6-11].

1. Key, J.; Leary, J. F. *International Journal of Nanomedicine***2014,** 9, 711-726.
2. Xie, J.; Lee, S.; Chen, X. Y. *Advanced Drug Delivery Reviews***2010,** 62, (11), 1064-1079.
3. De Sarno, F.; Ponsiglione, A. M.; Russo, M.; Grimaldi, A. M.; Forte, E.; Netti, P. A.; Torino, E. *Theranostics***2019,** 9, (6), 1809-1824.
4. Ponsiglione, A. M.; Russo, M.; Netti, P. A.; Torino, E. *Interface Focus***2016,** 6, (6).
5. Russo, M.; Bevilacqua, P.; Netti, P. A.; Torino, E. *Scientific Reports***2016,** 6.
6. Torino, E.; Aruta, R.; Sibillano, T.; Giannini, C.; Netti, P. A. *Scientific Reports***2016,** 6.
7. Torino, E.; Auletta, L.; Vecchione, D.; Orlandella, F. M.; Salvatore, G.; Iaccino, E.; Fiorenza, D.; Grimaldi, A. M.; Sandomenico, A.; Albanese, S.; Sarnataro, D.; Gramanzini, M.; Palmieri, C.; Scala, G.; Quinto, I.; Netti, P. A.; Salvatore, M.; Greco, A. *Nanomedicine: Nanotechnology, Biology, and Medicine***2018,** 14, (2), 483-491.
8. Vecchione, D.; Grimaldi, A. M.; Forte, E.; Bevilacqua, P.; Netti, P. A.; Torino, E. *Scientific reports***2017,** 7, 45121-45121.
9. Russo, M.; Ponsiglione, A. M.; Forte, E.; Netti, P. A.; Torino, E. **2017**.
10. Russo, M.; Grimaldi, A. M.; Bevilacqua, P.; Tammaro, O.; Netti, P. A.; Torino, E. **2017**.
11. Vecchione, D.; Aiello, M.; Cavaliere, C.; Nicolai, E.; Netti, P. A.; Torino, E. *Nanomedicine***2017,** 12, (18), 2223-2231.



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Drug delivery to solid tumors using nano-sized carriers: the mistakes we make.

Thursday, 17th October - 15:00: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 243

Dr. Timo L.M. ten Hagen [1]

1. *Erasmus MC*

Introduction

Delivery of active compounds to tumors is met by a number of hurdles, which are important reasons for failure of therapy. Chemotherapy is intrinsically toxic, not only to tumor cells but also healthy tissue. Additionally, most therapeutics are not tumor specific, distribute throughout the body and only a marginal amount arrives at the target. Increasing the dose is not an option because of the dose-limiting toxicity. Encapsulating drugs in nanoparticles shields toxicity, impairs distribution and clearance, and may improve accumulation at the tumor site. Lipid-based nanoparticles have thus far been the most successful, however, clinical outcome is less favorable. There are a number of reasons which affect nanoparticle-chemotherapeutic performance which need to be understood before we can start to formulate better nano-therapeutics.

Methods

Here we constructed several nanoparticles (NPs) of 90 nm, made of lipids in a composition specific for the proposed treatment: 1) standard, stable lipid-based NPs, pegylated and neutral of charge, 2) standard NPs, pegylated with a positive charge, 3) standard NPs pegylated with a targeting moiety, 4) trigger-sensitive NPs loaded with doxorubicin, and 5) trigger-sensitive NPs loaded with a drug with a higher uptake profile. Fate of these NPs was studied in vitro by standard assays and confocal microscopy, and intravitally in tumor-bearing mice by high resolution confocal microscopy.

Results

Administration of pegylated NPs is thought to result in higher tumor levels of the encapsulated drug due to the enhanced permeability and retention (EPR) effect. Also, it is thought that the drug is readily released from NPs outside of tumor cells. Here we show that this is not happening: NPs only distribute to tumors in low percentages and extravasation is low and heterogeneous. More so, drugs are only slowly released from standard NPs and after being taken up by tumor cells. Inside tumor cells NPs end up in lysosomes together with the drug and remain entrapped for days. Taken together, in vitro these NPs are a log-fold less active compared to free drug and in vivo, while delivery of drug is higher, availability of free drug is lower than free doxorubicin infused. Trigger-sensitive NPs have therefore been made to release contents when exposed to a trigger; here we use mild hyperthermia (41°C). When trigger-sensitive NPs (TSNPs) are used loaded with doxorubicin and mild hyperthermia is applied properly, massive intratumoral drug release can be achieved. However, we found that doxorubicin is not the best drug in this setting as a considerable amount does not enter tumor cells and diffuses back to the circulation.

Discussion

Nanotechnology and nanoparticle-mediated drug delivery to cancer has developed fast and delivered a number of clinical products. In spite of massive research results are however lagging. Results on performance of lipid-based nanoparticles indicate that better understanding of the interaction between NP, encapsulated drug, microenvironment and, if applicable, applied trigger is needed. Currently, TSNPs with doxorubicin are used in combination with radiofrequency ablation for primary liver cancer. In light of above mentioned aspects of drug delivery with NPs treatment options will be discussed.

Superparamagnetic iron oxide nanoparticles for MRI - new developments for clinical applications

Thursday, 17th October - 15:15: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 253

Dr. Rainer Tietze [1], ***Mrs. Eveline Schreiber*** [1], ***Ms. Marina Mühlberger*** [1], ***Dr. Harald Unterweger*** [1], ***Prof. Christoph Alexiou*** [1]

1. *Universitätsklinikum Erlangen, Department of Otorhinolaryngology, Head and Neck Surgery, Section of Experimental Oncology and Nanomedicine (SEON), Else-Kröner-Fresenius Stiftung-Professorship, Erlangen, Germany*

Superparamagnetic iron oxide nanoparticles (SPIONs) are of great importance in medical research due to their wide range of applications in diagnostics and therapy. The use of SPIONs as MRI contrast agents in particular has great potential since gadolinium containing contrast agents have been controversially discussed and a ban on the use of many of these substances by the European Medicines Agency was passed in 20181. We have developed a system of dextran-coated SPIONs (SEONDex) that is ideally suited for use as an MRI contrast agent.

Based on an alkaline precipitation reaction, SPIONs are in-situ coated with dextran and in a subsequent step, cross-linked with epichlorohydrin. By varying the educts, the hydrodynamic size of the particles can be well controlled. Direct correlations between particle size and relaxivity, as a quality feature for contrast enhancement in MRI, can be established. The SEONDex particles were comprehensively characterized. After the laboratory synthesis, a manufacturing process for the 2 litre scale was developed.

The particles have a size of 30 nm and a narrow size distribution, a relaxivity of 106 mM-1s-1 in T2* mode, excellent biocompatibility in vitro and in vivo and good signalling in animal experiments (Fig. 1). In addition, SEONDex is very stable and even after 2 years of storage there is no sign of agglomeration or sedimentation.

The current situation concerning gadolinium containing contrast media urgently requires the de-velopment of alternatives. Various paramagnetic complexes such as manganese or gallium are currently being tested, but cannot compensate for many disadvantages of the gadolinium that is still in use. Particle-based contrast agents made of iron oxide as developed by us can set new standards due to their convincing properties. They can be produced in large quantities in con-stant quality and offer the development perspective by selective modification of the surface with selective ligands to perform functional imaging in MRI.

Fig. 1:Wildtype mice treated with SEONDex (2.6 mg/ml Fe). T2 weighted images. From left side to the right: before, 1h after and 24h after application.

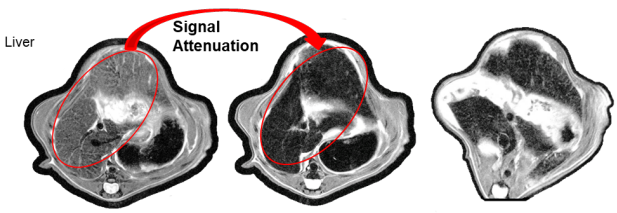


Fig. 1.png

Enhancement of Tumor Penetration and Efficacy of Liposomal Doxorubicin: What We Have Learned

Thursday, 17th October - 15:30: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 254

Dr. Leila Arabi [1]***, Prof. Mahmoud Reza Jaafari*** [1]

1. *Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad*

Introduction

Several liposomes targeting tumor have been developed to enhance the efficacy of chemotherapeutics. Despite reducing side effects and success in preclinical studies, most formulations show poor anti-tumor activity due to insufficient drug bioavailability at the tumor site. Inefficient tumor penetration caused by tumor microenvironment may be one of the main reasons. Hence, different approaches have been applied in our group to enhance the tumor penetration and therapeutic efficacy of liposomes, using penetration-promoting ligands, and modulating the tumor microenvironment.

Methods

During our research project aimed at improving of liposomal formulations, a number of liposomes were prepared to enhance either doxorubicin release or liposome uptake by the tumor cells. lipids with different melting points were used for preparation of liposomes by lipid film method to optimize the release of drug in vitro and in vivo in C26 mice model The measurement of the particles size and polydispersity index and zeta potential of liposomes was performed by dynamic light scattering (Nano-ZS;Malvern, UK). Flow cytometry (FCM) and confocal laser scanning microscopy (CLSM, Nikon Inc., Switzerland), were used to assess the cellular binding and uptake of targeted liposomal Doxorubicin. Therapeutic efficacy, biodistribution and pharmacokinetics of targeted- liposomal Doxorubicin were evaluated in mice bearing tumor. Survival data was analyzed by the log-rank (Mantel–Cox) test.

Results

Based on the FCM analyses and CSFM, liposomal formulation conjugated with monoclonal antibodies targeting the cancer stem cell markers, and liposomal formulation targeted with the matrix-modifying agents exhibited significantly higher concentration of doxorubicin inside the tumor cells and resulted in superior tumor growth inhibition in mice model.

Conclusion

Overall, using targeting ligand on the surface of liposome and optimizing lipid components, are valuable approaches resulting in a significant uptake and release of drug from liposome. These efforts have resulted in liposomes with enhanced therapeutic efficacy with ability to reach the tumor site in the more efficient manner. These results encouraged us to initiate new projects with different ligands against tumor associated antigens for development of promising liposomal Doxorubicin.

Keywords: Doxorubicin,Liposome, Targeting, Therapeutic efficacy, Tumor Penetration, tumor microenvironment

Yttrium oxide nanoparticles resolve acute pancreatitis by modulation of mitochondrial and endoplasmic reticulum stress

Thursday, 17th October - 15:45: Nanotechnology and translational medicine: from the bench to the bedside (Room N02.011) - Oral - Abstract ID: 241

Mr. Amit Khurana [1], ***Ms. Pratibha Anchi*** [1], ***Dr. Chandraiah Godugu*** [1]

1. *National Institue of Pharmaceutical Education and Research (NIPER), Hyderabad*

Introduction: Oxidative stress plays a major role in the pathology of acute pancreatitis (AP), leading to massive macrophage infiltration. Nanoyttria (NY), possesses potent free radical scavenging activity. As reactive oxygen species (ROS) and inflammation play major role in AP, we hypothesized that NY may alleviate cerulein induced AP by modulation of mitochondrial and endoplasmic reticulum stress.

Methods: The nanoparticles were characterized by dynamic light scattering, FTIR, pXRD, XPS, SEM and TEM. The anti-inflammatory potential of NY was evaluated by Griess assay and cytokines estimation whereas antioxidant effect was assessed by DCFDA, MitoSox and JC-1 staining in Raw 264.7 macrophages. The anti-pancreatitic potential of NY was evaluated at two dose levels (1 and 3 mg/kg) against cerulein challenged murine model of AP.

Results: The nanoparticles were in the size range of 159.25±7.45 nm. NY ameliorated LPS induced oxidative stress in vitro. It reduced ROS, superoxide radical generation and restored the mitochondrial membrane potential in macrophages (Figure 1). The TEM and ICP-OES based investigation indicated efficient internalization of the nanoparticles inside the cells. Interestingly, NY reduced plasma amylase and lipase levels and attenuated the mitochondrial stress and inflammatory markers. Further, NY reduced plasma amylase and lipase levels and significantly attenuated the levels of oxidative stress markers (MDA, GSH, SOD, catalase, nitrite) and significantly reduced the levels of inflammatory cytokines
(Figure 2). NY suppressed the recruitment of inflammatory cells around the damaged pancreatic acinar cells as evident from reduced expression of ICAM1 and PECAM1. Furthermore, NY intervention perturbed the course of AP via reduction of endoplasmic reticulum (ER) stress markers (BiP, IRE1 and Ero1-Lα), and molecular chaperones (Hsp27 and Hsp70) (Figure 3). Surprisingly, NY intervention showed promising effects by modulation of epigenetic modifications by H3 acetylation.

Discussion: We, to the best of our knowledge, report for first time that NY can attenuate experimental AP by restoration of mitochondrial and ER homeostasis through Nrf2/NFκB pathway modulation. The figure 4 shows the graphical representation of our novel findings.

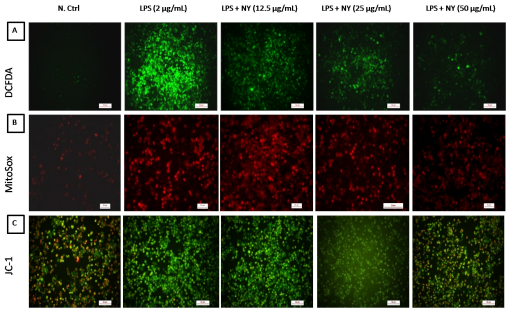


Fig 1.jpg

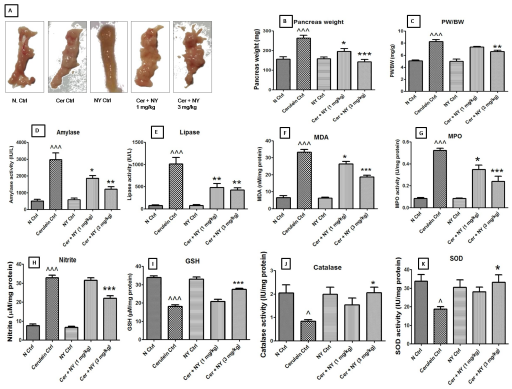


Fig 2.jpg

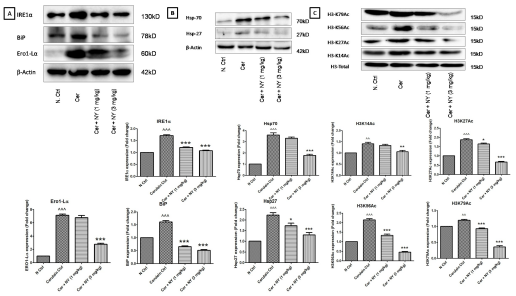


Fig 3.jpg

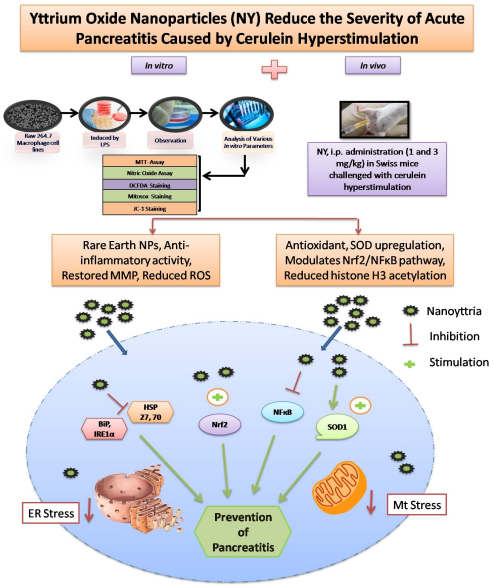


Fig 4.jpg

My MSCA-funded postdoc project: A modular nanocarrier platform for siRNA and prodrug delivery

Thursday, 17th October - 16:45: ERC + Marie Curie session: How to successfully apply for a grant (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 226

Dr. Roy van der Meel [1]

1. *Eindhoven University of Technology*

In 2015, I obtained Marie Skłodowska-Curie Action (MSCA) funding from the European Commission and a Veni grant from the Netherlands Organization for Scientific Research (NWO) to conduct a postdoctoral research project in Pieter Cullis' lab at the University of British Columbia, Vancouver, BC, Canada and Raymond Schiffelers' lab at the University Medical Center Utrecht, Utrecht, The Netherlands.

Technologies developed in the Cullis lab have enabled the clinical translation of the first siRNA therapeutic called Onpattro®, recently approved for treatment of hereditary amyloidogenic transthyretin amyloidosis[1]. Therapeutically applying siRNA is crucially dependent on delivery systems to prevent degradation and ensure cytosolic delivery in target cells. In the case of Onpattro®, lipid nanoparticle (LNP) technology facilitates siRNA delivery to hepatocytes where it inhibits the production of disease-causing mutant transthyretin protein[2].

During my postdoc project, I leveraged LNP and drug derivatization technology to develop a modular delivery platform for stable co-loading siRNA and lipophilic prodrugs[3] (*Figure 1*). As a proof-of-concept, I designed LNPs containing siRNA against the androgen receptor (AR), a fundamental prostate cancer driver, and taxane chemotherapeutic prodrugs as a combination treatment (*Figure 2*).

LNPs were composed of DSPC, cholesterol, PEG-lipid and the ionizable cationic lipid DLin-MC3-DMA for efficient siRNA encapsulation. To formulate LNPs, lipids and prodrugs were dissolved in ethanol and nucleic acids in buffer and rapidly mixed using a T-junction mixer[4] (*Figure 3*). Following physicochemical analysis, LNPs' therapeutic effects *in vitro*were determined by cell viability assays and AR target gene knockdown. Formulations with radiolabeled lipids and prodrugs were designed to determine the LNPs' pharmacokinetic parameters and biodistribution in mice.

In this interactive session, I will share my experience with grant applications, how successfully obtaining research funding has shaped my research career and how it has positively impacted my personal life.

Funding

This research was supported by funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 660426 and a Veni STW Grant (#14385) from the Netherlands Organization for Scientific Research (NWO).

References

1. Adams, D. *et al.* Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *N Engl J Med* 379, 11–21 (2018).

2. Kulkarni, J. A., Witzigmann, D., Chen, S., Cullis, P. R. & van der Meel, R. Lipid nanoparticle technology for clinical translation of siRNA therapeutics. *Acc. Chem. Res.* acs.accounts.9b00368 (2019). doi:10.1021/acs.accounts.9b00368

3. Chen, S. *et al.* Dexamethasone prodrugs as potent suppressors of the immunostimulatory effects of lipid nanoparticle formulations of nucleic acids. *J. Control. Release* 286, 46–54 (2018).

4. Kulkarni, J. A. *et al.* On the Formation and Morphology of Lipid Nanoparticles Containing Ionizable Cationic Lipids and siRNA. *ACS Nano* 12, 4787–4795 (2018).

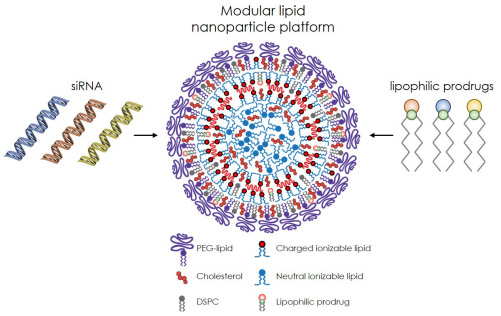
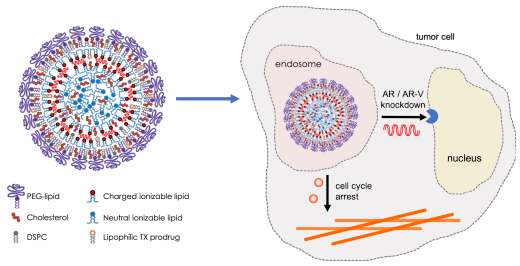


Figure 1.jpg



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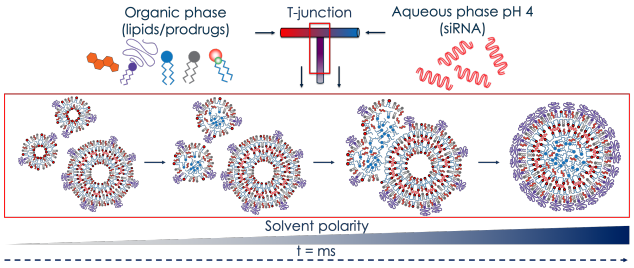

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Securing a prestigious personal grant

Thursday, 17th October - 17:45: ERC + Marie Curie session: How to successfully apply for a grant (Amphitheatre N02.040) - Workshop - Abstract ID: 283

Prof. Twan Lammers [1]

1. *ExMI – Experimental Molecular Imaging*

Obtaining a prestigious personal grant, such a Marie Curie Individual Fellowship (MSCA) or a Starting or Consolidator Grant from the European Research Council (ERC), is becoming increasingly important in academic career development. In the present lecture, I'll discuss several key considerations when intending to apply for such a prestigious personal grant, and I'll share some personal thoughts, tips, tricks, and experiences with regard to grant writing and panel interviews. The lecture is intended to be interactive and there will be ample time in the end for comments and questions.

From encoded combinatorial libraries to targeted therapeutics

Friday, 18th October - 09:00: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 22

Prof. Dario Neri [1]

1. *ETH Zürich*

Most pharmaceutical agents are either small molecules or proteins, which may display a beneficial action for patients by binding to one or more protein targets of biomedical interest. Thus, the discovery of binding molecules (be them large or small) of fundamental importance for pharmaceutical applications.

Over the past 20 years, my laboratory has mainly used two technologies for ligand discovery:

- antibody phage-display libraries (pioneered by the laboratories of Sir Gregory Winter in the UK and of Richard Lerner in California) for the isolation of fully-human monoclonal antibodies
- DNA-encoded chemical libraries for the isolation of small organic molecules, capable of binding to specific protein targets of interest

Both technologies have common feature, as they require the construction of large encoded sets of molecules (be them antibodies or small organic molecules), followed by the identification of specific ligands by affinity capture procedures on immobilized protein targets. In both cases, DNA serves as an amplifiable identification barcode.

In this lecture, I will present:

(i) how we have practiced, developed and applied antibody phage technology and DNA-encoded chemistry for the discovery of ligands to tumor-associated antigens

(ii) how we have converted ligands, capable of selective localization at the site of disease, into therapeutic agents

(iii) emerging preclinical and clinical results, obtained with targeted therapeutics developed in my laboratory, in collaboration with the Philogen group (www.philogen.com)

Nano-Ghosts: A cancer delivery platform or an inflammatory therapeutic?

Friday, 18th October - 09:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 282

Prof. Marcelle Machluf [1]

1. *Technion*

Mesenchymal stem cells (MSC), have been extensively investigated as cell carriers or cell therapies for treating a wide range of malignant and inflammatory diseases. Despite promising preclinical results, MSCs have largely failed to translate into broadly-applicable clinical application due to MSCs' susceptibility to host-induced changes, limiting their ability to deliver a long-lasting effect. Recent studies show that MSCs' preferential accumulation in malignant and inflamed tissues as well as their immunomodulatory capacity, both are largely governed by direct cell-cell interactions facilitated by membrane-bound proteins. Building on this knowledge' we have developed a scalable and cGMP compliant technology for the production of nano-vesicles from MSC membranes, and their loading with diverse bioactive compounds including proteins, small drugs, and nucleic acids. These nano-vesicles, are termed Nano-Ghosts (NGs). Our data highlight the NGs as a delivery platform that can be effectively loaded and used to selectively deliver diverse therapeutics including biological drugs, small molecules, and nucleic acids. Never the less' surprising data demonstrate that the NG by themselves can modulate inflammation via cell-cell interaction. Their abundance of natural targeting mechanisms allows the NGs to penetrate the entire tumor bulk and rapidly deploy their payload directly into the target cells' cytoplasm and nucleus led to unprecedented tumor growth inhibition and increased animals' survival in *established* metastatic lung cancer, pancreatic and prostate tumor models. The NG also interact with the cancer stem cells modulating the response of the tumor to chemotherapy. On the other hand, our studies in a mice model for Multiple Sclerosis show that the NGs can bypass the BBB and retain MSCs' tropism towards inflamed tissues and function as immunomodulators. The question arises whether these NG are acting as a smart delivery platform, which can change the inflammatory process in the tumor niche as well as in other pathologies such as MS manifested by sever inflammatory process. Our results, so far, clearly demonstrate the translational potential of NG, both as targeted carriers and as a novel immunomodulatory biologic, for oncological and immunological applications.

RNA delivery: between extracellular vesicles and lipid nanoparticles

Friday, 18th October - 10:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 270

Prof. raymond schiffelers [1]

1. *University Medical Center Utrecht*

Nucleic acids can be considered the holy grail of drug development as the active ingredient is essentially always the same, from a physicochemical point of view, only the nucleotide sequence differs. As a result the same formulation principles may be applied across many applications. In the past, electrostatic interactions between the negatively charged nucleic acids and positively charged carrier materials was used to drive complexation into nanoparticles that would aid tissue distribution and cellular entrance. The recent clinical success of OnPattro(R) has put the ionizable lipid-based lipid nanoparticles to the forefront. Although much better than many of the systems explored in the past, even these particles leave room for improvement. An exciting new development is the use of endogenous cell-derived extracellular vesicles. These nanoparticles are released by cells and contain many biomolecules, including RNA. It is tempting to speculate that these vesicles are Nature's way of delivering complex cargo between cells. In this presentation, I will discuss our experiences with both systems.

Developing Nanomedicines for Innovative Therapies - Industrial Perspective

Friday, 18th October - 11:00: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 246

Dr. Marianne Ashford [1]

1. *AstraZeneca*

There is a huge array of nanomedicines in pre-clinical research for a breadth of applications, yet few have progressed to late stage clinical development and commercialisation. This talk will focus on the drivers for nanomedicines and some of the challenges with the translation of nanomedicines into clinical development
It will share some of the learnings and experiences in exploring three very different nanoparticle systems with different drug modalities.
A case history showing the factors considered in the design & translation of a polymeric nanoparticle into clinical development will be shared.
In addition, it will highlight some of the additional challenges with intracellular delivery systems which are critical for nucleic acid-based drugs and share some of the work done exploring LNP as a delivery system for mRNA

Tuning Particle Deformability in Drug Delivery Systems

Friday, 18th October - 11:30: Plenary Speeches (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 285

Prof. Paolo Decuzzi [1]

1. *Nanotechnology for Precision Medicine, Italian Institute of Technology, Genova, Italy.*

Multifunctional nanoconstructs are particle-based nano-scale systems designed for the 'smart' delivery of therapeutic and imaging agents. The Laboratory of Nanotechnology for Precision Medicine at IIT-GE synthesizes multifunctional polymeric nanoconstructs with different *sizes*, ranging from a few tens of nanometers to a few microns; *shapes*, including spherical, cubical and discoidal; *surface* properties, with positive, negative, neutral coatings; and mechanical *stiffness*, varying from that of cells to rigid, inorganic materials, such as iron oxide. These are the *4S parameters* – size, shape, surface, stiffness – which can be precisely tuned in the synthesis process enabling disease- and patient-specific designs of multifunctional nanoconstructs. In this lecture, the role of manipulating these 4S parameters over different temporal and length scales will be elucidated in the context of future nanomedicines with applications in cancer, cardiovascular and anti-inflammatory diseases. Particular attention will be given to the role of particle deformability in modulating US-triggered drug release, vascular transport, mechanical support and the therapeutic efficacy of implantable drug delivery systems.

Inhibition of hyperactive protein kinases using targeted therapy of solid breast cancer

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 19

Ms. Zuzana Skubalova [1], ***Dr. Simona Dostalova*** [2], ***Ms. Marketa Charousova*** [3], ***Ms. Zuzana Bytesnikova*** [4], ***Dr. Jan Pribyl*** [5], ***Dr. Akila Weerasekera*** [6], ***Prof. Tomas Eckschlager*** [7], ***Prof. Marie Stiborova*** [8], ***Dr. Zbynek Heger*** [9]

1. *Department of Chemistry and Biochemistry, Mendel University in Brno,Zemedelska 1, Brno,CZ-61200,* ***2.*** *(1)Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, Brno, CZ-613 00, (2) Central European Institute of Technology, Brno University of Technology, Purkynova 123, Brno, CZ-612 00,* ***3.*** *(1)Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, Brno, CZ-613 00, (2) Central European Institute of Technology, Brno University of Technology, Purkynova 123, Brno, CZ-612 00,,* ***4.*** *(1)Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, Brno, CZ-613 00, Czech Republic, (2)Central European Institute of Technology, Brno University of Technology, Purkynova 123, Brno, CZ-612 00, Czech Republic,* ***5.*** *Central European Institute of Technology, Masaryk University, Kamenice 5/A35, CZ-62500 Brno,* ***6.*** *Biomedical MRI unit/MoSAIC, Department of Imaging and Pathology, KU Leuven, Leuven, Belgium,* ***7.*** *Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, Prague 2, CZ-128 40,* ***8.*** *Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030/8, CZ-128 43 Prague 2, Czech Republic,* ***9.*** *1) Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, Brno, CZ-613 00, 2) Central European Institute of Technology, Brno University of Technology, Purkynova 123, Brno, CZ-612 00*

Introduction

Current cancer treatment is for patients really painful, tormenting and lengthy; above all the therapy is not too specific. Due to this nonspecific treatment, patients are exposed to various serious side effects.

In our project, we focus on a targeted therapy of solid breast carcinoma by tyrosine kinase inhibitors. For these purposes, three different types of tyrosine kinase inhibitors were selected, as approved chemotherapeutic agents used in clinic. These include lenvatinib, vandetanib and cabozantinib. In spite of the fact that these agents are very effective in cancer cure, they cause a number of serious side effects, such as bleeding to the gastrointestinal tract, fistula formation or vomiting. Therefore, we encapsulated the inhibitors into protein cage ferritin and modified the protein surface by folic acid (FA) to target folate receptor-overexpressing cancer cells.

Methods

Prepared nanoconstructs with FA-surface modification were characterized using AFM, TEM, DLS, NMR and native PAGE. We tested their toxic properties*in vitro*conditions in following malignant breast cell lines: MCF-7 (PR+, ER+,HER2-, FR-), T-47D (PR+, ER+, HER2-,FR+) and also normal breast cell line HBL-100. The uptake kinetics and efficacy was assessed via flow cytometer and CLSM.

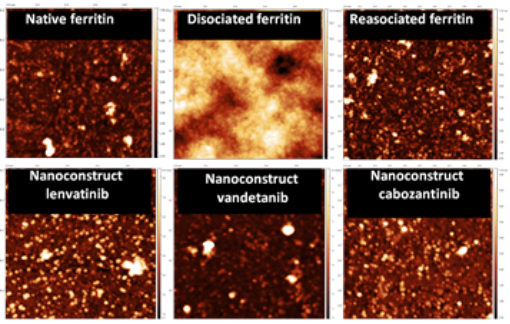
Results

It was found that formed nanoconstructs have almost spherical shape and size around 12 nm. The structure of ferritin was successfully reassociated after process of encapsulation with yield of encapsulation from 20 % to 60 %, as well as an acceptable long-term stability of 3 weeks. FA-mediated targeting fruitfully promoted uptake to the target cell lines and increased its efficacy.

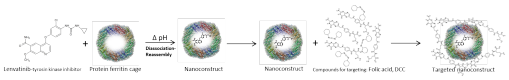
Discussion

Obtained results provide a promising basis for gaining a folate receptor-targeted nanotherapy of solid breast malignancies, with pronounced safety for surrounding healthy tissue.

The authors gratefully acknowledge financial support from the GACR project 18-10251S and AF-IGA2019-IP073, League Against Cancer Prague.



Characterization of nanoconstrut by afm atomic force microscopy .png



Scheme of preparation of folate receptor-targeted nanoconstruct carrying tyrosin kinase inhibitor.png

Gold nanocages as drug and gene delivery systems

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 47

Mr. Jamal Almowalad [1], ***Dr. Christine Dufes*** [1]

1. *Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde*

Gold nanocages are a novel class of gold nanomaterial with cage shape, hollow interiors and porous wall. Their unique optical and physiochemical properties make them an appropriate candidate for nanomedicine applications. Their hollow interiors and porous wall allow them to load and release drugs or biomolecules. In addition, gold nanocages have the ability to convert the near-infrared light to heat that can be applied into the desired tissue (photothermal therapy). They can be synthesized by the galvanic replacement reaction between silver nanocubes and cholorauric acid solution. However, only small amounts of gold nanocages (2–4 mL) can be isolated through this method. The aim of this project is therefore to optimize the synthesis of gold nanocages and to modify them with tumour-targeted ligands for increasing their drug and gene delivery efficacy to cancer cells.

We have successfully synthesized gold nanocages in uniform shape, large quantities and controlled size. All gold nanocages samples (blue, grey and teal) had particles size lower than 60 nm, which is ideal for drug delivery applications. These samples also exhibited UV-Vis spectra in the near-infrared region, which makes them appropriate for photothermal therapy. Grey and blue gold nanocages showed colloidal stability in water, phosphate buffer solution and cell culture medium. Moreover, two concentrations of grey gold nanocages (1 and 4 nM) showed minimum cytotoxicity on the melanoma cell line B16F10 s. In addition, we have modified the surface of the gold nanocages with thiol-PEG-amine to obtain positively charged PEGylated nanocages, which can be utilised to carry DNA and can be functionalized with tumour-targeting moieties. In conclusion, PEGylated gold nanocages could be a promising drug and gene nano-carrier for cancer therapy.

In vitro evaluation of siRNA loaded hNPs for the treatment of cystic fibrosis

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 105

Ms. Domizia Baldassi [1], ***Dr. Gabriella Costabile*** [1], ***Ms. Gemma Conte*** [2], ***Dr. Ivana D'Angelo*** [2], ***Prof. Francesca Ungaro*** [3], ***Prof. Olivia M. Merkel*** [1]

1. *Ludwig-Maximilians University Munich,* ***2.*** *University of Campania,* ***3.*** *University of Napoli Federico II*

Introduction

Nowadays, the downregulation of genes involved in the pathogenesis of severe lung diseases through local siRNA delivery appears an interesting therapeutic approach. In the case of Cystic Fibrosis (CF), it can be used to downregulate the NF-kβ gene, one of the most critical signals in evoking the inflammatory response in this disease. In this context, hybrid lipid/polymer nanoparticles (hNPs) can be exploited as a safe and effective pulmonary delivery system for siRNA [1].

Methods

In this project, we investigated the biological behavior and the *in vitro* effects of siRNA-loaded hNPs on human bronchial epithelial cells. The hNPs studied here consist of an inner core of poly(lactic-co-glycolic) acid (PLGA) combined with an outer lipidic corona of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DPSE-PEG), with or without polyethylenimine (PEI) as third component to assist siRNA encapsulation in the hNPs [2].

The efficacy of the hNPs was evaluated *in vitro*in terms of cytotoxicity, intracellular delivery and gene silencing ability. The cytotoxicity was tested in terms of cell viability, LDH release in the cell supernatant and pro-inflammatory effect after incubation with different hNPs concentrations and different exposure times. The intracellular delivery was measured by flow cytometry and confirmed via confocal microscopy, while the gene downregulation was determined via western blot analysis.

Results

The cytotoxicity studies consisted of MTT and LDH assays at different time points: the results of both experiments agreed that no relevant cytotoxicity was detectable for any tested formulation. We also performed an ELISA assay to check if TNF-α, a main mediator of inflammation, was released after incubation with hNPs. The ability of hNPs to deliver siRNA was investigated by flow cytometry using hNPs encapsulated with AlexaFluor647 labelled siRNA and was then confirmed by confocal microscopy. From this study, we observed that PEI plays a key role: in fact, only the formulations containing PEI as third constituent showed a significant uptake without affecting the cellular viability. Finally, the ability to downregulate NF-kβ gene was assessed via western blot analysis. From this experiment, we observed that PEI-containing formulations were able to achieve significant silencing efficacy.

Discussion

The formulations showed an overall safe profile even after long exposure times and at high concentrations. Among them, the ones containing PEI retained the best parameters in terms of cellular uptake and gene down-regulation.

In conclusion, we were able to characterize *in vitro* a formulation that could represent an interesting new therapeutic strategy for the treatment of inflammation in cystic fibrosis patients, suitable for a following *in vivo* study.

[1] I. d'Angelo et al., J. Aerosol Med. Pulm. Drug Deliv., 31, 170-181 (2018)

[2] D. Pandita, Drug Discovery Today, 20 (2015)

Preparation of water insoluble hyaluronic acid nanofiber layers for potential use in regenerative medicine

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 119

Ms. Kristýna Skuhrovcová [1], ***Ms. Adela Kotzianova*** [1], ***Mr. Ondřej Židek*** [1], ***Mr. Marek Pokorný*** [1], ***Dr. Vladimír Velebný*** [1]

1. *Contipro a.s.*

Introduction

Hyaluronic acid (HA) is for its great biocompatibility and ability to bind water often used in tissue engineering and regenerative medicine. Because of its specific behavior in water solution, its transformation into nanofiber layers or scaffolds is challenging. Uniform and homogeneous layers of oriented or non-oriented nanofibers can be produced by the electrospinning process.

A drawback of nanofibrous layers made of HA is their high hydrophilicity. As HA nanofibers instantly dissolve in water-based solutions, these materials are not suitable for longer use during medical treatment. Therefore, we optimized the electrospinning process to prepare nanofibrous layers using two different types of HA with chemically modified structure. These modifications increased its lipophilicity by acylation of UV crosslinkable (1) or fatty acid (2) groups and thus we were able to prepare water stable nanofiber layers.

Methods

Nanofiber layers made of two different HA derivatives (dHA) – furanyl HA and lauroyl HA were prepared by the electrospinning process. HA derivatives were spun along with polyethylene oxide (PEO) to enhance the electrospinning process. Various ratios between dHA and PEO as well as different degree of substitution of dHA were used, so we were able to prepare materials with various properties (e.g. swelling index). All layers were tested for their swelling index and solubility in phosphate buffer solution up to 72 hours and were analyzed for their possible structural changes during the electrospinning process and swelling tests by SEM and Raman spectroscopy.

Results and discussion

Using the UV crosslinkable dHA, we were able to prepare nanofibrous layers solely from water solution, thus there is no need to use potentially hazard solvents. The swelling behavior of such layers could be controlled by the time of exposure, the crosslinking time increase fiber diameter. In case of lauroyl HA, there is a necessity to use also other solvents, but there is no need to post process layers to make them hydrophobic. Swelling is related mainly to ratio between dHA and PEO and thus there are no other changes in fiber morphology. In both cases, our results showed the possibility to prepare homogeneous hydrophobized nanofibrous layers from both hyaluronan derivative. It was shown, that concentration of the solution, degree of substitution and ratio between hyaluronan and PEO significantly affect both the microscopic quality and application properties of nanofibrous layers.

References

1. Marcela Dušánková, Gloria Huerta-Angeles, Kristina Nešporová. Self-Supporting, Biodegradable Film Based on Hydrophobized Hyaluronic Acid, Method of Preparation and Use Thereof. US20180105610A1 Contipro a.s., 3. 9. 2016.

2. Gloria Huerta-Angeles, Martin Bobek, Eva Příkopová. Nanofibers Containing Photocurable Ester Derivative of Hyaluronic Acid or Its Salt, Photocured Nanofibers, Method of Synthesis Thereof, Preparation Containing Photocured Nanofibers and Use Thereof. US20160312385A1 Contipro a.s., 21. 11. 2014.

Utilizing Tumor Microenvironment pH to Enhance Drug Delivery by Hydrazone Containing Polymeric Micelles

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 141

Mr. Xiangyang Bai [1], ***Ms. Qingxue Sun*** [1], ***Prof. Fabian Kiessling*** [1], ***Prof. Twan lammers*** [1], ***Dr. Yang Shi*** [1]

1. *RWTH Aachen University Clinic*

Nanomedicines have shown their promise for cancer treatment, which can significantly improve drug accumulation in tumors due to the enhanced permeability and retention effect (EPR). However, after tumor accumulation, most of nanomedicines are trapped by extracellular matrix (ECM) and/or taken up by macrophage, and only a minimal portion can enter cancer cells. We attempt to tackle this issue of nanomedicine by pH sensitive nanocarriers which are rapidly degraded in the tumor tissues due to the slightly acidic pH. This strategy has been exploited previously by other pH sensitive materials, however, they mostly have incomplete or slow release at the tumor microenvironment pH or premature release at neutral pH. We design a super pH sensitive polymer with hydrazone bond linked side groups to form micelles with triggered drug release at the tumor microenvironment pH. The polymer was synthesized by reversible addition fragmentation chain-transfer (FAFT) polymerization and formed micelles in PBS. Docetaxel (DTX) could be efficiently loaded in the micelles (loading capacity>20 wt%) and the size was around 60 nm. Micellar degradation was analyzed by GPC, HPLC and DLS. Empty and DTX-loaded micelles showed complete degradation and drug release in 8-12 hours at pH 6.5 and 37 ^{O}C. Meanwhile, the micelles were stable at pH 7.4 and 37 ^{O}C for >24 hours. Furthermore, the micelles loaded with DTX or a photosensitizer induced significant cytotoxicity in 4T1 cells in 6-8 hours at pH 6.5 due to the rapid drug release rate, however, the cytotoxicity was negligible at neutral pH. In conclusion, the designed super pH sensitive polymeric micelles containing hydrazone groups are promising carriers to improve tumor drug delivery by exploiting acid-triggered drug release.

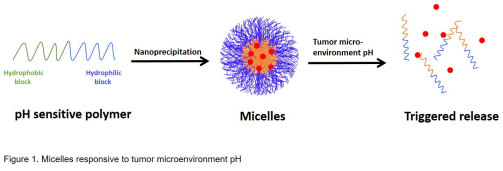


Figure 1. Micelles responsive to tumor microenvironment pH

Picture1.jpg

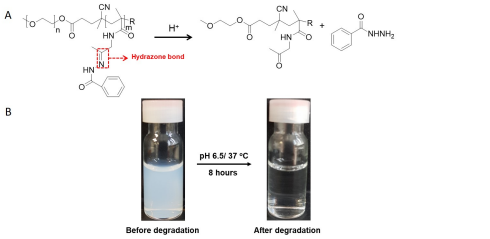


Figure 2. Degradation of polymeric micelles. A) Polymer degradation. B) Photograph of polymeric micelles before and after degradation.

Picture2.jpg

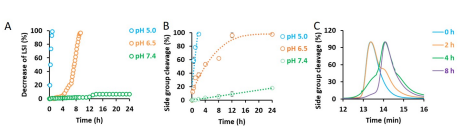


Figure 3. Characterization of polymer micelles degradation at different pHs. A) DLS. B) HPLC. C) GPC.

Picture3.jpg

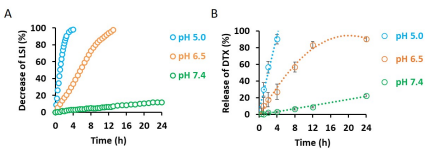


Figure 4. Characterization of degradation of DTX loaded polymeric micelles at different pHs. A) DLS. B) HPLC.

Picture4.jpg

Nanoencapsulation of chemotherapeutics in smart Polyurethane/Polyurea Nanoparticles as a novel tool to enhance cancer targeting and improve safety profile

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 166

Dr. Cristina Cuscó [1], Dr. Marta Pérez [2], Mr. Joaquin Bonelli [1], Prof. Roberto Quesada [3], Prof. Vanessa Soto [2], Prof. Ricardo Pérez [2], Dr. Josep Rocas [1]

1. *EcopolTech,* ***2.*** *University of Barcelona,* ***3.*** *University of Burgos*

Most of the current Cancer therapies derives in several problems for patients, leading to death in some cases. Novel targeted nano-therapies against different types of Cancer have been a strategic pillar in last years for Nanomedicine Community.

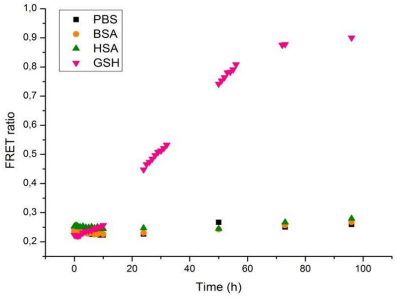
From Ecopol Tech S.L., in collaboration with Department of Pathology and Experimental Therapeutics of University of Barcelona, we are developing new one-pot and industrially scalable process for the synthesis of targeted-Nanoparticles against Lung Cancer.

These type of nanoparticles have been performed to accumulate and internalize preferentially in the acidic media of Tumor Micro-Environment (TME), as well as to release the drug inside cancer cells due to the high concentration of Glutathione, which promotes the degradation of the shell in Nanoparticles. In terms of surface charge, these Nanocapsules exhibit different behavior depending on the pH value of the media because of the presence of amphoteric moieties in the pre-designed polymer: in TME media, with slightly acidic values about 6.8-7.0 compared with physiological media value of 7.4, Nanoparticles becomes positively charged thus facilitating its penetration through cell membrane. This fact has been proved *in vitro* by internalization experiments at different pH values.

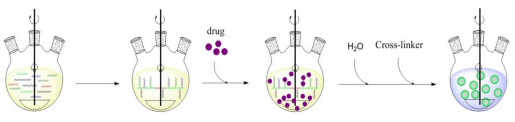
Thus, we are able to decrease the accumulation in other locations of human body and preventing the undesired release in these locations. We have tested the preferential release in different media by FRET analisys with Nanoparticles loaded with different fluorophores.

This study has been carried out with Nanocapsules loaded with Tambjamine analog. This type of drug is highly hydrophobic, hampering its administration by intravenous via. This new method of nanoencapsulation allows to disperse it and stabilize it in a crosslinked oil in water dispersion between 15-20 nm.

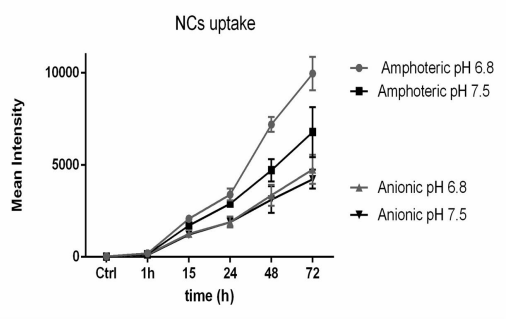
In vivo tests show a promising safety profile compared with the same doses administration program for free drug, demonstrating that encapsulation of Tambjamine analog decreases the systemic toxicity caused by the administration of free drug (which has to be administered intraperitoneally due to its poor solubility).



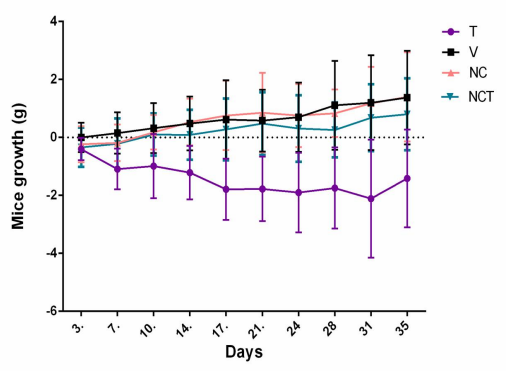
Fret-release.jpg



Synthetic-scheme.jpg



Ncs-uptake-different-ph.tif.jpg



Safety-assay.tif.jpg

Scalable flame synthesis of superparamagnetic iron oxide nanoparticles for triggered drug release from colloidal capsules

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 178

Mrs. Shno Asad [1], ***Dr. Jamal Khan*** [1], ***Prof. Christel Bergström*** [1], ***Prof. Alexandra Teleki*** [1]

1. *Uppsala University*

Introduction: Microcapsules self-assembled from colloidal particles are promising oral drug delivery vehicles for therapeutics of low gastrointestinal stability.[1] Capsule functionality can be tuned by incorporating nanoparticles with various physicochemical properties in their shell.[1] An example of such a functional nanomaterial are superparamagnetic iron oxide nanoparticles (SPION) that act as MRI contrast agents or trigger hyperthermic drug release. However, the lack of scalable and reproducible synthesis methods of SPION have hindered the successful translation into clinics. Flame spray pyrolysis (FSP) is an industrially established synthesis process that allows large scale manufacture of SPION and close control over particle properties. We have developed a single-step flame process for encapsulating SPION with a biocompatible SiO_2 coating[2,3] that facilitates their subsequent assembly into colloidal capsules. This study aims to develop colloidal capsules loaded with biological drugs for oral delivery. We coat the capsules with biopolymer layers to improve drug encapsulation efficiency and to control release properties. Finally, we explore the incorporation of functional, flame-made nanoparticles in the shell of these capsules.

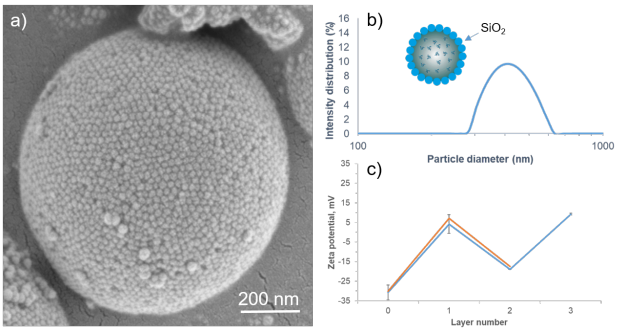
Methods: SiO_2-coated SPION were made by FSP.[2,3] Magnetic hyperthermia performance of particles was measured by recording the temperature evolution of an aqueous suspension (10 g/L) exposed to an alternating magnetic field. Colloidal capsules were synthesized using a water-in-oil (W/O) emulsion stabilized by commercially available SiO_2 nanoparticles or flame-made SiO_2-coated SPION. Capsules were assembled by ultrasonication, followed by transfer of capsules to a water phase through centrifugation.[4] Hydrodynamic size and zeta potential of nanoparticle suspension and colloidal capsules were measured by dynamic light scattering and electrophoretic light scattering, respectively. Capsule morphology was visualized by scanning electron microscopy (SEM). SiO_2 capsules were coated with alginate and chitosan using the layer-by-layer (LBL) technique.[5] The encapsulation efficiency of a model compound (fluorescein) in the colloidal capsules was measured by microplate reading.

Results: Superparamagnetic iron oxide nanoparticles with a primary particle size of about 20 nm and encapsulated with a nanothin (2-3 nm) silica coating were produced by scalable flame spray pyrolysis and exhibited hyperthermia in an alternating magnetic field. Silica colloidal capsules with an average hydrodynamic diameter of 335 nm were successfully synthesized using an emulsion-based method. SEM images showed homogenously assembled nanoparticles in the capsule shell with a pore size between particles of about 7 nm. The capsules were successfully coated with alternating layers of alginate and chitosan and each coating layer reversed the capsule surface charge. Coated capsules exhibited a lower variability in the encapsulation efficiency (about 60%) of a model compound (fluorescein).

Conclusions: Colloidal capsules made of SiO_2 nanoparticles were synthesized using Pickering emulsion-based method. The SiO_2-capsules were coated with alginate and chitosan through the LBL technique, which proved to enhance the encapsulation efficiency of the model drug. Our ongoing work focuses on assembling colloidal capsules using flame-made SiO_2-coated SPION for controlled oral delivery of biological drugs.

[1] Bollhorst *et.al.***Chem. Soc. Rev**. (46) 2017
[2] Teleki *et.al.* **RCS. Adv**. (6) 2016
[3] Teleki *et.al.* **Chem. Mater.** (21) 2009
[4] Bollhorst *et.al.* **Chem. Soc. Rev.** (25) 2013

[5] Yang *et.al.***Acta. Biomater.** (6) 2010



Colloidal capsules pre data.png

Multi-responsive nanogels for biomedical applications

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 239

Dr. Sebastian Spain [1], ***Ms. Emma Owens*** [1], ***Ms. Marissa Morales-Moctezuma*** [1]

1. *Department of Chemistry, University of Sheffield*

The ability to control a material's properties in response to external stimuli potential applications applications from recycling of plastics to biomedical sensors. We are currently developing a range of nanogels that respond to multiple stimuli (e.g. pH, redox, salts) separately or synergistically. Here, I will present our recent work in two areas: oxidation-responsive nanogels for the detection or treatment of inflammation; and calcium-responsive nanogels for wound care.

Nanogels have been synthesis via polymerisation-induced self-assembly (PISA) using reversible addition-fragmentation chain transfer polymerisation. For oxidation-responsive nanogels, a poly(N-hydroxyethyl)acrylamide stabiliser block is chain extended with N-isopropylacrylamide (NIPAM), an oxidation-responsive acrylate and bisacrylamide (BIS). Nanogel size and morphology can be tuned by various the relative block lengths, and swell and shrink in response to temperature. Oxidation-responsive nanogels also display a dose-dependent swelling in response to hydrogen peroxide as a model oxidant.

pH-responsive and thermoresponsive nanogels have synthesised using poly(acrylic acid) or poly(2-(dimethylamino)ethylmethacrylate) as a stabiliser block with crosslinked polyNIPAM as the core-forming block. Nanogels behave synergistic responses to temperature and pH or the presence of metal ions.

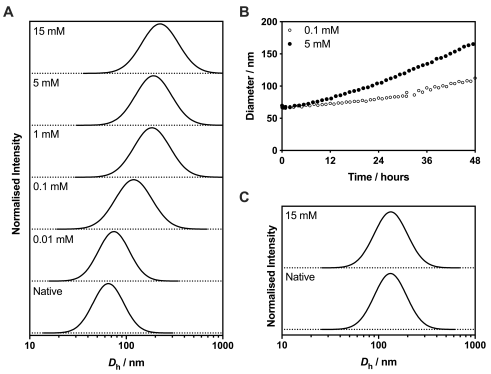


Figure 2 - oxidation response of nanogels.png

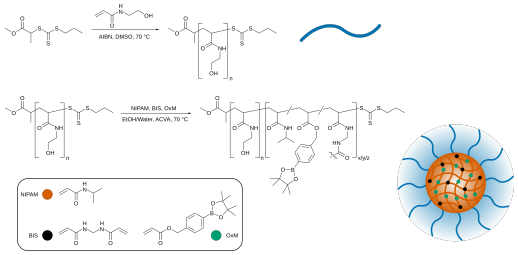


Figure 1 - synthesis of oxidation reponsive nanogels.png

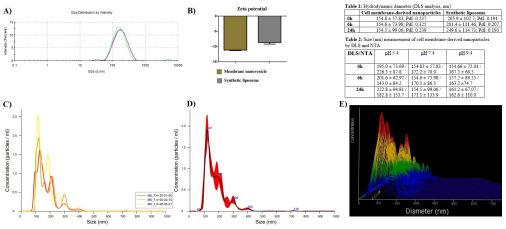
Nanoparticle synthesized from the main components of cancer cell membrane functionalized with c-myc siRNA modulate the antitumor mechanisms of macrophages.

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 252

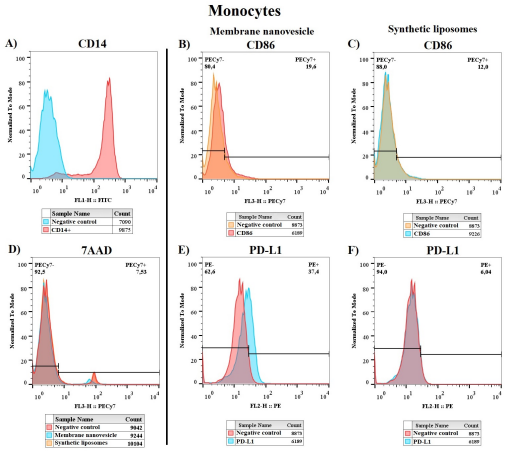
Mr. Edson Comparetti [1], ***Prof. Valtencir Zucolotto*** [2]

1. *Physics Institute of São Carlos, University of São Paulo,* ***2.*** *Physics Institute of São Carlos, USP - University of São Paulo*

Cancer is the most recurrent chronic disease in the world. Human hepatocellular carcinoma (HCC) is the second type of cancer with highest number of deaths in the world and their frequency of relapses and metastasis require the development of new diagnostic and therapeutic approaches. Neoplastic cells have the ability to evade immune surveillance, modulating tumor microenvironment, to favor tumor progression in the body. Nanocomposites can be used to prevent the expression of immunosuppressive proteins and cytokines, increasing the activity of the main mechanisms of cellular immunity. This study aims to develop a nanocarrier to transport immunomodulatory agents into cancer and immunocompetent cells. Nanoparticles were synthesized with lipids and proteins from plasma membrane of neoplastic cells to deliver a large amount of antigenic material to professional antigen-presenting cells (APC). To establish a pro-inflammatory response, lipid nanoparticles were incorporated with monophosphoryl lipid A and oligonucleotide sequence (siRNA) to silence c-Myc oncogene. Following, nanoparticle was exposed to HCC cells, peripheral blood monocytes from healthy donors and macrophages generated *in vitro*. The nanocarriers were tested for: a) nanoparticle internalization into cancer and immunocompetent cells, b) immunomodulatory activity, observing the expression of cell surface markers and cytokine production, and c) cytotoxicity of nanocarriers. Uptake was analyzed by fluorescence microscopy and cytotoxicity by flow cytometry. We aim at increasing the immunogenicity of the tumor cell, upon positively modulating the action of the antigen-presenting cells (APCs).



Nanoparticle size and charge. .jpg



Monocytes.jpg

A Full Surveillance Internet of Bio-Nano Things Based on Feynman-Path-Integral-Based Cognitive Radio Theory and Machine Learning

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 267

Dr. Huber Nieto-Chaupis [1]

1. *Universidad Privada del Norte*

We present a full surveillance Internet of Bio-Nano Things that is based on the combination of the theories of Feynman path integral and cognitive radio **[1].** In addition we add the Mitchell's criteria in order to guarantee the continuous learning of the system and to improve its performance along the time where the measurements are carried out **[2]**. We focus on the clinic variables: (i) cardiac pule, (ii) blood pressure, (iii) glucose, and (iv) cholesterol. When all these variables are fully interconnected among them the probability to anticipate strokes might be substantial.

Usage of the Cognitive Radio (CR) Model:

Consider top panel in FIG-1 where for q nano devices labeled by h_q acquire a signal from x_m. It should be noted that e assume that all signal are electromagnetic waves in the range of allowed frequencies. Once the sensing and measurement has been performed, then all devices send their signal to the fusion center y. In turn, this has an intrinsic perturbation that alters the output in y. With respect to the h_q we can associate each of the them a specific function as for example given from (i) to (iv) above. Thus CR model this as:

y = x_m \beta_q h_q + n (1)

Usage of the Path Integral Feynman Theory

Eq (1) can be written as a probability amplitude that demands of an well-defined initial and final state by allowing to compute the probability of the transitions among the allowed states. Clearly the concept of CR applies in the sense that the best path is the one where the best scenario is the one by which the sensing of a certain signal is n such manner that it does not find any obstruction during its trajectory to the fusion center. Thus we Eq (1) in terms of the Feynman theory gives

$$< y_f \mid y_i > = \sum_m \sum_n \int\int dx_1\, dx_2\, \Phi(x_1)\ \ Exp[\, -i H_1\, \Delta T/\, h\,]\ \ Exp[\, -i H_2\, \Delta T/\, h]\, \Phi(x_2) \quad (2)$$

With H_1 and H_2 the Hamiltonian functions. It is noteworthy that these Hamiltonians are depending on the instantaneous expended energy of system, electromagnetic energy and batteries of nano-devices.

Usage of the Mitchell's Criteria

Both CR and Feynman path have yielded a compact expression as given in Eq (2) where the sub index m and n indicates us the number of available nano devices that are expected to perform the measurement, that means that **n** runs from 1 to 4 for the present study. Once the replacement of initial and final states is done, the full integral Eq (2) is computationally operated. The resulting surveillance is shown in Fig-2. The apparition of peaks is counted as alarms.

[1] Huber Nieto-Chaupis, Theory of Cognitive Ratio Through the Feynman Path Integral, 2019 International Workshop on Antenna Technology (iWAT).

[2] Huber Nieto-Chaupis , The Feynman Path Integral and Machine Learning Algorithms to Characterize and Anticipate Bacteria Chemotaxis in a Host Healthy Body 2019 IEEE 9th Annual Computing and Communication Workshop and Conference (CCWC).

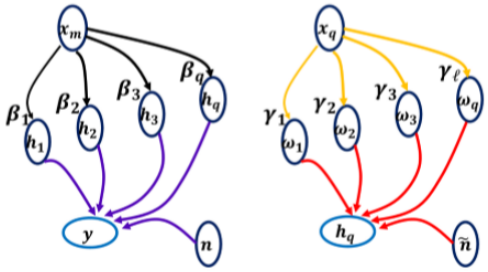


Fig-1.png

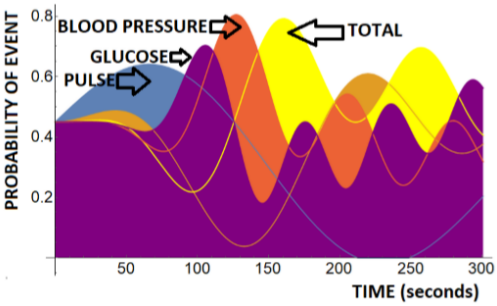


Fig-2.png

Nose-to-Brain Delivery of Biologics

Friday, 18th October - 13:30: Poster Session (Foyer N02.083) - Poster - Abstract ID: 79

Ms. Bettina Schwarz [1], ***Dr. Aditi Mehta*** [1], ***Dr. Friederike Adams*** [1], ***Dr. Christian L. Ried*** [2], ***Dr. Thomas Merdan*** [3], ***Prof. Olivia Merkel*** [4]

1. *Ludwig-Maximilians-University Munich,* ***2.*** *AbbVie Deutschland GmbH & Co. KG,* ***3.*** *AbbVie Deutschland GmbH,* ***4.*** *Ludwig-Maximilians University Munich*

Introduction:
The selective permeability of the blood-brain-barrier (BBB) prevents the utilization of many therapeutic agents, especially proteins and peptides, for treating CNS disorders. A very promising option to deliver particularly macromolecular drugs to the CNS is bypassing the BBB by using the nose-to-brain route. In the last few decades, the nasal route has attracted wide attention as a reliable, safe and non-invasive route to achieve faster and higher levels of drug absorption in the brain. Despite various benefits, such as rapid onset and avoiding first-pass metabolism, it presents certain limitations such as short drug residence time and insufficient drug transport due to mucociliary clearance.
This project aims to develop a novel drug delivery system for the intranasal delivery of macromolecules to the brain with a controlled drug release rate for sufficient therapeutic CNS concentrations. Therefore, we use low molecular weight chitosan that is known to be biodegradable and non-toxic facilitating paracellular transport of peptides and proteins across the nasal mucosal barrier.
Methods:
Chitosan nanoparticles (NP) were prepared by ionotropic gelation method using pentasodium tripolyphosphate as crosslinker. (Fig.1) Physicochemical characterization included measurements of size using dynamic light scattering technique, nanoparticle tracking analysis and atomic force microscopy. Furthermore, the overall charge was assessed by laser Doppler anemometry and shape and morphology by transmission and scanning electron microscopy. The encapsulation efficiency was determined using the model protein BSA coupled to fluorescein isothiocyanate (FITC). The NP cytotoxicity was evaluated via MTT assay in the human nasal epithelial cell line RPMI2650. For the evaluation of NP cellular uptake, free protein was compared to protein encapsulated in NPs at 4°C and 37°C. To specifically target nasal epithelial cells and enhance the cellular uptake, a conjugate of chitosan with transferrin as targeting ligand was synthesized using click chemistry.
Results:
The NPs have a mean size of approx. 100 nm together with a positive surface charge of +40 mV. Scanning electron microscopy showed that the particles are spherical in shape with a smooth surface. (Fig.2) The NPs were not found to inhibit cellular proliferation and demonstrated an encapsulation efficiency of FITC-BSA of about 20-45% depending on chitosan and FITC-BSA concentration.(Fig. 3) The uptake in human nasal epithelial cells was significantly higher for protein encapsulated in NP compared to free protein. (Fig. 4) The two different temperatures show that the NPs were internalized (37°C) and not just adsorbed to the cellular surface (4°C).
Discussion:
The prepared chitosan NPs have a size of about 100 nm, hence, favoring uptake into olfactory neurons in the nasal cavity together with potential paracellular transport through the tight junctions of the nasal epithelial cells. The positive surface charge supports a mucoadhesive effect based on electrostatic interactions with negatively charged mucins in the nasal cavity. We were able to show that the NPs did not affect cell proliferation of nasal epithelial cells but mediated enhanced cellular uptake of a model protein, which makes them a promising delivery system for macromolecules. We are currently developing chitosan-transferrin to enhance cellular uptake by active targeting.

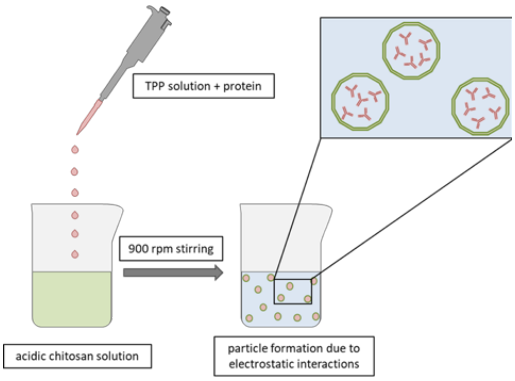


Figure 1- chitosan nanoparticle preparation by ionotropic gelation.png

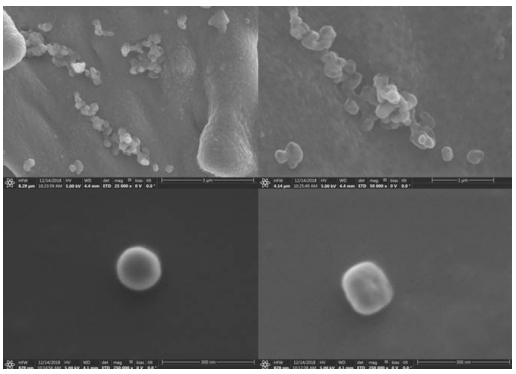


Figure 2- scanning electron microscopy of chitosan nanoparticles.png

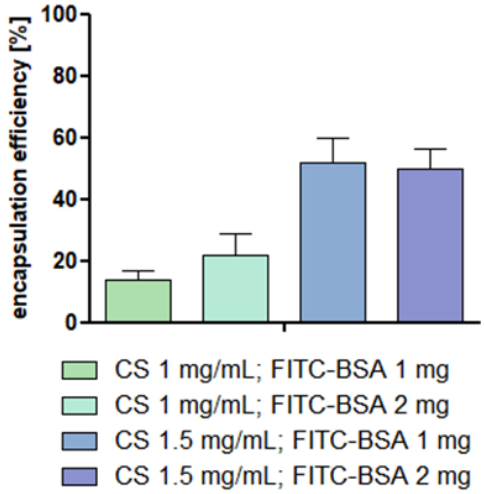


Figure 3- encapsulation efficiency of model protein fitc-bsa in chitosan nanoparticles.png

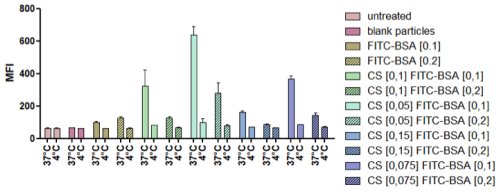


Figure 4- cellular uptake of chitosan nanoparticles in rpmi2650 cell line at 4 c and 37 c.png

Pattern-generating fluorescent molecular probes for chemical biology

Friday, 18th October - 14:15: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 4

Dr. leila Motiei [1]

1. *Weizmann Institute of Science*

Fluorescent molecular probes have become a powerful tool in protein research. However, these probes are less suitable for analyzing specific populations of proteins in their native environment. In this talk I will give an overview of a new class of fluorescent molecular probes[1-5] recently developed in our group, and show how they can be used to detect individual proteins, protein combinations, as well as binding interactions and dynamic changes that occur on their surfaces. In the second part of this talk, I will present a new class of fluorescent molecular sensors that combines the properties of small molecule-based probes and cross-reactive sensor arrays (the so-called chemical nose/tongue') and explain how these pattern-generating probes could expand the fluorescent toolbox currently used to detect and image proteins.[6] Specifically, I will show how such systems can be used to identify combinations of specific protein families within complex mixtures and to discriminate among protein isoforms in living cells, where macroscopic arrays cannot access.[6]

References

- Rout, B.; Milko, P.; Iron, M. A.; Motiei, L.; Margulies, D. *J. Am. Chem. Soc.***2013**, 135, 15330.
- Unger-Angel, L.; Rout, B.; Ilani, T.; Eisenstein, M.; Motiei, L.; Margulies, D. *Chem. Sci.* **2015**, 6, 5419.
- Nissinkorn, Y.; Lahav-Mankovski, N.; Rabinkov, A.; Albeck, S.; Motiei, L.; Margulies, D. *Chem. Eur. J.***2015**, 21, 15981.
- Motiei, L.; Pode, Z.; Koganitsky, A.; Margulies, D. *Angew. Chem. Int. Ed.* **2014**, 53, 9289.
- Hatai, J.; Motiei, L.; Margulies D. *J. Am. Chem. Soc.* **2017**, 139, 2136.
- Pode, Z.; Peri-Naor, R.; Georgeson, J. M.; Ilani, T.; Kiss, V.; Unger, L.; Markus, B.; HM, B.; Motiei, L.; Margulies, D. *Nature Nanotechnol.* **2017**, 12, 1161.

All Organic Ultrabright Red to Near-Infrared Nanoparticles for Single Particle Tracking and Bioimaging

Friday, 18th October - 14:30: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 14

Dr. Paolo Pagano [1], Dr. Morgane Rosendale [2], Ms. Jessica Flores [1], Dr. Chiara Paviolo [3], Dr. Jonathan Daniel [2], Dr. Laurent Cognet [3], Dr. Jean-Baptiste Verlhac [2], Dr. Mireille Blanchard-Desce [2]

1. *(formerly) Institute of Molecular Sciences - University of Bordeaux,* ***2.*** *Institute of Molecular Sciences - University of Bordeaux,* ***3.*** *Institut d'Optique Graduate School - University of Bordeaux*

Nanotechnologies have the potential to revolutionise our understanding of devastating diseases such as cancer or neuropathologies through the design of nano-carriers for drug delivery and of fluorescent nano-emitters for bioimaging. In this latter field, we have now reached the era of nanoscopy where one can dynamically follow the fate of a single molecule inside a living cell thanks to a class of techniques known as single particle tracking (SPT). To date, quantum-dots are the most widely used nanoparticles for such applications thanks to their unparalleled brightness and photostability. Moreover, the size of these semiconductor nanocrystals can be modulated to finely tune their spectral properties such that their emission can cover the entire visible range and more. However, their inorganic core is inherently water insoluble. They thus and have to be passivated with a shell of metal chalcogenides and further coated, typically with silica or polyethylene-glycol, to increase their solubility. Moreover, most contain toxic heavy metals such as arsenic or cadmium, which raises environmental concerns on the long-term and drastically limits their potential of ever being used in the clinic.

To circumvent these limitations, our lab develops Fluorescent Organic Nanoparticles (FONs). Prepared by self-aggregation of rationally designed hydrophobic dyes in water, FONs show remarkable colloidal and optical stability, making them a promising, all organic, spontaneously water soluble alternative to quantum-dots. Their bottom-up molecular design makes it possible to rationally tune their properties for specific applications. Through engineering of excitonic coupling effects, we have previously reported on green ultrabright FONs (Daniel *et al.*, J Phys D Appl Phys., 2016). However, biological tissues scatter light and exhibit some level of auto-fluorescence. These observations led to the definition of an imaging window in the red to near infra-red (NIR) spectral region, *i.e.* 600-1000 nm, that is most favourable for deep tissue imaging (Weissleder, Nat. Biotechnol., 2001). Our previous work in this direction, based on push-pull conjugated systems optimising intramolecular charge transfer by electron delocalisation, led to the development of near-infra-red emitting FONs of gigantic brigtness (Genin *et al.*, Adv Mat., 2014). These FONs had the property of non-specifically interacting and entering living cells such that they are potential candidates for drug delivery systems. However, stealth emitters can be of interest for single particle tracking of cell-surface receptors, a class of biomolecules suspected to be dysregulated in disease states including neuropsychiatric disorders(Jézéquel *et al.*, Trends Neurosci., 2018).

In this work, we describe stealth, ultrabright, red to near-infrared emitting FONs made from novel quadrupolar dyes. We report on the characteristics and properties of these FONs and show that they have no unspecific interactions with living cells. Moreover, we demonstrate that some candidates have sufficient brightness and photostability to achieve single particle tracking in solution. From these combined properties, we conclude that these novel FONs are promising candidates for the next generation of tools for super resolution bioimaging.

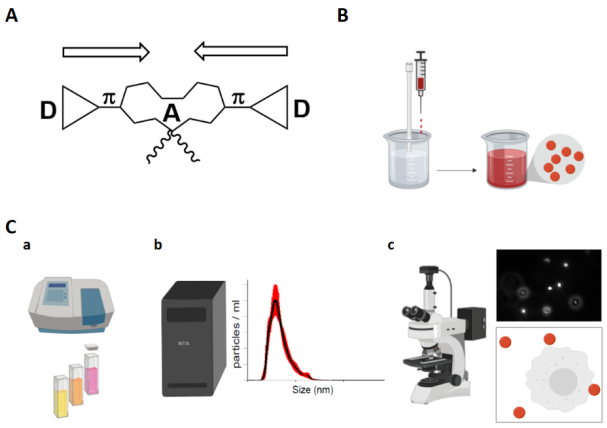


***Figure 1*: From dye design to FONs preparation and characterisation**
A Schematic representation of the quadrupolar dyes designed for this study. Two symmetrically arranged donor groups (D) are linked to an acceptor core motif (A) *via* conjugated systems (π). The length and chemical nature of the conjugated systems influence charge transfers along the molecule, resulting in a red-shifted emission. Arrows symbolise dipolar moments and wavy bonds indicate bulky side chains. **B** One-pot preparation of FONs: a minute amount of concentrated dye dissolved in THF is added to a large volume of water under sonication resulting in self-organised nanoparticles. **C** Systematic characterisation of FONs: **a** Spectral properties are determined by spectrophotometry, **b** particle size is determined by Nanoparticle Tracking Analysis (Malvern Panalytics) and **c** single particle tracking in solution and assessment of interactions with cells after 24h incubation are performed by fluorescence microscopy.

Figure 1 from dye design to fons preparation and characterisation.png

The density of cell targeting antibodies on sarcosine-based peptobrushes correlates with the extent of unwanted liver accumulation associated with diminished cell targeting efficiency

Friday, 18th October - 14:45: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 56

Ms. Cinja Kappel [1], Dr. Meike Schinnerer [2], Mr. Gabor Kuhn [1], Mrs. Ingrid Tubbe [1], Dr. David Paßlick [1], Ms. Dominika Hobernik [1], Dr. Rebekka Bent [1], Dr. Katharina Haas [1], Ms. Evelyn Montermann [1], Dr. Kerstin Walzer [2], Dr. Mustafa Diken [3], Dr. Matthias Barz [4], Prof. Manfred Schmidt [4], Prof. Volker Mailänder [5], Dr. Matthias Bros [1], Prof. Stephan Grabbe [1]

1. *Medical University Mainz,* ***2.*** *Biontech AG,* ***3.*** *TRON–Translational Oncology at the University Medical Center of the Johannes Gutenberg University GmbH,* ***4.*** *Johannes Gutenberg University,* ***5.*** *Medic*

The development of nanocarriers that deliver cargo specifically to leukocyte populations is considered an innovative immunotherapeutic strategy. The suitability of antibody-decorated nanocarriers to target immune cell populations has been assessed in numerous studies. So far, most types of functionalized nanocarriers accumulate in the liver after systemic application, which constitutes an obstacle for the perspective of nanocarrier based therapy. The exact mechanisms that contribute to unwanted liver accumulation of nanocarriers have not been fully elucidated yet. Here we asked for the role of antibody density per nanocarrier as a determinant of liver trapping *in vivo*.

We employed sarcosine-based peptobrushes which are characterized by a long circulation time, and low organ accumulation as well as little unspecific cell binding. This nanocarrier was conjugated with defined numbers (2, 6 and 12) of an antibody specific for the surface receptor DEC205 that is specifically expressed by conventional dendritic cells type 1 (cDC1). In parallel assays, we tested the time-dependent biodistribution (*in vivo* & *ex vivo* imaging) of antibody-functionalized peptobrushes and their cell binding (flow cytometry) and cell uptake (cLSM) properties.

Our data indicate a direct correlation between the number of antibodies per peptobrush and the extent of peptobrush accumulation in the liver. We demonstrate that peptobrushes with intermediate and high antibody density are efficiently engaged and internalized by liver sinusoidal endothelial cells (LSEC), whereas Kupffer cells as the main liver macrophage population play a minor role. Blocking experiments revealed that LSEC bind antibody-decorated peptobrushes via their Fc receptors. Consequently, peptobrushes with intermediate/high antibody density engaged cDC1 only at low extent. In contrast, conjugation of peptobrushes with 2 antibodies resulted in pronounced binding to cDC1 target cells in the spleen and only low liver accumulation.

Altogether, our results suggest that low densities of antibodies for specific cell targeting are preferable regarding the design of nanocarriers for immune-therapeutic purposes to prevent unwanted liver accumulation.

Multi-Detector Field-Flow Fractionation - A powerful analytical tool in the field of Nanomedicine

Friday, 18th October - 15:00: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 103

Dr. Gerhard Heinzmann [1]

1. *Postnova Analytics GmbH*

Field-Flow Fractionation (FFF) belongs to the flow-based separation techniques, where separation of dissolved, suspended and dispersed sample constituents in the size range of 1 nm to approx. 50 µm is achieved within a thin, ribbon-like channel without stationary phase (Fig. 1) [1].

In FFF, separation of different sample constituents is induced by a force field that is applied perpendicular to the channel flow, which transports the sample toward the channel outlet and further to the respective detectors. Common FFF detectors such as UV/Vis, RI and ICP-MS thereby enable access to the mass and concentration of the fractionated sample, but also size-sensitive detectors based on Multi-Angle- (MALS) and Dynamic Light Scattering (DLS) are frequently applied turning FFF into a multi-detector analytical platform that facilitates the comprehensive physico-chemical characterization of the sample. Depending on the applied force field, FFF can be divided into different subtechniques. In Asymmetrical-Flow FFF (AF4), a second flow, the so-called cross flow, enables separation according to hydrodynamic size. By superimposing the cross flow field with an electrical field, Electrical Asymmetrical-Flow FFF (EAF4) additionally enables the separation according to charge thus gaining access to electrophoretic mobility and Zeta potential of the sample. In Centrifugal FFF (CF3), a centrifugal force field induces separation according to mass respectively density of the sample. Due to its wide applicability and gentle separation conditions, FFF has become a powerful characterization tool for nano-sized samples particularly in the field of bio- [2] and nanomedicine [3].

This presentation shall display the benefits of multi-detector FFF to propel the field of nanomedicine highlighting its application toward the characterization of liposomal drug formulations, polymeric drug delivery systems and nano-sized antimicrobial agents.

Fig. 1: Separation principle of Field-Flow Fractionation.

References:

[1] J.C. Giddings, Separation Science, 1(1), 123-125 (1966).

[2] X. Zhang, Y. Li, S. Shen, S. Lee, H. Dou, TrAC Trends in Analytical Chemistry, 108, 231-238 (2018).

[3] M. Wagner, S. Holzschuh, A. Traeger, A. Fahr, U.S. Schubert, Analytical Chemistry, 86(11), 5201-5210 (2014).

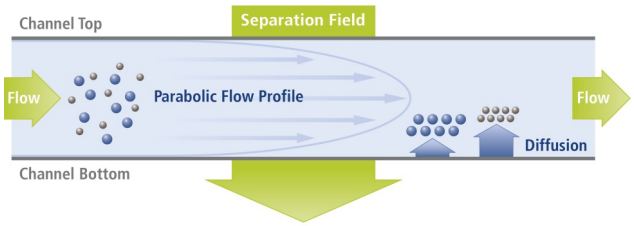


Figure 1.jpg

Microfluidic assembly of siRNA embedded nanoparticles

Friday, 18th October - 15:15: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 111

Mr. Christoph Zimmermann [1], ***Dr. Daniel P. Feldmann*** [2], ***Prof. Paola Luciani*** [3], ***Prof. Olivia M. Merkel*** [1]

1. *Faculty for Chemistry and Pharmacy LMU,* ***2.*** *Wayne State University,* ***3.*** *University of Bern*

Introduction

Nanoparticles have been shown to be successful drug delivery systems and are useful for encapsulating drugs, enabling more precise targeting with a controlled release. Different approaches can be used to form these nanoparticles. The classical procedures comprise top-down or bottom-up techniques. However, if electrostatic interactions are involved in particle formation, the self-assembly of nanoparticles can be achieved via simple bulk mixing of both fluids. Microfluidic mixing techniques, bringing cationic polymers and nucleic acids together at a constant ratio during the entire mixing process, have the potential for a more homogeneous and smoother complexation, leading to a more uniform charge distribution and increased colloidal stability. Microfluidic mixing can be conducted based on two techniques: active micromixers use applied forces to create a higher diffusion rate and turbulent flow, whereas passive micromixers change the fluid flow within the channels to reduce the diffusion time or increase the contact area of the fluids. In this work, siRNA containing polyplexes were formed by electrostatic interactions using the Micromixer Chip from Dolomite as a passive micromixer system.

Method

The particle preparation was performed by pumping positively charged br-PEI-g-PCL-b-mPEG triblock copolymer solution and siRNA solution through the Dolomite Micromixer Chip. The flow rate was set by a syringe pump. Fixed parameters were siRNA concentration, triblock copolymer concentration and buffer (5% glucose, pH 7.4). The N/P ratio was set at 6. Variable parameters to be tested were tube length (10 cm, 20 cm) and flow rates (0.1 – 1 mL/min). The preparation and implementation of the mixing process was kept the same for every run. The resulting polyplexes were characterised using dynamic light scattering (DLS) and dye exclusion assays. Selection criteria for subsequent experiments were particle size, PDI, and zeta potential. Furthermore, parameters were compared with polyplex formulations prepared by simple batch reaction.

Results

The results show that independently of tubing length, no difference could be found in size or PDI. However, both parameters were affected by the flow rate resulting in smaller sizes (<130 nm) and more monodisperse particles (PDI < 0.3) obtained with higher flow rates (0.5 mL/min). In comparison, the batch reaction resulted in sizes of 260 nm and a PDI of 0.38. The zeta potential of the polyplexes assembled by both mixing techniques were positive and below +10 mV.

Discussion

Microfluidic mixing was found to promote conducive characteristics for cellular transfection. The freedom in design of the different mixing devices offers almost unlimited possibilities to obtain the most suitable settings. As shown in Figure 1, even preparation of functionalised or hybrid nanoparticles, consisting of siRNA mixed with both polymers and lipids or different types of polymers or lipids, is possible. Additional proof-of-concept experiments will be performed to optimise siRNA formulations made from biodegradable and biocompatible materials for further *in vitro*and *in vivo*screening.

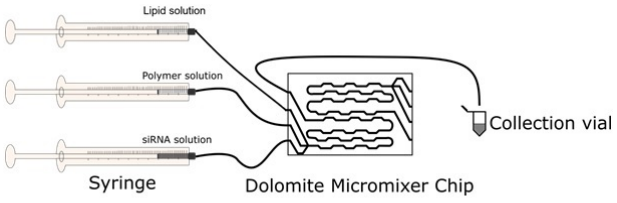


Figure 1: Scheme of microfluidic mixing using differently charged solutions to form polyplexes adapted from D. Feldmann et al., 2018, Nanotechnology

Figure 1 - microfluidic assembly of sirna embedded nanoparticles.jpg

Selective self-assembly of bioelectrocatalysts for functional biofuel cells.

Friday, 18th October - 15:30: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 171

Mr. Alexander Trifonov [1], ***Dr. Ran Tel-Vered*** [1], ***Prof. Andreas Stemmer*** [1]

1. *ETH Zürich*

Introduction

Self-assembly considered one of the foremost ways of nature to promote the ordering of chemical structures and the controlled growth of biological systems. Through a set of local interactions, structures of growing dimensions are assembled, showing in many cases an emergence of functionality. The self-assembly process has consequently become a prominent nanofabrication tool in many fields such as switchable catalytic surfaces and synthesis of complex materials exhibiting improved electrical or structural properties.

Enzymatic biofuel cells are well recognized as clean energy biogenerators, possessing intrinsic selectivity towards the substrate molecules, and avoiding the use of toxic and/or expensive catalysts. In recent years, a new class of enzymatic biofuel cells was developed, enabling direct electron transfer (DET) between redox enzymes and their binding electrodes and showing inherent advantages in terms of design and bioelectrocatalytic efficiencies.

In this work, we describe a design of an all-DET, membrane-less biofuel cell whose active components self-assemble on stationary electrode surfaces following infusion of mixed catalysts-modified nanoparticles into the electrolyte.

Methods

The general concept behind the construction of the biofuel cell employing self-assembly is introduced in Figure 1. Two conductive surfaces were chemically modified with receptor units, R_1 and R_2, showing high affinity to ligands L_1 and L_2, respectively. The latter were individually functionalized on carbon coated magnetic nanoparticles (ccMNPs), co-modified with fructose dehydrogenase (FDH) or bilirubin oxidase (BOD) as an oxidation or reduction processes-catalyzing enzymes, respectively. Starting from a mixed suspension, the specific enzyme-coated ccMNP hybrids showed selective binding to the receptor-modified electrodes within few minutes, thus self-assembling the anodic and cathodic elements required for the functioning of a biofuel cell.

The self-assembling anodic layer relies on the strong host-guest complexation between the R_1 and L_1 , whereas the cathodic compartments interact through electrostatic forces between R_2 and L_2 termini.

Results and discussion

The anodic and cathodic compartments were constructed and investigated separately, including monitoring of self-assembly kinetics, which was found to occur within the first 5 minutes of active components interaction; biocatalytic activities of both compartments, revealing high turnover rates at DET communication of both enzymes in presented configurations; and the detachment of the cathodic and anodic catalytic carriers from the electrodes using external magnetic field.

The resulted two electrodes (the anode and the cathode) were combined into a self-assembling all-DET biofuel cell. The self-assembled biofuel cell was discharged versus decreasing resistor loads, showing very impressive results, reflected in characteristic polarization curve. Very impressive power density was obtained at a maximal current density, obtained at 80 mM of fructose. The cell demonstrated good power generation reproducibility upon cyclic magnetic removal/self-assembled restoration of fresh hybrids on the electrode surface. The resulted biofuel cell was further operated under harsh biological conditions, namely viscous biomass mixture, revealing yet lower, but stable power output.

Miniaturized Nanopore Reader for single Nanoparticle detection and analysis

Friday, 18th October - 15:45: Biological & medical nanodevices and biosensors (Amphitheatre N02.040) - Oral - Abstract ID: 193

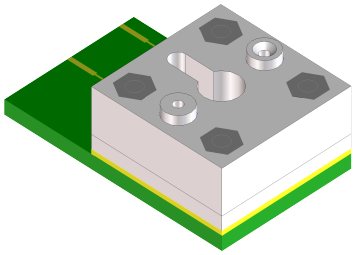
Mr. Marcus Pollard [1], ***Dr. Angelika Holzinger*** [2], ***Dr. Micheal Scanlon*** [2], ***Dr. Federico Thei*** [3], ***Prof. Mark Platt*** [1]

1. *University of Loughborough,* ***2.*** *University of Limerick,* ***3.*** *Elements srl*

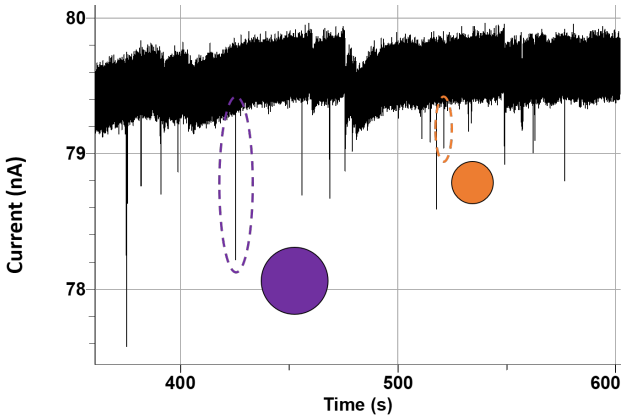
Resistive pulse sensing (RPS) is a technique which can provide a full characterization of an analyte. In an RPS system, a nanopore is sandwiched between two fluid cells, one cell contains a ground electrode and the other contains a working electrode. A potential is applied across the nanopore which causes ions to migrate resulting in a current which is measured when an analyte passes through the pore it causes exclusion of these ions reducing the current which is termed a pulse. Using these pulses, we can characterize the analyte's size, concentration and charge. Herein we present using a simple approach to characterize nanoparticle size and concentration using a Nanopore Reader developed by Elements SRL (Italy), a miniaturized ultra-low noise current amplifier and Nanopore flowcell, a small cartridge which hosts the nanopore chip. Measurements were done using a solid-state Silicon Nitride nanopore of a fixed size to identify the limits of detection for the pores. Possible applications of this sensing technology are in the early detection of marker disease.

The figure shows a current trace of a run containing 400 and 800 nm particles, one peak of each size has been highlighted, 800 nm (Purple) and 400 nm (Orange). The different sized particles can be observed by the differences in blockade magnitude, caused by an increase in the volume excluded within the pore by a larger particle. By analyzing these blockades a distribution of the blockade magnitudes for a particle can be characterized and by using a known calibrant the size can then be determined.

Nanopore reader.png



Nanopore flowcell.png



Particle translocations.png

Complex macromolecular architectures for promoting drug penetration across biological barriers

Friday, 18th October - 14:15: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 43

Prof. Francesco Cellesi [1]

1. *Politecnico di Milano*

Recent advances in polymer chemistry have led to a fine control of key properties of polymer nanocarriers, such as size, drug loading and functionality, with the purpose of an efficient site-specific drug delivery[1]. However, a major challenge in nanomedicine is to maximise drug transport across biological barriers,which are necessary to prevent undesired access of molecules to sensitive organs, tissues and cells in healthy conditions.

In this study, synthetic polymers of controlled molecular architecture were designed to obtain size-tuneable, amphiphilic and multifunctional drug-loaded nanocarriers with therapeutic and diagnostic properties (Figure 1). Controlled living polymerisations (ROP and ATRP [2]) of polyester-, PEG-, glycerol- based monomers/initiators, and possible peptide conjugation by Michael-type addition[3], allowed the development of bioactive and traceable nanomaterials which selectively cross specific barriers, including the glomerular filtration barrier to treat chronic kidney diseases, the blood brain barrier to target brain tumours, and specific cell membranes to obtain intracellular drug delivery and/or controlled immunostimulation. When required, fluorinated repeating units were introduced to the macromolecules in order to obtain nanoscopic ^{19}F magnetic resonance imaging agents, which were also capable of in situ drug release.

Acknowledgments

The financial support from Regione Lombardia (POR FESR 2014 – 2020) within the framework of the NeOn project (ID 239047) is gratefully acknowledged.

References

1. Ordanini, S.; Cellesi, F. Complex polymeric architectures self-assembling in unimolecular micelles: Preparation, characterization and drug nanoencapsulation. Pharmaceutics 2018, 10, (4).

2. Bruni, R.; Possenti, P.; Bordignon, C.; Li, M.; Ordanini, S.; Messa, P.; Rastaldi, M. P.; Cellesi, F. Ultrasmall polymeric nanocarriers for drug delivery to podocytes in kidney glomerulus. Journal of Controlled Release 2017, 255, 94-107.

3. Celentano, W.; Battistella, J.; Silvestri, I. P.; Bruni, R.; Huang, X.; Li, M.; Messa, P.; Ordanini, S.; Cellesi, F. Engineered polyester-PEG nanoparticles prepared through a “grafting through” strategy and post-functionalization via Michael type addition. Reactive and Functional Polymers 2018, 131, 164-173.

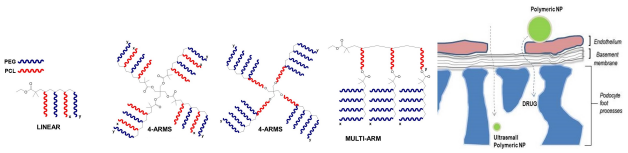


Figure1 final.jpg

Effect of protein source on nanoparticle-protein corona and cellular uptake

Friday, 18th October - 14:30: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 69

Ms. Keni Yang [1], ***Dr. Anna Salvati*** [1]

1. *University of Groningen*

The application of nanotechnology for medical purposes has attracted extensive research over the last decades, but relatively few nano-formulations have reached the market so far. One of the main obstacles is the limited understanding of the interactions between nanomaterials, cells/tissues and the biological environment in which they are applied. Generally, in order to evaluate nanomedicine efficiency, *in vitro* experiments are performed using human cell lines cultured in FBS supplemented medium. However, little attention has been paid to the effects of the protein source in the medium on the uptake behavior of nanomaterials. To this aim, in this work, liposomes composed of DOPG and DC-cholesterol lipids (DOPG-DC) have been chosen as a model nanomedicine. DOPG-DC was prepared by rehydrating a mixed lipid film and extrusion through a 100 nm pore membrane, followed by physicochemical characterization by DLS and zeta potential measurements. The cell uptake of DOPG-DC in medium supplemented with FBS and HS was studied by flow cytometry and confocal microscopy. Additionally, the liposome-corona complexes formed in FBS and HS were separated from free proteins by size exclusion chromatography, followed by qualitative and quantitative analysis of corona proteins by SDS-PAGE and LC-MS/MS. To further study the effects of proteins of different source on the uptake behavior, the corona-coated DOPG-DC formed in FBS and HS were isolated and incubated with cells in serum free medium and medium supplemented with either FBS or HS. This allowed us to discriminate effects due to the source of the adsorbed corona proteins from those due to the source of free proteins in solution.

The results showed that the uptake of DOPG-DC liposomes incubated in FBS and HS supplemented medium differed strongly, and the proteomics results confirmed that a different corona was formed in the respective media. However, when the different hard corona-coated liposomes were exposed to cells in serum free medium, they showed similar internalization efficiency. Similar results were observed when liposome-corona complexes were re-introduced in FBS or HS medium. This suggested that the protein source can affect nanomaterial-cell interactions not only because of the absorbed corona proteins but also via effects related to the presence of free proteins in the medium. Similar effects should be taken into account when testing nanomedicines in order to reduce the differences between*in vitro* and *in vivo* studies.

The impact of Nylon-3 copolymer composition on the efficiency in siRNA delivery

Friday, 18th October - 14:45: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 100

Dr. Friederike Adams [1], ***Mrs. Natascha Hartl*** [1], ***Prof. Runhui Liu*** [2], ***Prof. Olivia M. Merkel*** [1]

1. *Faculty for Chemistry and Pharmacy, LMU Munich,* ***2.*** *East China University of Science and Technology*

Introduction

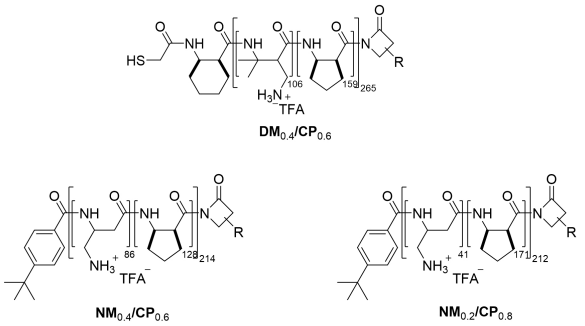
RNA interference by small interfering RNA (siRNA) is an efficient strategy to silence genes and thus to prevent translation. However, the application of siRNA faces challenges, since siRNA is a negatively charged macromolecule. It cannot cross cell membranes, the cellular uptake is limited and siRNA is unstable in blood due to rapid enzymatic degradation. Consequently, several carriers for siRNA have been developed in which polymeric vectors showed advantages over viral vectors regarding safety, immunogenicity, production costs and risk of mutagenicity. In these systems, siRNA is condensed with polycationic polymers through electrostatic interactions to so-called polyplexes. Amine-containing polymers, such as poly(*L*-lysine) (PLL), linear or branched poly(ethylenimine) (PEI) or poly(2-dimethylaminoethyl methacrylate) are positively charged at physiological pH, form ion pairs with nucleic acids, and mediate transfection in a variety of cell lines. Despite their common use, however, high molecular weight PEI and PLL are significantly cytotoxic. For use *in vivo*, new cationic polymers have to be designed incorporating hydrolyzable or biodegradable moieties such that the polymers readily degrade into nontoxic byproducts. In addition, hydrophobic modification of cationic polymers, e.g. in phospholipid-modified PEI and in PEI-poly(caprolactone)-poly(ethylene glycol) polymers was shown to improve the performance in siRNA delivery coupled with considerably decreased cytotoxicity. Our group developed polymeric vectors for siRNA encapsulation and delivery based on Nylon-3 polymers, which have a similar backbone to biocompatible and biodegradable peptides and a cationic side chain. These cationic subunits derived either from β-lactam monomer DM or NM. In addition, hydrophobic subunits were introduced to the polymer which derived from β-lactam monomer CP. $DM_{0.4}/CP_{0.6}$, $NM_{0.4}/CP_{0.6}$and $NM_{0.2}/CP_{0.8}$polymers were designed to study the impact of the ratio between the hydrophobic and cationic subunit as well as of the use of different cationic monomers related to the *in vitro* siRNA delivery into glioblastoma cells.

Methods

Nylon-3 polymers were synthesized via anionic ring-opening polymerization (ROP) through statistical copolymerization of various β-lactams using lithium bis(trimethylsilylamide) (LiHMDS) as initiator. The obtained siRNA-polymer polyplexes were characterized regarding physicochemical characteristics such as siRNA encapsulation ability (SYBR gold assay), polyplex stability (Heparin assay), particle size and surface charge (DLS and zeta potential measurements). Furthermore, cell internalization capability, route of uptake and gene knockdown efficiency were assessed by flow cytometry and cell tolerability was examined by MTT and LDH assays.

Results and Discussion

Efficient siRNA condensation was demonstrated for all tested polymers even with low polymer concentrations. These led to particles with hydrodynamic diameters of < 250 nm and slightly positive surface charges. $NM_{0.4}/CP_{0.6}$ built up the most stable polyplexes under physiological conditions leading to the assumption that NM subunits interact more strongly with siRNA molecules than DM subunits. Nevertheless, all polyplexes were able to release acceptable amounts of siRNA under acidic conditions, mimicking endosomal compartment conditions. Furthermore, polyplexes with the highest hydrophobic content displayed significantly higher cellular internalization in comparison to more cationic formulations and successful knockdown capabilities. Taken together with convenient toxicity profiles; hydrophobic Nylon-3 polymers provide a potential siRNA delivery agent for therapeutic approaches.



Nylon-3 polymers derived from monomers dm nm and cp.jpg

Bacteria-derived vesicles show low cytotoxicity but inherent antimicrobial activity against gram-negative and gram-positive pathogens

Friday, 18th October - 15:00: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 106

Dr. Gregor Fuhrmann [1], ***Ms. Eilien Schulz*** [1], ***Ms. Adriely Goes*** [1]

1. *Helmholtz Institute for Pharmaceutical Research*

Bacterial infections with antibiotic-resistant pathogens are becoming a major healthcare issue. Indeed, the WHO has declared these infections as one of the top three threads to humanity, which amplifies the need to develop new therapeutic avenues. Encapsulation of clinically established antibiotics into nanoparticles is promising to enhance their targeted delivery to the site of infection and could reduce potential side-effects, such as kidney toxicity.

In this work, we characterised a new group of naturally occurring nanoparticles, so-called outer membrane vesicles (OMVs) shed by bacteria, as carriers for antibiotics. Bacteria use OMVs for intercellular communication and as defence strategy. We assessed OMVs from myxobacteria, a group of non-pathogenic, soil-living bacteria. In recent years, myxobacteria have been explored for their capability of providing natural compounds that have a great potential for many uses, especially antimicrobial therapy

We studied myxobacterial strains Cystobacter velatus (Cbv34) and Cystobacter ferrugineus (Cbfe23). Cbv34 and Cbfe23 are producers of antimicrobial products that we hypothesised to be shed into their OMVs. OMVs were isolated by differential centrifugation and purified using size-exclusion chromatography. The myxobacterial OMVs showed a mean size of 150-200 nm which we visualised by both nanoparticle tracking analysis and cryo-electron microscopy. Interestingly, when OMVs were incubated with human epithelial A549 lung cells or human macrophage THP-1 cells we did not see any influence on cell viability or cytotoxicity, even at concentrations of 10,000 OMVs/cell. OMVs also showed low endotoxin concentrations and comparable to buffer controls. We further studied the interaction of OMVs with Escherichia coli model bacteria by confocal microscopy and saw that OMVs co-localise with bacteria better than plain liposomes. When assessing their antimicrobial activity, we found that OMVs were inherently antibiotic against gram-positive Staphylococcus aureus and gram-negative Escherichia coli model pathogens. This antibiotic effect was elicited by a cystobactamid, a potent inhibitor of the bacterial topoisomerase, which is packaged into the bacterial OMVs. The antibacterial effect of Cbv34-derived OMVs remains potent upon storage in 4° C up to 4 weeks, indicating cargo protection by vesicles.

In ongoing work, we are studying how OMVs interact with bacterial membranes and whether their uptake into prokaryotes is selective over mammalian cells. In this work, we report a new type of natural vesicle shed by non-pathogenic bacteria. These OMVs were not toxic towards human cells in our simple assays but they showed promising activity against gram-positive and gram-negative bacteria.

Imaging the stability and extravasation of micelles using a microfluidic platform mimicking tumor microenvironment

Friday, 18th October - 15:15: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 146

Ms. Natalia Feiner Gracia [1], ***Ms. Adrianna Glinkowska Mares*** [2], ***Ms. Marina Buzhor*** [3], ***Dr. Sílvia Pujals*** [2], ***Dr. Roey Amir*** [4], ***Dr. Josep Samitier*** [2], ***Dr. Lorenzo Albertazzi*** [1]

1. *Eindhoven University of Technology,* ***2.*** *Institute for Bioengineering of Catalonia,* ***3.*** *Center for Nanoscience and Nanotechnology. Tel-Aviv University,* ***4.*** *University Center for Nanoscience and Nanotechnology, Tel-Aviv University*

Supramolecular structures potential use as drug delivery systems is closely related to their stability in complex biological media. Due to their dynamic behavior, it is necessary to understand the state of the self-assembled nanocarriers in conditions mimicking the *in vivo* environment and barriers they will interact with. Current 2D *in vitro*models do not provide exhaustive information due to the lack of complex environments such as flow-induced shear stress, endothelial barrier, 3D tumor microenvironment (TME) and cancer cells distribution in extracellular matrix (ECM)[1–3]. These deficiencies in pre-clinical complexity are the main reason of the low clinical translation of nanocarriers. Therefore, a comprehensive study of supramolecular systems stability in complex and dynamic environment requires a reliable, developed and versatile method.

Herein, we present a real-time monitoring study of stability and extravasation of self-assembled micelles, combining spectral confocal microscopy and tumor-on-a-chip. We developed a 3D microfluidic chip recapitulating the TME. It consists of a perfusable blood vessel channel lined with endothelial cells forming a wall which separates it from the cancer cells distributed in the ECM in spheroids. In the present work we investigate in the tumor-on-a-chip model three amphiphilic PEG-dendron hybrids that change their fluorescent properties upon assembly into micelles[4]. This property allows us to resolve the stability state of the hybrids in space and time, in correlation with the barrier they encountered, by monitoring changes in their fluorescence emission.

We observed a time dependent extravasation of monomer followed by micelles when cancer cells were present in the ECM. Moreover, no micelle extravasation occurred if cancer cells were not co-cultured in the chip within the time of the experiment. Interestingly, we observed that small differences in number of spheroids or the proximity between spheroid and endothelial monolayer impairs their retention capacity. These observations reflect the heterogeneity of the EPR effect which has been extensively discussed recently and is related not only to the type of cancer but also to the stage of the diseases and varies from patient to patient[1]. Overall, we demonstrated the high stability of our micelles against flow shear stress, and interactions with the ECM. However, we observed a low stability in contact with cancer cells forming spheroids which impedes the assembled state to penetrate the solid tumor, in contrast to micelle internalization verified in previous studies in 2D cancer monolayers.[4] Moreover, we observed big differences in stability and extravasation within hybrids with minor structural modifications.

Our results highlight the interplay between micelle stability in biological media and their pathway to extravasate to the target cell, a key step towards drug delivery, and demonstrate the importance of molecular control on nanostructures for their successful clinical translation.

1. Björnmalm, M. *et. al*., *ACS Nano* **11**, 9594–9613 (2017).
2. Shi, J. *et. al.*, *Nat.Rev. Cancer* **17**, 20–37 (2017).
3. Lazzari, G., Couvreur, P. & Mura, S. *Polym.Chem.* **8**, 4947–4969 (2017).
4. Feiner-Gracia, N. *et al.*, *J. Am. Chem. Soc.* **139**, 46, 16677-16687 (2017).

A Nanotoxicology Investigation: Exploring the size effects of silver and gold nanoparticles on lung cells

Friday, 18th October - 15:30: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 158

Ms. Hanouf Bafhaid [1], ***Dr. Hanene Ali-Boucetta*** [1], ***Dr. Zubair Ahmed*** [2], ***Dr. Youcef Mehellou*** [3]

1. *University of Birmingham,* ***2.*** *University of Birminhgam,* ***3.*** *Cardiff University*

Unfortunately, nanotoxicology literature does not present a homogenous finding in regards to the cytotoxicity of nanoparticles and discrepancies are still present between the different conclusions. While the biological responses and cellular-uptake of nanoparticles are predominately influenced by their different physiochemical properties, a systematic nanotoxicology framework is still lacking.

This study aims to systematically compare the impact of different composition and different sizes (10, 40 and 80nm) of spherical silver (AgNP) and gold (AuNP) nanoparticles on cellular toxicity and assess the changes mediated by these nanoparticles following their exposure to human adenocarcinomic alveolar basal epithelial cells (A549) and normal lung fibroblasts (MRC-5). The size and charge of the nanoparticles were characterised using dynamic light scattering and transmission electron microscopy. Their cytotoxicity effects were then studied using MTT, mLDH assays and the mechanism(s) of cell death investigated using Annexin-V/PI, reactive oxygen species (ROS) and glutathione depletion. On A549 cells, we found that CC50 (50% cytotoxic concentration) of Ag10 and Au10 after 24hrs are 11.7 and 42.2µg/ml respectively which suggests that AuNP are safer than AgNP. It was interesting to see that the smaller sizes (10,40nm) of AgNP and AuNP demonstrate more cytotoxicity in comparison to their bigger counterparts (80nm). This could be due to their enhanced cellular uptake due to their small size, as we found the amount of silver -taken-up by A549 cells after 24hrs exposure to Ag10 and Ag80 measured by ICP-MS are 4 and 3µg/ml, respectively. Necrotic cell death was observed in a size, concentration and time dependent manner following exposure to AgNP for 24 and 48hrs (more than 15% Annexin (+)/PI (+)) in both cell lines. Induction of oxidative stress was also found after 24hrs post- exposure to all sizes of AgNP on both cell lines, while AuNP showed an increase in ROS generation only at the highest concentration (40µg/ml). After 24hrs exposure to AgNP, the amount of reduced glutathione was significantly depleted in a size and dose-dependent manner with up to 80% decrease in reducing glutathione levels after A594 exposure to Ag10. AuNP illustrated a reduction effect on reduced glutathione in A549 and MRC-5 cells at the highest concentration which correlates with the detected ROS levels.

In summary, our findings highlight that the size of nanoparticles can influence their interaction with cells. AgNP of smaller sizes (10 and 40nm) showed detrimental effects on lung cells after 24 and 48hrs of exposure. AuNP demonstrated a good safety profile comparing to AgNP of the same size, emphasising that nanoparticle composition has also an impact on their cellular toxicity. However, MRC-5 and A549 cells display similar trend of toxicity toward AgNP, which is associated with the release of silver ions.

While size and chemical composition were the key determinant factors in defining the toxicity of our silver & gold nanoparticles, the ultimate goal of this work is to create a systematic nanotoxicology framework in order to validate current literature and to pave the development nanoparticles for biomedical applications.

Cell penetrating liposomes enable the oral delivery of vancomycin

Friday, 18th October - 15:45: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 264

Dr. Philipp Uhl [1], ***Dr. Max Sauter*** [1], ***Dr. Tobias Hertlein*** [2], ***Dr. Dominik Witzigmann*** [3], ***Dr. Knut Ohlsen*** [2], ***Prof. Gert Fricker*** [4], ***Prof. Walter Mier*** [1]

1. *Department of Nuclear Medicine, Heidelberg University Hospital, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany,* ***2.*** *Institute for Molecular Infection Biology, University of Würzburg, Josef-Schneider-Straße 2/D15, 97080 Würzburg, Germany,* ***3.*** *Nanomedicines Research Group, Life Sciences Institute, University of British Columbia, Vancouver BC, V6T 1Z3, Canada,* ***4.*** *Ruprecht-Karls-University, Institute of Pharmacy and Molecular Biotechnology, Im Neuenheimer Feld 329, 69120 Heidelberg, Germany*

Introduction:
Oral delivery of liposomally encapsulated peptide drugs is limited due to their instability in the gastrointestinal tract and their low mucosa penetration. Tetraether lipids are considered to stabilize liposomes under harsh conditions while cell penetrating peptides (CPPs) can enhance the mucosa penetration. Therefore, the oral availability of peptide drugs such as vancomycin incorporated into novel liposomes containing cell penetrating peptides and tetraether lipids was examined.
Methods:
Tetraether lipid isolation of *S. acidocaldarius*was performed by Soxhlet extraction. The cyclic cell penetrating peptide (CPP) R9C was synthesized by solid-phase synthesis and coupled to maleimide-functionalized phospholipids. Purification of the novel conjugates was achieved by preparative HPLC and confirmed by HPLC/MS analysis. Preparation of the liposomal formulations was performed by dual assymetric centrifugation (DAC). For in vivo studies, the oral uptake of the peptide antibiotic vancomycin was determined by radiolabelling. Therapeutic trials in a mouse '(MRSA) systemic infection model' were performed according to Hertlein et al [1].
Results:
The novel liposomes were characterized by Zetasizer measurements. The increase in the zeta potential indicated the successful incorporation of the CPP-phospholipid-conjugates into the liposomes. TEM and cryo-TEM micrographs confirmed the appropriate liposomal size and morphology. The preparation of the liposomes by DAC enabled high encapsulation efficiencies (up to 60%) as verified by HPLC and FCS measurements [2, 3]. Microscopy studies using Caco-2 cells showed that a strongly enhanced binding of the CPP-liposomes in contrast to standard liposomes was obtained. Furthermore, a high increase in the transport of vancomycin over rat mucosal tissue in ussing chamber studies in contrast to the free peptide could be shown. Oral application in a rat model revealed that the novel liposomal formulation led to a fivefold increase in the bioavailability of vancomycin. The functional efficiency of this uptake enhancement was proven by a strong increase of the antibiotic efficiency in a mouse '(MRSA) systemic infection model'.
Discussion:
The promising results clearly raise the hope that this novel liposomal formulation can be used as platform-technology for oral application of a variety of peptide drugs. Bioavailability studies in dogs are ongoing to investigate the transferability from rodents to higher mammals. Additionally, the applicability for further peptide drugs is currently examined.
[1] Hertlein, T., et al. "Bioluminescence and 19F magnetic resonance imaging visualize the efficacy of lysostaphin alone and in combination with oxacillin against Staphylococcus aureus in murine thigh and catheter-associated infection models." Antimicrobial agents and chemotherapy 58.3 (2014): 1630-1638.
[2] Uhl, P., et al. "A liposomal formulation for the oral application of the investigational hepatitis B drug Myr-

cludex B." European Journal of Pharmaceutics and Biopharmaceutics 103 (2016): 159-166.
[3] Uhl, P., et al. "Oral delivery of vancomycin by tetraether lipid liposomes." European Journal of Pharmaceutical Sciences 108 (2017): 111-118.

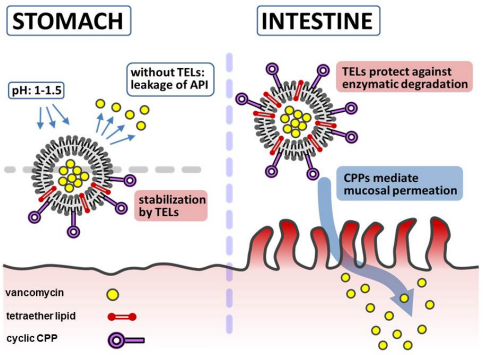


Figure 1. cpp-liposomes enable the oral delivery of vancomycin.jpg

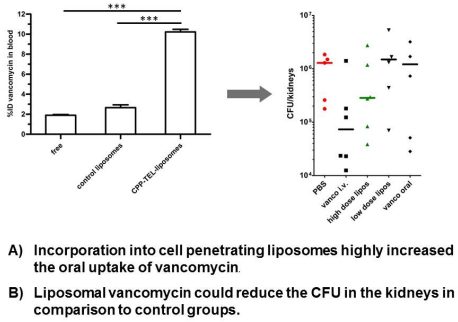


Figure 2. animal study.jpg

Multimodal Image-Guided folic acid targeted Ag2S quantum dots for photothermal therapy and selective methotrexate delivery

Friday, 18th October - 16:00: Targeted drug delivery, organ biodistribution and nanocarriers (Room N02.011) - Oral - Abstract ID: 45

Ms. Mahshid Hashemkhani [1], ***Prof. Havva Yagci Acar*** [1], ***Prof. Alphan Sennaroğlu*** [1], ***Mr. ABDULLAH MUTI*** [1]

1. *Koc university*

Introduction:
The use of Ag_2S near-infrared quantum dots (AS NIRQDs) as a sensitizer in photothermal therapy (PTT) is relatively new. Emission of AS QDs in near infrared I (NIR-I) allows better signal/noise ratio and deeper penetration depth for optical imaging of biological tissues. Here, we developed a multifunctional AS NIRQDs tagged with folic acid (FA) to deliver methotrexate (MTX) which is an anticancer drug to folate receptor overexpressing cancer cell lines for image guided combination therapy.
Method: AS QDs with glutathione (GSH) coating was prepared in aqueous solutions. It was conjugated with FA using a PEG spacer via amidation reactions. Then, MTX was covalently conjugated to AS-GSH-PEG-FA to form a multifunctional nanocarrier for NIR imaging, PTT and targeted chemotherapy. Hydrodynamic size and surface charge of nanoparticles were measured by Malvern Zetasizer. In the in vitro studies, FR-positive human cervical carcinoma cells (HeLa) cells and FR-negative A549 and colorectal adenocarcinoma (HT29) cells were used. Particle internalization was determined by ICP. MTT was used for determination of cytotoxicity (with and without laser irradiation). The PTT effect of AS-GSH and AS-GSH-PEG-FA QDs were first tested in aqueous solutions as a function of QD concentration, irradiation time and input power. To evaluate PTT efficiency of AS-GSH, AS-GSH-PEG-FA and AS-GSH-PEG-FA-MTX QDs, HeLa and HT29 cells were treated with different concentrations, which was determined as safe, and then irradiated with 795 nm laser at 500 mW for 10 minutes. Cell viability after laser irradiation was determined using MTT assay.
Results and discussions:
Aqueous colloidal AS-GSH QD with excellent stability was achieved with emission maxima between 800-900 nm. The absorption peak around 370 nm with a slight red shift compared to free FA clearly confirmed the FA and MTX conjugation (Figure 1). Based on a calibration curve prepared from the concentration dependent absorption of FA and MTX separately, binding efficiency of FA and MTX to AS-GSH were obtained 93.6% and 76%, respectively. QDs showed no cytotoxicity up to 200 µg/ml Ag concentration in either cell lines but induced significant dark toxicity when conjugated with FA and MTX in HeLa due to the presence of folate receptors. QDs were well internalized by HeLa cells and provided strong optical signal in the NIR. Using 795 nm laser irradiation induced significant cell death in AS-GSH QDs (Figure 2).

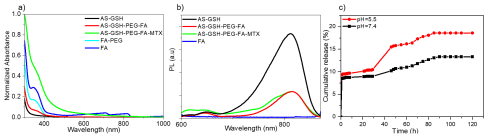


Figure 1- a the uv-vis b pl spectra of different conjugates of as qds and c in vitro release profiles of as-gsh-mtx in pbs solution at ph 7.4 and 5.5 37 .png

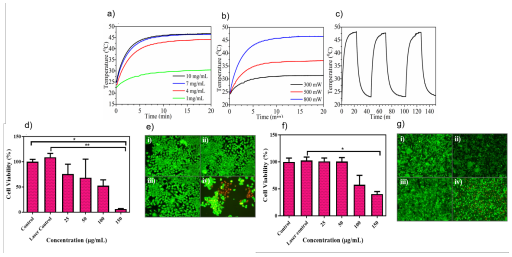


Figure 2-time dependent change in the temperature of as-gsh a at different qd concentrations b at different powers c stability d e cell viability after laser.png

The CRC 1066: From a center for nanoparticle based tumor immunotherapy to novel approaches in oncology

Friday, 18th October - 16:45: Large projects session: How to run a successful bio-nano-pharmaceutical consortial project? (Amphitheatre N02.040) - Plenary Speech - Abstract ID: 262

Dr. Matthias Barz [1]

1. *Johannes Gutenberg University*

In 2013 the center of nanoparticle based tumor immunotherapy (CRC 1066: Nanodimensional polymer therapeutics for tumor therapy) was established at the Johannes Gutenberg University and the Medical Center of the JGU in Mainz with the goalm to develop novel therapeutic interventions for melanoma therapy. After its elongation in 2017 the center hosts 32 groups from chemistry, physics, biology and medicine. The interdisciplinary character of this CRC is also reflected by the steering committee, which includes Rudolf Zentel (Organic/Polymer chemistry, JGU), Stephan Grabbe (Department of Dermatology, University Medical Center), Katharina Landfester (Max-Planck-Institute for Polymer Research), Detlef Schuppan (University Medical Center) and Matthias Barz (Organic/Polymer chemistry, JGU).
Therefore, the presentation will include a general introduction to the funding scheme of CRC (Collaborative Research Center, SFB), an overview on the development of the CRC1066 and a personal overview on the related research from our group.

MINDED – Multiscale Technologies for NeuroDevelopmental Disorders

Friday, 18th October - 17:07: Large projects session: How to run a successful bio-nano-pharmaceutical consortial project? (Amphitheatre N02.040) - Workshop - Abstract ID: 287

Prof. Paolo Decuzzi [1]

1. *Nanotechnology for Precision Medicine, Italian Institute of Technology, Genova, Italy.*

The societal and economic impact, the growing incidence, and the lack of any cure call for radical changes in the way Neurodevelopmental Disorders (NDDs) are studied and treated. This lecture will elaborate on synergistic and highly interdisciplinary approaches aiming to revolutionize the life of NDD patients. MINDED uses a multiscale technology approach integrating across Nanomedicine, Molecular Neurosciences, and Cognitive Neuroscience Robotics, for the development of novel diagnostic, imaging and therapeutic interventions for NDDs. Nanomedicine for the precise delivery of therapeutic and imaging agents; RNA-technologies for gene silencing, modulation and repair; laboratories-on-chip for high throughput screening of novel therapeutic strategies; molecular imaging for the early detection and localization of diseased cells; and advanced cognitive robotics for patient stratification and rehabilitation are integrated together to improve the classification, diagnosis, imaging and treatment of NDDs. Training in these three advanced technological areas is expected to foster the establishment of a new class of scientists, technologists, administrators and entrepreneurs.

Authors Index