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BOOK OF ABSTRACTS

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Table of Contents

Bio-inspired Nanomaterials for Biomedical Applications

Monday, 25th September - 09:05 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 500

Prof. philippe Barthelemy [1]

1. *University of Bordeaux, ARNA laboratory, INSERM, U1212, UMR CNRS 5320, F-33000 Bordeaux, France.*

Advances in nanotechnology, biotechnology, and materials sciences are leading to novel approaches in the field of health science and technology. Effective integration of biological units in these new technologies is critical for interfacing biological functions. As an alternative to synthetic polymers, biomolecules such as nucleic acids, amino acids, sugars represent valuable bioorganic material for the construction of biomedical devices due to their general biocompatibility and their supramolecular properties. While the utility of synthetic biomolecules for biomedicine has been demonstrated through a number of reports, an undeveloped potential for more effectively using the molecular and supramolecular capabilities of bioconjugates in nanomedicine remains to be explored.

In this presentation we will see how the synthetic combination of biomolecules, (i.e. nucleic acids sugars, lipids) provides an efficient approach to prepare well-defined nano systems with tunable physico-chemical properties and functions. The new bioinspired systems are currently under investigation in our lab for i) drug delivery applications (therapeutic, theranostic), and ii) biomaterials.[1]

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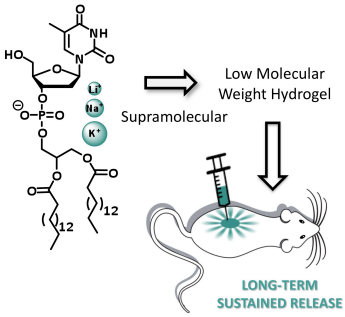

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Formulation of biological drugs using nanotechnology approaches

Monday, 25th September - 09:40 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 501

Prof. Maria Alonso [1]

1. *University of Santiago de Compostela*

The progress over the last decades in the field of nanomedicine has led to the improved understanding of the biological barriers, the availability of new complex bioactive molecules and the design of new biomaterials and nanotechnologies. The nanopharmaceutical technology has greatly contributed to this progress by making feasible the nanoencapsulation and controlled delivery of complex molecules, as well as defining ways to scale-up the production of nanomedicines. Nevertheless, a significant amount of this research has relied in developing “mee too” delivery carriers, while move innovative initiatives been oriented to the design of drug carriers, which suffer of safety concerns and/or poor pharmaceutical quality assessment.

Our group, being committed with the translation of ideas from the university through novel pharmaceutical nanotechnology, has designed novel nanostructured materials intended to transport drugs and antigens across biological barriers and to deliver them to the target tissue. During my presentation I would like to focus on specific applications of the nanotechnologies we have designed until now, notable in the area of cancer immunotherapy as well as in the area oral and ocular delivery of complex macromolecules. I will present specific formulation approaches for the delivery of peptides, monoclonal antibodies and polynucleotides. I will also highlight that our experience in this field has greatly benefited from integrative approaches adopted by specifically designed consortia. Hopefully, the results of these cooperative efforts will help to accelerate the progress on the rational design of nanomedicines.

More information about these projects can be found at:

http://www.usc.es/grupos/mjalonsolab/

ACKNOWLEDGMENTS

The research activity has been founded by:

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- The European Comision Horizon 2020 Programme (grant agreement n° 646142 – NANOPILOT and grant agreement No. 642028-NABBA) (see http://nabbaproject.eu and http://www.nanopilot.eu)
- The Ministry of Economy of Spain, Ref. Ref. RTC-2016-4823-1 (GLAUKUS) and, Ref. RTC-2016-4884-1 (SEKEYE)

Nanotechnology based materials to repair and rebuild human organs

Monday, 25th September - 10:45 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 2

Prof. Alexander Seifalian [1]

1. *Nanotechnology & Regenerative Medicine Commercilisation Centre (Ltd)*

Nanotechnology is revolutionising the repair and replacement of human organs. Nanomaterials have the unique physical, chemical, mechanical, and optical properties that naturally occur at that nano scale. We have developed a family of nanocomposite materials for clinical application. The nanocomposite materials have been used in repair and development of human organs. The materials have been fabricated to the 3D scaffold using coagulation, casting, electrospinning as well as 3D printing. Then the scaffold has been functionalised with bioactive molecules and antibodies for capturing and differentiation of stem cells to mature cells either in vitro or in vivo using the body as a bioreactor. In this talk present to development as well as route of taking laboratory research to patients and commercialisation. The organs will be discussed in details world first synthetic trachea, tear ducts, small diameter bypass graft for replacement of coronary and vascular arteries. Taking organs to patients and commercialisation is challenging in term of regulatory as well as manufacturing under GMP/GLP. In this talk will discuss the pathway and time scale taking the organs to the clinical trial from laboratories products. Finally, will discuss our experience in development of nanoparticles and its application in diagnostic and treatment of cancer. These nanoparticles include quantum dots, magnetic nanoparticles and carbon nanotube.

The Nanopharmacokinetic Gap

Monday, 25th September - 11:25 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 171

Prof. Victor Puntes [1]

1. *Vall d Hebron Institut of Research*

Despite the tremendous potential of nanomedicine and hundreds of millions poured from funding institutions, it could be acknowledged that little progress has been made towards matching expectations. Thus few years ago, in 2012, Bayer announced that when they tried to replicate results of 67 studies , nearly two-thirds failed, which is in part attributed to poorly described nanomaterials along with the lack of publication of negative results in the scientific community. Also in Nature Nanotechnology there was a call for better described NPs and Harald Krug after analyzing about 10.000 nanotoxicology papers did find serious problems in the state and purity of the employed NPs. More recently, Derek Lowe's commentary on drug discovery and the pharma industry in the Science Magazine Blog, commenting on *Analysis of Nanoparticles Delivery to tumours*, recognized it again: "Working out that delivery and pharmacokinetics aspects of these things (nanoparticles) was already known to be a challenge, but it's proven to be even more of one than anybody thought.

Accumulation and Effects of Pb, Cr and Cd on the growth of Zea mays Seedlings

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 474

Ms. Zaima Afzal AWAN [1]

1. *CEMB University of the Punjab, Lahore*

Heavy metals caused adverse effects on the plant growth and development, microbial growth and survival, productivity of soil and quality of soil. Heavy metal toxicity caused death of plants and soil microbes; therefore the removal of heavy metals from soil is necessary to keep microbes and plant healthy along with clean and pure environment. Prescribed study was conducted at Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan to access the ability of maize genotypes (SWL-2002 and Raka-poshi) to grow and survive under heavy metals (chromium, cadmium and lead) in different treatments (control, 0.5mM $CrCl_3$, 0.5mM $CdCl_3$, 0.5mM $Pb(NO_3)_2$, 0.5mM+0.5mM $CrCl_3$+$CdCl_2$, 0.5 mM+0.5mM+0.5mM $CrCl_3$+$CdCl_2$+$Pb(NO_3)_2$). It was found from results that both of the maize genotypes showed almost similar behavior under heavy metals treatments for different morphological studied traits. Strong and positive correlation was reported among the morphological traits and among the uptake of heavy metals by leaves, roots and stem. From principal component analysis it was revealed that most of the variation was due to the uptake of heavy metals by root, leaves and stem. The lowest uptake of chromium, cadmium and lead was reported under control and 0.5 mM+0.5mM+0.5mM $CrCl_3$+$CdCl_2$+$Pb(NO_3)_2$ treatment which indicated the ability of heavy metals to be up taken by roots from root zone area of maize plants. It was concluded form results that the heavy metals caused adverse effects on morphological traits of maize which ultimately reduced production and productivity of maize. It was also found beside the adverse effects of heavy metals maize may be used for the removal of heavy metals from soil biosphere.

Amine-functionalised multi-walled carbon nanotubes as drug delivery nanovector into the brain

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 365

Dr. Muhammad Huzaimi Haron [1], ***Prof. Nicholas Barnes*** [1], ***Dr. Hanene Ali-Boucetta*** [1]

1. *University of Birmingham*

The utilisation of carbon nanotubes (CNTs) in the biomedical field is being realised. Short multi-walled CNTs (MWNTs) has the better toxicological profile, thus is preferable over its single-walled counterpart. Functionalisation of MWNTs (producing fMWNTs) confer modifiable functional groups with which therapeutic molecules can be attached; employment of fMWNTs as drug delivery nanovectors could be evaluated. Plenty of potentially beneficial neurological therapeutics have been hampered by poor penetration of the blood-brain barrier (BBB). Therefore, the objective was to demonstrate cellular internalisation of fMWNTs into cells of the BBB.

Pristine MWNTs were functionalised to produce MWNT:PF127, MWNT-COOH and MWNT-$NH3^+$. All MWNT preparations were dispersed in water before used in experiments and were characterised using transmission electron microscopy (TEM), Fourier-transform infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA). Three types of cells were used for the internalisation experiments: human brain microvascular endothelial cells (hBEC-5i, ATCC CRL-3245), human pericytes (hPeric) and human astrocytes (hAstro). Internalisation of MWNTs were assessed using flow cytometry analysis (FCA) of HBEC-5i monolayers and confocal microscopy (CFM) of fluorescently-labelled heterocellular spheroidal model of the BBB. Measures to indicate internalisation are increased side-scatter (FCA) and reduction in fluorescence intensity fraction (CFM).

All fMWNT preparations were easily dispersible in water. Compared to MWNT:PF127, both MWNT-COOH and MWNT-$NH3^+$were shorter. FTIR confirmed the presence of the functional groups on MWNT-COOH and MWNT-$NH3^+$. TGA showed that whilst pristine MWNTs were thermally stable, MWNT-COOH and MWNT-$NH3^+$ lost 33% and 80% of their weight, respectively, after heated to 1000°C. The quantitative Kaiser test performed on MWNT-$NH3^+$ showed amine group loading of 674 µmol/g of MWNT. HBEC-5i monolayer exposed to MWNT:PF127 for 24 and 48 hours did not show any dose-dependent difference in side scatter nor AUC. However, exposure of the same cell line to MWNT-COOH and MWNT-$NH3^+$showed a dose-dependent increase in cellular side scatter. Exposure of the heterocellular spheroidal model of the BBB to the fMWNTs resulted in reduced fluorescence intensity fraction in both hBECs and hPerics.

fMWNTs were shown to be safely internalised into cells of the BBB, with MWNT-NH3+ possessing the higher potential as a drug-delivery nanovector due to its modifiable functional group.

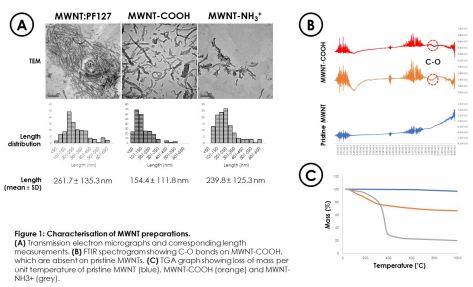

Figure 1: Characterisation of MWNT preparations. **(A)** Transmission electron micrographs and corresponding length measurements. **(B)** FTIR spectrogram showing C-O bonds on MWNT-COOH, which are absent on pristine MWNTs. **(C)** TGA graph showing loss of mass per unit temperature of pristine MWNT (blue), MWNT-COOH (orange) and MWNT-NH3+ (grey).

Figure characterisation mwnt muhammad huzaimi haron.jpg

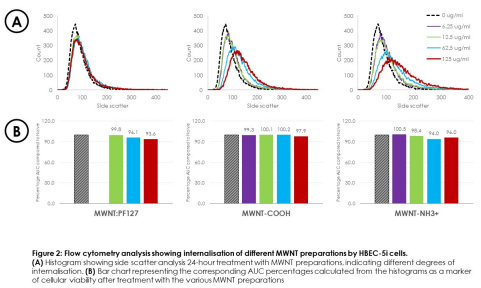

Figure 2: Flow cytometry analysis showing internalisation of different MWNT preparations by HBEC-5i cells. **(A)** Histogram showing side scatter analysis 24-hour treatment with MWNT preparations, indicating different degrees of internalisation. **(B)** Bar chart representing the corresponding AUC percentages calculated from the histograms as a marker of cellular viability after treatment with the various MWNT preparations

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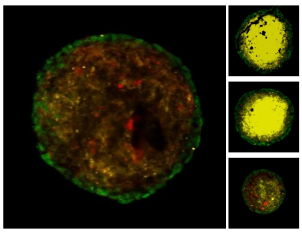

Figure 3: Confocal microscopy pictures of heterocellular blood-brain barrier spheroids.
hBEC-5i (green), hPeric (red) and hAstro (yellow) configuration in the spheroidal BBB model.
Clockwise from left: Untreated spheroid, and spheroid treated with 50 ug/ml of MWNT:PF127, MWNT-COOH and MWNT-NH3+, respectively.

Figure bbb spheroids muhammad huzaimi haron.jpg

Biocompatible upconverting core/shell/shell nanoparticles with enhanced red emission for application in bioimaging and cancer therapy

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 72

Mrs. Dovile Baziulyte-Paulaviciene [1], ***Ms. Greta Jarockyte*** [2], ***Prof. Ricardas Rotomskis*** [2], ***Dr. Vitalijus Karabanovas*** [2], ***Dr. Simas Sakirzanovas*** [1]

1. *Vilnius University, Faculty of Chemistry and Geoscience,* ***2.*** *Biomedical Physics Laboratory, National Cancer Institute*

Cancer therapy and diagnostics have become one of the most promising and studied topics in terms of nanomedicine. Focusing on therapeutic applications, the application of nanoparticles (NPs) as drug delivery vehicles or multimodal contrast agents is of major importance [1]. In this work, we report the thermal decomposition synthesis of $NaGdF_4:Er^{3+}@NaYbF_4@NaGdF_4$ core/shell/shell upconverting nanoparticles (UCNPs) as multimodal imaging agents. X-ray diffraction (XRD), scanning electron microscopy (SEM) and photoluminescence (PL) spectroscopy were used to characterize UCNPs (Fig. 1). Further surface functionalization was subsequently accomplished using a well-known Tween80 nonionic surfactant which converts hydrophobic nanoparticles to hydrophilic ones. The concentration of Er^{3+} in core $NaGdF_4$ matrix was varied and optical properties of obtained nanoparticles was investigated by Edinburgh Instruments FLS980 spectrometer. Compared with $NaGdF_4:Er^{3+}$ active-core NPs, the two order of magnitude enhancement of total integrated emission intensity was obtained in the core/shell/shell NPs. Importantly, the red UC emission of 653 nm in core/shell/shell NPs is greatly increased compared to the core and core/shell samples under 980 nm excitation at room temperature. In contrast to green and blue light, red light (600–700 nm) can deeply penetrate into biotissues owing to the lack of efficient endogenous absorbers [2]. Furthermore, cell viability assay XTT was performed to measure cellular metabolic activity after 24 hours treatment with different concentrations of UCNPs. Nanoparticles have no significant influence on viability of cells after incubation with 0.01 mg/mL and 0.5 mg/mL UCNPs solutions; however a slight decrease of viability was observed after incubation with 0.1 mg/mL UCNPs solution. Smart design of multifunctional nanocomposite and further studies are still essential to generate sufficient data for better understanding of long-term toxicity and safety of the UCNPs before introducing their wide applicability in medicine.

Reference:

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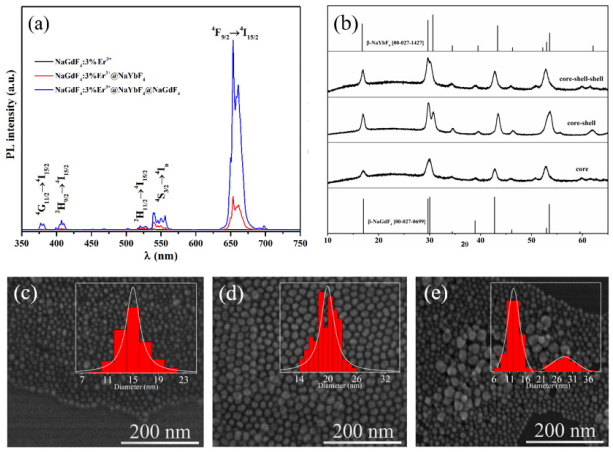

Upconversion luminescence spectra of UCNPs (a). XRD patterns of the hexagonal $NaGdF_4$, $NaYbF_4$ and as prepared UCNPs (b). SEM images of core (c), core/shell (d) and core/shell/shell (e) UCNPs.

Figure 1.jpg

Combining photodynamic therapy and chemotherapy: Nanotechnology improving breast cancer treatment

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 51

Dr. Marilia Calmon [1], ***Ms. Natalia Candido*** [1], ***Ms. Maryanne Melo*** [2], ***Prof. Fernando Primo*** [1], ***Prof. Antonio Claudio Tedesco*** [2], ***Prof. Paula Rahal*** [1]

1. *São Paulo State University,* ***2.*** *University of Sao Paulo*

Breast cancer is a serious public health problem, not only because of the high incidence and mortality, but also because of the often ineffective treatment. Different nanostructured systems demonstrate advantages over other delivery systems due to their nanometric scale structure with unique properties. The aim of this study were to synthesize and characterize nanoemulsion containing chloroaluminium phthalocyanine (ClAlPc) and doxorubicin (Doxo) (NPcDoxo) and to investigate its action associated with photodynamic therapy (PDT) on murine breast cancer cell line 4T1. In addition, differential gene expression analysis of apoptosis-related genes and anti-cancer drug target genes in 4T1 cells treated with NPcDoxo associated with PDT were performed. NPc-Doxo presented a size of 180.1 ± 0.7, polydispersity index of 0.28 and zeta potential of -75 ± 0.12 mV. The three-dimensional analysis of NPcDoxo by atomic force microscopy showed that NPcDoxo has a spherical shape and low polydispersity. Confocal microscopy analysis confirmed the efficient internalization of AlClPc and Doxo by 4T1 cells after 3 hours of incubation with NPcDoxo. 4T1 cells incubated with NPcDoxo and empty nanoemulsion presented high cell viability (80% and 90%, respectively) prior to PDT. Cell viability remained high for the empty nanoemulsion but decreased to 10.3% after incubation of NPcDoxo associated with PDT (1000 mJ / cm 2). The analysis of apoptosis assay of 4T1 cells after NPcDoxo incubation associated with PDT presented a significant increase ($p < 0.001$) in the amount of apoptotic cells after 1.0 $J.cm^{-2}$laser light dose. In order to identify the possible genes related to apoptosis and targets for anticancer therapeutics, changes in the gene expression profile of the 4T1 cells before and after treatment with NPcDoxo associated with PDT were analyzed and 23 target genes of anticancer drugs and 15 genes related to apoptosis showed low expression and 2 target genes of anticancer drugs and 5 genes related to apoptosis showed increased expression in 4T1 cells incubated with NPcDoxo associated with PDT. The phthalocyanine and doxorubicin loaded within nanoemulsions presented appropriated physical stability, improved photophysical properties, and remarkable activity *in vitro* to be considered as promising formulations for PDT and chemotherapeutic use in breast cancer treatment.

Customization of recombinant BabA-Lewis b adherence domain as a novel gastric-targeted drug delivery system

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 462

Mr. AYMEN MOHAMMED [1], ***Dr. Naim Hage*** [1], ***Mr. Waleed Alharbi*** [1], ***Mr. Abdulaziz Alouffi*** [2], ***Dr. Nora Francini*** [1], ***Ms. Vasiliki Paraskevopoulou*** [1], ***Dr. Paul Gellert*** [3], ***Dr. Ross Overman*** [4], ***Dr. Geoffrey Holdgate*** [4], ***Dr. Sebastiaan Winkler*** [5], ***Dr. Snow Stolnik-Trenkic*** [1], ***Dr. Franco Falcone*** [1]

1. *Division of Molecular Therapeutics and Formulations, School of Pharmacy, University of Nottingham,* ***2.*** *Division of Molecular Therapeutics and Formulations, School of Pharmacy,The University of Nottingham,* ***3.*** *Pharmaceutical Development, AstraZeneca R&D, Charter Way, Macclesfield, Cheshire SK10 2NA,* ***4.*** *Discovery Sciences, Innovative Medicines and Early Development, AstraZeneca R&D, Alderley Park, Cheshire SK10 4TG,* ***5.*** *Biomolecular Science and Medicinal Chemistry Division, School of Pharmacy, University of Nottingham*

Introduction: Lectin-mediated cytoadhesion is a promising targeting concept for gastric drug delivery. *Helicobacter pylori*, a natural inhabitant of the human stomach, inspired us to use its gastric-adhesion proteins as a novel gastro-retention drug delivery platform. Our recently published co-crystal structure of *H. pylori* BabA (Blood group Antigen binding Adhesin), with its physiological ligand Lewis b (Le^b), revealed the existence of a distinct binding domain, the BabA-Crown. However, gastric degradation by pepsin is a major obstacle to any protein-based gastric delivery system. As the ligand-binding region of BabA appears to offer partial resistance to pepsin degradation, we assessed the feasibility of using only the BabA crown for particle targeting.

Methods: The BabA-Crown DNA sequence was amplified by PCR and cloned into pOPE101 periplasmic expression vector. An AviTag sequence was added by site-directed mutagenesis to enable enzymatic monobiotinylation with BirA and subsequent nano/microparticle binding. The resulting constructs were transformed into chemically competent *E.coli* XL-10 Gold cells. After extraction from bacterial periplasmic space, rBabA-Crown protein was used for assessment of cellular and molecular binding interactions, resistance to pepsin digestion using simulated gastric juice, and microparticle coating.

Results: Our data show binding of the complete extracellular region of BabA ($BabA_{547}$) results in submicron-sized particles able to bind specifically to Le^b. However, $BabA_{547}$ protein was rapidly degraded by pepsin. Attempts to express various truncated forms of rBabA which included the crown region, failed to yield recombinant protein. In contrast, expression of the core binding crown region was successful, but only after addition of chloramphenicol during expression. The resulting protein had partial stability to pepsin digestion. Unexpectedly, circular dichroism data indicated that it may not be folded correctly. Binding to Le^b neoglycoconjugates was revealed at low pH, but this was not specific for Le^b-HSA, as the crown also bound to Le^a-HSA, lacdiNac-HSA and unglycosylated HSA, suggesting non-specific adhesion.

Discussion: Currently, gastric pepsin is a powerful obstacle to protein-based gastric delivery. The use of small, pepsin-resistant polypeptides recognising specific ligands expressed on the gastric epithelium may be a viable approach. However, difficulties with the expression of a correctly folded protein ligand will first need to be overcome.

Design and Synthesis of Light-Responsive Amphiphilic Block Copolymers as Drug Carriers

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 156

Dr. Binnur Aydogan Temel [1]

1. *Department of Pharmaceutical Chemistry, Bezmialem Vakif University, Fatih, 34093 Istanbul, Turkey*

Introduction: In recent years, a number of strategies have been devoted in the use of nanoparticles as drug carriers. Drugs may be encapsulated, adsorbed or dispersed in nanoparticles, which can be colloidal-sized particles, possessing diameters ranging between 1 and 1000 nm.[1] A wide variety of nanoparticles composed of a range of materials including lipids, polymers and inorganic materials have been developed, resulting in delivery systems that vary in their physicochemical properties and thus their applications. Among them amphiphilic block copolymers with hydrophilic and hydrophobic segments have attracted a great deal of attention in terms of their ability to form various types of nanoparticles.[2-4] The stability of polymeric micellar systems is a crucial condition especially for the encapsulation of active compounds and various approaches have been proposed to stabilize polymeric micelles. Among them, the core-crosslinked micelle and the shell-crosslinked micelle are attractive ways to maintain the structural integrity of micellar object.[5]

Methods: Herein, we investigated the synthesis and characterization of light-responsive amphiphilic block copolymers via ring opening polymerization and click reaction. The light responsiveness of polymers was tested using UV-Vis spectroscopy. The structures of starting materials and corresponding copolymer were confirmed by ^{1}H NMR and GPC analyses. Micelle formation of amphiphilic copolymers were also investigated and characterized by DLS.

Results and Discussion: We have demonstrated the successful synthesis of light-responsive amphiphilic block copolymers and their self-assembling behaviour fort he formation of small polymeric nanoparticles with a narrow size distribution in an aqueous medium. The simplicity of preparation and stimuli response of polymeric nanoparticles make them attractive for various potential applications as a tailor-made advanced functional drug carrier.

References:

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Design of recombinant small antibodies for siRNA delivery

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 345

Mr. Daisuke Miura [1], ***Dr. Takamitsu Hattori*** [1], ***Dr. Teppei Niide*** [1], ***Dr. Hikaru Nakazawa*** [1], ***Prof. Mitsuo Umetsu*** [1]

1. *Tohoku University*

RNA interference, which suppresses gene expression by small interfering RNA (siRNA), has been utilized in the medical field, but specific delivery of siRNA to cells or tissues remains challenging. Now, main method of siRNA delivery is the method of using liposome. But liposomes can't reach deep in the tumor tissue because the size of liposomes is several ten nm to several hundred nm. In contrast, recombinant small antibodies, such as single-chain fragment of variable region (scFv) and diabody consisting of the variable regions of antibody which has high specificity to targeted cells and tissues, can more highly permeate tissues and reach various disease sites. In this study, we aim to design the small antibodies bearing the siRNA to construct a drug delivery system (DDS) of siRNA (Fig. 1).

First, we prepared 20 recombinant small antibodies (10 scFvs and 10 diabodies) with cationic peptide fragments as RNA carrier at C-terminus via 2 types of linkers by means of *E.coli* expression system. The charged peptides electrostatically interact with negatively-charged siRNAs. SDS-PAGE and western blot analyses revealed low expression of the antibodies with LK15 peptide and protamine in *E.coli*, whereas the antibodies with Arg9, TAT and truncated protamine (tp) peptides were expressed and secreted in culture supernatant. The expressed scFv with tp was purified by immobilized metal affinity chromatography followed by size exclusion and cation exchange chromatographies; as a result, pure scFv with tp was obtained, but the yield of purified scFv with tp was 4 μg/L-culture, which was significantly low. Next, we tried to prepare recombinant small antibodies with RNA carrier by chemical conjugation using amine coupling. Antibodies after chemical conjugation with RNA carrier were fractionated by cation exchange chromatography. As a result, many small antibodies remained un-conjugated and also caused multimerization (Fig. 2).

In conclusion, the fusion of RNA carrier to small antibody decreased the expressed yield in *E.coli*. We are in progress of preparing small antibody-RNA carrier complex by means of site-specific chemical conjugation using enzyme with small antibody and RNA carrier.

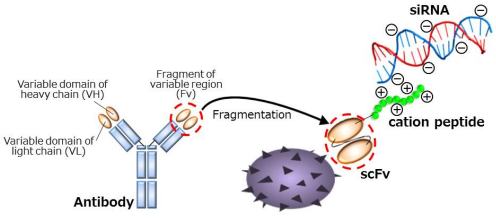


Fig. 1 sirna delivery by small antibody..jpg

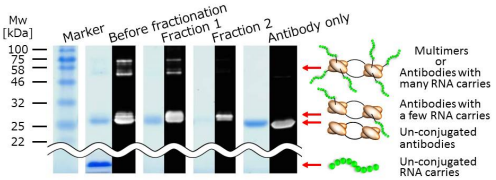


Fig. 2 chemical conjugation of antibodies with rna carriers..jpg

Development of material-binding proteins fused device to improve sensitivity of biosensor.

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 350

Mr. Tatsuki Miyaki [1], ***Mr. Takuma Sujino*** [1], ***Dr. Hikaru Nakazawa*** [1], ***Prof. Mitsuo Umetsu*** [1]

1. *Tohoku University*

Biosensor is used early diagnosis in medical setting. Many biosensors use antibodies for recognizing marker proteins and the antibodies should be immobilized on sensor chips that transduce capturing action to optical or electrical signals. High density and homogeneous orientation of immobilized antibody increases sensitivity for biosensor. In general, antibodies are chemically immobilized on sensor chips; however, the chemical method can't control the orientation of antibody on the chips and disrupt there's construction.

Recently, we devised a single variable domain of the heavy chain camel antibody (VHH) with high affinity for inorganic material surface. Use of the VHH for an interface between sensor chip and recognition molecule is expected for highly sensitive detection, because it can immobilize antibody with high density on the surface of material chip and it also needs no chemical modification.

In this study, we constructed a fusion protein by fusing various antibodies to C-terminal of material -binding VHH and tried to make the wide use biosensor which can detect various maker proteins sensitivily. Consequently, sensitivity of target detection increases by using fusion protein compared with physical adsorption.

Dry versus hydrated collagen scaffolds: are dry states representative of hydrated states?

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 330

Dr. Monika Supova [1], ***Dr. Tomas Suchy*** [1], ***Dr. Martin Bartos*** [2], ***Dr. Radek Sedlacek*** [3], ***Dr. Marco Piola*** [4], ***Dr. Monica Soncini*** [4], ***Dr. Giafranco Fiore*** [4], ***Dr. Pavla Sauerova*** [5], ***Dr. Marie Hubalek Kalbacova*** [5]

1. *Department of Composites and Carbon Materials, Institute of Rock Structure and Mechanics, The Czech Academy of Sciences, Prague,* ***2.*** *Department of Stomatology, First Faculty of Medicine, Charles University and General University Hospital in Prague,* ***3.*** *Laboratory of Biomechanics, Department of Mechanics, Biomechanics and Mechatronics, Faculty of Mechanical Engineering, Czech Technical University in Prague,* ***4.*** *Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano,* ***5.*** *Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University in Prague*

Abstract

Introduction

Collagen composite scaffolds have been used for a number of studies in tissue engineering. The hydration of such highly-porous and hydrophilic structures may influence mechanical behaviour and porosity due to swelling. The differences in physical properties following hydration would represent a significant limiting factor for the seeding, growth and differentiation of cells *in vitro* and the overall applicability of such hydrophilic materials *in vivo*.

The aim of the present work, however, is to describe the study of the influence of eight differing scaffold material compositions, focusing on collagen content and their final properties such as internal structure, porosity and mechanical properties in both the dry and hydrated states. It was anticipated that the differences in physical properties following hydration would represent a significant limiting factor for the seeding, growth and differentiation of mesenchymal stem cells and the overall applicability of such hydrophilic materials.

Methods

Scaffolds based on collagen matrix, poly(DL-lactide) nanofibers, calcium phosphate particles and sodium hyaluronate with 8 different material compositions were characterized in the dry and hydrated states using X-ray micro-computed tomography, compression tests, hydraulic permeability measurement, degradation tests and infrared spectrometry.

Results

Hydration, simulating the conditions of cell seeding and cultivation up to 48 hours and 576 hours, was found to exert a minor effect on the morphological parameters and permeability. Conversely, hydration had a major statistically significant effect on the mechanical behaviour of all the tested scaffolds. The elastic modulus and compressive strength of all the scaffolds decreased by approx. 95%. The quantitative results provided confirm the importance of analysing scaffolds in the hydrated rather than the dry state since the former more precisely simulates the real environment for which such materials are designed. The measurement of scaffolds in the dry state is, however, useful regarding to the basic characterisation of the selection of preparation methods; however, it is important that real conditions not be neglected, especially with concern to hydrophilic polymers.

Acknowledgements

This study was supported by a grant project awarded by the Ministry of Health of the Czech Republic (15-25813A).

Emergence of PEI as a Valuable Reducing Agent and Cationic Coating in the Production of Gold Nanoparticles as Transfection Vectors

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 158

Ms. Ozge Cavuslar [1], ***Ms. Cagnur Celaloglu*** [1], ***Dr. Fatma Demir Duman*** [1], ***Dr. Yeliz Utku Konca*** [1], ***Dr. Baris Yagci*** [1], ***Dr. Havva Yagci Acar*** [1]

1. *Koc University*

Introduction

Gold nanoparticles (GNPs) attract researchers from both fundamental and applied sciences. Due to inert and nontoxic nature, they are very valuable nanomaterials for in vivo biomedical applications including imaging and therapy. One attractive composition would be GNPs coated with polyethyleneimine (PEI), which is the golden standard for non-viral gene delivery. Here, we aimed to study the one step, aqueous synthesis of gold nanoparticles by using branched polyethyleneimine (bPEI). The influence of PEI protonation on particle size and stability is investigated for the first time. Impact of the molecular weight was also studied in detail. High gene transfection efficiency coupled with simple one pot synthesis make this process a valuable alternative for the generation of new GNP based transfection agents.

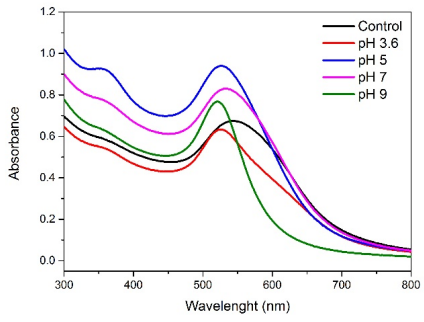
Methods

GNPs were synthesized by thermal reduction of $HAuCl_4$ with bPEI as capping and reduction agent. Molecular weight of the PEI (25, 10, 1.8, 0.6 kDa), reaction time and pH of the solution were changed systematically. MTT assay was used to analyze cytotoxicity of particles in HEK-293T cells. For the transfection analysis GNPs were loaded with pMax-GFP.

Results and Discussion

The surface plasmon band (SPB) of GNPs was observed at around 520 nm. At longer reaction times, peak maxima shifted to 530-540 nm due to particle growth. The influence of reaction pH and PEI molecular weight influenced particle size and aggregation dramatically. Best synthetic and storage pH will be discussed.

These PEI coated gold nanoparticles are cationic and they successfully condensed and transfected pMax-GFP to HEK-293T. Toxicity and transfection potential will be discussed in detail.



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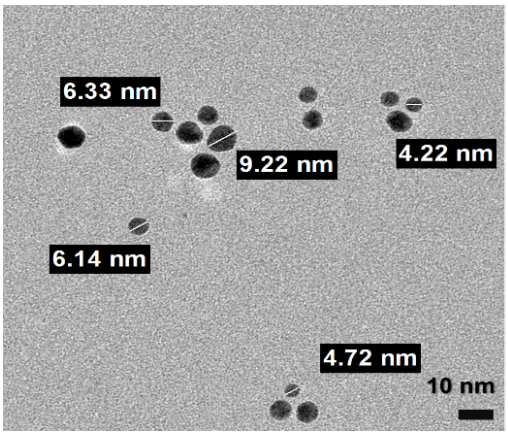

Tem.png

Evaluation of biocompatibility of magnetic silica nanoparticles and magnetic hyperthermia - induced drug release in a cell culture system

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 188

Mr. Sami Ullah [1], ***Ms. Katja Siedel*** [2], ***Dr. Mario Köster*** [1], ***Prof. Andreas Krischning*** [2], ***Prof. Dagmar Wirth*** [1]

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Mesoporous silica nanoparticles (MSN) have been extensively used in numerous effective therapeutic strategies as novel drug delivery systems. Their large loading capability, flexible surface modification and excellent biocompatibility are ideal properties for pharmaceutical drug carriers. However, there is an urgent need to improve MSN target specificity in vivo and to achieve subsequent controlled drug release at the target site. To address these questions we characterized a MSN-based drug delivery vehicle containing a Fe_3O_4 magnetic core. By subjecting these magnetic particles to an alternating magnetic field hyperthermia is produced. We proposed to combine magnetic core MSNs and thermo-sensitive linker surface modification to generate a novel drug delivery system. We have used different phagocytic cell lines and primary cells to investigate biocompatibility and efficient uptake. Upon subjecting cells loaded with magnetic core MSNs to an alternating magnetic field, magnetic hyperthermia is produced which induces cell death by an apoptotic pathway. We also show that the extent of cell death upon magnetic hyperthermia is cell line dependent with highest effect on primary cells. Next, an Ansamitocin derivative, as a model drug was linked to magnetic core MSNs via a thermo-sensitive linker. In vitro studies show that the drug can be cleaved from the particles upon exposure to magnetic field and drops the viability of exposed cells. Together our results indicate that controlled drug release from nanoparticles can be achieved upon exposure of cells to an alternating magnetic field.

Exploitation of Immune Cell Iron Oxide Nanoparticle Interaction for Cancer Immunotherapy

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 390

Ms. Idoia Mikelez Alonso [1]***, Dr. Ane Ruiz De Angulo*** [1]***, Prof. Francisco Borrego*** [2]***, Prof. Juan C. Mareque-Rivas*** [1]

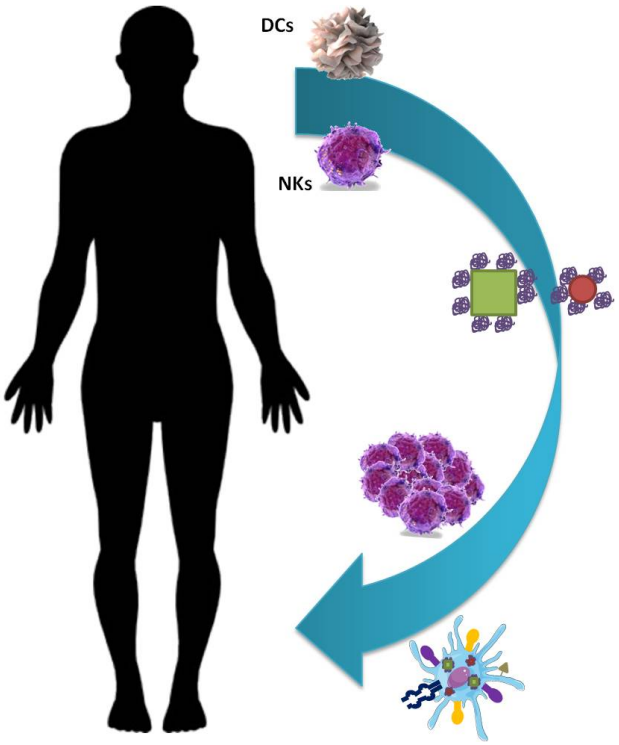
1. *Theranostic Nanomedicine Laboratory, CIC biomaGUNE, 20014 Donostia/San Sebastián, Gipuzkoa, Spain,* ***2.*** *Immunopathology group, Bio Cruces Health Research Institute, Bizkaia*

Idoia Mikelez-Alonso[1], Ane Ruiz-de-Angulo[1], Francisco Borrego Rabasco [2,3,*], Juan C. Mareque-Rivas[1,3,*]
[1]Theranostic Nanomedicine group, CIC biomaGUNE [2]Immunopathology group, Bio Cruces Health Research Institute, [3]Ikerbasque, Basque Foundation for Science 3, 48013 Bilbao, Bizkaia, Spain, [*]Corresponding Authors: F. Borrego, *francisco.borregorabasco@osakidetza.eus*; J.C. Mareque, *jmareque@cicbiomagune.es*

Nanoparticles (NPs) have a clinical interest because of their usefulness for non-invasive imaging, diagnosis and therapy. Using NPs in cancer immunotherapy is becoming very promising. Important challenges are targeting the therapy to specific locations[1] and the induction of specific cytotoxic activity against cancer cells. Dendritic cells (DCs)[2] and natural killer (NK) cells are two immune cell types currently used in cancer immunotherapy.

Harnessing these two cell types with NPs could be an effective new approach to generate robust and potent immune responses against cancer.
Several types of iron oxide NPs (IONPs) were used, with different sizes and shapes and PEG-phospholipid coatings. Mouse bone marrow derived dendritic cells (BMDCs) were generated and NK cells were obtained from human blood.
Activation and maturation of BMDCs was explored by incubating the cells with the described IONP-based systems. Migration assays were carried out incubating the cells within a transwell 24-well plate coupled to a magnetic plate. For NK cells, the proliferation rate in response to interleukin (IL)-2 was analyzed using the IONPs in a 48-well plate.
21 nm IONPs enhanced the migration of the BMDCs and induced their maturation as shown by cell surface maturation markers. They promoted the production of pro-inflammatory cytokines, such as TNF-α, IL-12 and IL-6. In addition, these 21 nm IONPs were not toxic and cooperated in the IL-2 mediated proliferation of NK cells[3].
Cube shaped 21 nm IONPs coated with PEG-PLs efficiently induced activation and migration of DCs and NK cell proliferation, showing their potential to use them in cancer immunotherapy.

[1]Ruiz-de-Angulo, A.; Zabaleta, A.; Gómez-Vallejo, V.; Loop, J.; Mareque-Rivas, J.C., ACS Nano, 2016, 10, 1602-1618.
[2]Palucka, K.; Banchereau, J., Nature, **2012**, 12, 265-277.
[3]F. Borrego, M.C. Alonso, M.D. Galini, J. Carracedo, R. Ramirez, B. Ostos, J. Peña, R. Solana., Experimental Gerontology 34 (1999) 253–265



General scheme iconan 2017.jpg

Facile and Novel Synthesis of MR/NIR Dual Modal Contrast Agent for In Vivo Non-invasive Molecular Imaging

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 131

Dr. Hye Sun Park [1], ***Mrs. mi young cho*** [1], ***Mr. Hyunseung Lee*** [1], ***Dr. Kwan Soo Hong*** [1]

1. *Korea Basic Science Institute, Korea Research Institute of Bioscience & Biotechnology*

Nanoprobes that are used for biomedical applications usually require surface modifications using amphiphilic surfactants or inorganic coating materials to enhance their biocompatibility. We proposed a facile synthetic approach for the phase transfer of hydrophobic magnetic nanoparticles by the direct adherence of fluorescent probes, without any chemical modifications, for use as a magnetic resonance (MR)/near-infrared (NIR) fluorescence bimodal imaging contrast agent. Indocyanine green (ICG) was used not only as an optical component for NIR imaging, but also as a surfactant for phase transfer with no superfluous moiety, which we call 'ICGylation'. Cell labeling and tracking *in vivo* with ICGylated magnetic nanoparticles were successfully performed by MR/NIR dual-modal imaging for three days, which shows great biostability without any additional surface functionalization. We expect that this novel MR/NIR contrast agent with sensitive detection and simultaneous imaging capability can be used in diverse fields, such as the imaging and tracking of immune cells to confirm immunotherapeutic efficacy. This approach could also be applied to other kinds of nanoparticles, and it would promote development of advanced functional multimodal nanobioprobes.

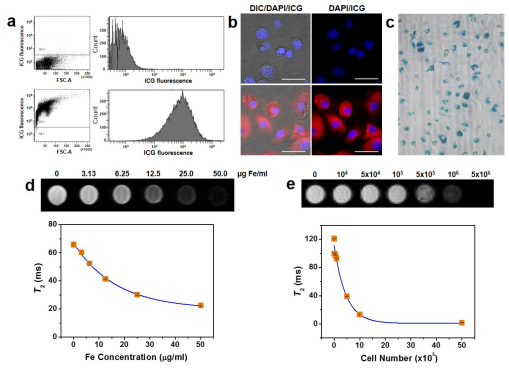


Figure1 iconan2017.jpg

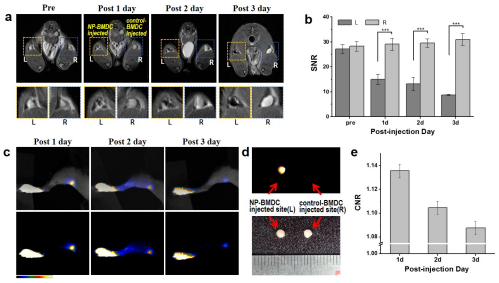


Figure2 iconan2017.jpg

Gold Nanostar-based Point-of-Care Diagnostics of Carbapenemase-Producing Enterobacteriacae

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 73

Mr. Kang Wei Cherng Malvin [1], ***Ms. Wong Yen Lynn*** [2], ***Dr. Marek Piotrowski*** [3], ***Mr. Miguel Reyes*** [4], ***Ms. He Shuai*** [2], ***Dr. Teo Jeanette*** [5], ***Dr. Kah Chen Yong James*** [2]

1. *NUS Graduate School for Integrative Sciences and Engineering,* ***2.*** *National University of Singapore,* ***3.*** *International Iberian Nanotechnology Laboratory,* ***4.*** *Massachusetts Institute of Technology,* ***5.*** *National University Health System*

The ever-increasing spread of antibiotic resistance poses a serious threat to global public health. With the existence of Carbapenem-resistant Enterobacteriaceae (CRE), it renders the use of carbapenems, the last-resort class of β-lactam antibiotics, useless against combating against bacterial infections. Such infections reduce the ability to treat complex infections due to the lack of antibiotic options for treatment, leading to CRE-associated mortalities. Current methods of detection, like CarbaNP test and Modified Hodge's Test, have significant limitations that the time taken for detection of carbapenemase activity ranges between hours to days, and are non-specific in phenotypic profiling, making it challenging to isolate patients rapidly and devise appropriate treatment for infected patients. We propose a methodology by utilizing Surface Enhanced Raman Spectroscopy (SERS) to study bacterial β-lactamase activity. This is done via the use of gold nanostars (AuNS), which have reported excellent SERS properties, conjugated with a β-lactam antibiotic ceftriaxole, as a proof-of-concept study to analyse the changes in the SERS spectra associated with cleavage of the β-lactam ring upon interaction with the New Delhi Metalloproteinase (NDM)-producing *Escherichia coli*.

Methods

1) Synthesis of AuNS

The synthesis of AuNS was done using the one-pot seedless protocol, involving 3 reagents: gold (III) chloride, silver nitrate & ascorbic acid (AA), which have been optimised and reported for maximum SERS enhancement signal.

2) Conjugation of Ceftriaxole onto AuNS

100uL of 5mM CTX solution was added to 100uL of 50pM washed AuNS solution, and incubated for 30 minutes at room temperature. Colloidal stability of the conjugate was tested via addition of 100uL of 0.5X PBS, where the optical properties of the conjugates was characterised using UV-Vis Spectroscopy.

3) Bacterial Studies

30uL of NDM- and non-NDM-producing *E. coli* suspension was added to cuvettes containing 200uL of AuNS-CTX solution separately. SERS spectra was acquired every 5 minutes up to 45 minutes.

Results & Discussion: SERS Study on β-lactam cleavage

We are able to obtain detection of carbapenemase activity within 25 minutes, with the associated changes in SERS spectra being diminishing of SERS peaks at 1358cm^{-1} and 1495cm^{-1}.

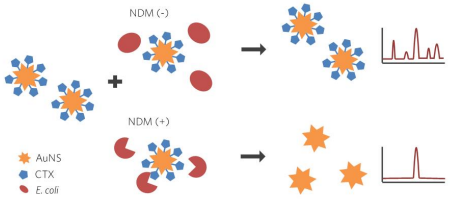


Fig1 reaction schematic.jpg

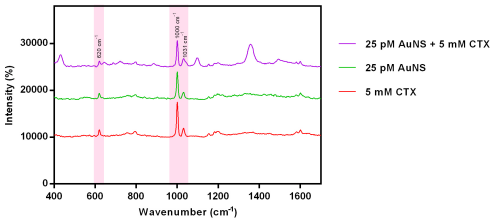


Fig2 sers spectra of ctx auns and auns-ctx.jpg

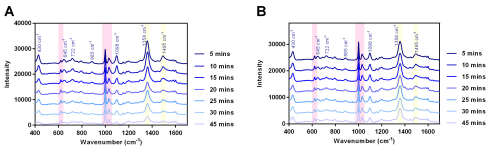


Fig3 changes of sers spectra in auns-ctx by non-ndm-producing left and ndm-producing bacteria r .jpg

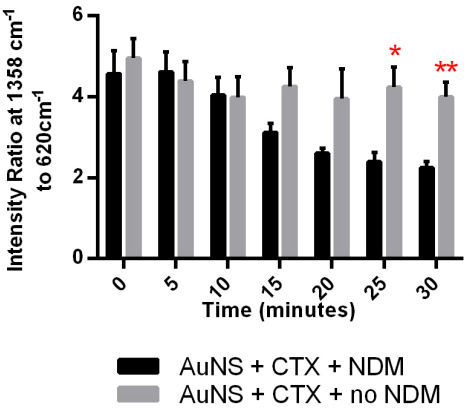


Fig4 ratio of sers intensity.jpg

Non-ionic surfactant based in-situ forming nanovesicles as controlled parenteral delivery systems

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 319

Ms. Rehab Shamma [1]***, Dr. Azza Mahmoud*** [2]***, Ms. Nada Elhouffi*** [2]***, Prof. Magdy Ibrahim*** [3]***, Prof. Hussein Ammar*** [2]

1. *Faculty of pharmacy, Cairo university,* ***2.*** *Faculty of Pharmacy, Future University in Egypt,* ***3.*** *Force Microscopy for Inserm*

Introduction: Non-ionic surfactant (NIS) based *in-situ* forming vesicles (ISVs) present an attractive alternative to the traditional vesicular systems for the parental control of drug release.
Methods: NIS based ISVs encapsulating tenoxicam were prepared using the emulsion method. TX-loaded ISVs were prepared using a $2^2.3^1$ full factorial experimental design, where three factors were evaluated as independent variables; type of NIS; Span®60 or Brij®52 (A), molar ratio of NIS:Tween® 80 (B) and phase ratio of the internal ethyl acetate to the external Captex® oil phase (C). Percentage drug released after 1hr, particle size of the obtained vesicles and mean dissolution time were chosen as the dependent variables. Selected formulation was subjected to morphological investigation, injectability, viscosity measurements and solid state characterization. **Results and Discussion**: Optimum formulation showed spherical nano-vesicles in the size of 379.08 nm with an initial drug release of 37.32% in the first hour followed by a sustained drug release pattern for 6 days. DSC analysis of the optimized formulation confirmed the presence of the drug in an amorphous form with the nanovesicles. Biological evaluation of the selected formulation was performed on New Zealand rabbits as IM injection. The prepared ISVs exhibited a 45 and 28 fold larger AUC and MRT-values, respectively, compared to those for drug suspension. The obtained findings boost the use of ISVs for the treatment of many chronic inflammatory conditions.

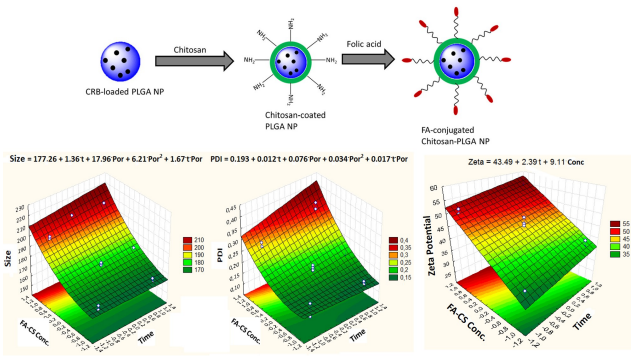
Preparation and optimization of folic acid conjugated-chitosan functionalized PLGA nanoparticles for delivery of carboplatin

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 401

Mr. Daniel Profirio [1], ***Prof. Francisco Pessine*** [1]

1. *University of Campinas (UNICAMP)*

Carboplatin, a platinum-based antitumoral drug, represents a chemotherapeutic agent for newly diagnosed malignancies and it is effective for testicular, ovarian, bladder, head and neck cancers. However, low uptake of carboplatin by tumor cells is considered a key reason for its limited therapeutic efficacy [1]. In this work nanoparticles composed of poly(D-L-lactic-*co*-glycolic) acid (PLGA) were prepared to produce nanocarriers for carboplatin. The carboplatin-loaded PLGA nanoparticles were formulated by nanoprecipitation method, using TPGS (D-α-tocopheryl polyethylene glycol succinate) as stabilizer [2]. In order to improve the delivery of carboplatin to cancer cells, folic acid-conjugated chitosan-coated (FA-CS) PLGA nanoparticles were also prepared using experimental design [3]. Briefly, PLGA was dissolved in acetone while an aqueous solution of carboplatin was prepared. Drug solution was added to the PLGA solution and the mixture was sonicated to give a primary w/o emulsion. This emulsified mixture was added to a solution containing TPGS and sonicated again to give a double emulsion (w/o/w). Following evaporation of acetone, a suspension of NPs was obtained and purified. For surface modification, carboplatin-loaded PLGA nanoparticles suspension was mixed with FA-CS solutions in 1% acetic acid. For unmodified nanoparticles, the results showed that optimized formulation (mean particle size = 121.0 nm, PDI = 0.120 and zeta potential = -34.0 mV) was stable over a period of 60 days when stored at 10°C, with entrapment efficiency = 5% and nanoparticle yield = 77%. Also, it was possible to improve both parameters (EE = 37% and Yield = 87%) by reduction of dialysis (24 h to 2 h) and acetone evaporation (24 h to 1 h) time. Encapsulation of carboplatin was confirmed by UV-Vis spectroscopy using a derivatization technique with o-phenylenediamine. Mean particle size and polydispersity index are dependent of stirring time and concentration of FA-CS solution (according to a quadratic model), while zeta potential is also dependent but according to a linear model. **REFERENCES** – [1] S. Jose *et al*, Colloids and Surfaces B: Biointerfaces 142 (2016) 307-314. [2] O. S. Muddineti *et al*, Expert Opinion on Drug Delivery 14 (2017) 715-726. [3] J. Ji *et al*, Nanoscale Research Letters (2015) 10:453.



Response surfaces.jpg

Rational Design of Novel Nanosystems for Needle-free Vaccination

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 387

Dr. José González [1]***, Ms. Carla Molina*** [2]***, Mr. Juan Bussio*** [2]

1. *1. Departamento Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile. 2. Centro de Investigación en Nanotecnología y Materiales Avanzados "CIEN-UC", Pontificia Universidad Católica de Chile, Santiago, Chile.,* ***2.*** *Departamento Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile.*

Needle-free vaccination is one the most important strategies to improve the immunization coverage and get better patient comfort which leads to increased compliance, without the requirement of trained personnel and the potential safety risks related to the traditional parenteral route. On the other hand, the skin is an organ attractive for vaccination because it has abundant APC found in their structure, leading to the use of lower amounts of antigen and the ability to be self-administered and a global decrease of the costs associated with immunization. The major obstacle to reach immunocompetent cells is the permeation characteristics of the stratum corneum. Overcoming this barrier is the aim for a successful transcutaneous vaccination. Nanosystems have shown efficient ability to get over biological barriers and they are striking means for transdermal delivery. Considering this, the aim of this work is the design and development of new nanosystems based on biocompatible materials. These materials are arranged in nanocapsular or nanoemulsion structures, bearing therefore adjuvant properties. These nanocarriers were prepared by solvent displacement technique. These systems can include different materials such as polysaccharides (e.g: chitosan, hyaluronic acid), triterpenic saponins (obtained from an endemic Chilean tree (*Quillaja saponaria*)) and oils already licensed in some adjuvant formulations (e.g. α-tocopherol).

As shown in **Figure 1**, the different developed systems present nanometric size with a monomodal distribution and adequate surface charge. TEM images showed spherical shape and homogenous population without particle aggregation. These nanosystems allowed an efficient association of ovalbumin as a model antigen in its bioactive form. Stability studies showed that aqueous nanoparticle suspensions maintain their nanometric size over 12 months in storage conditions (4ºC) (**Figure 2**). Cell viability studies in RAW264.7 macrophages showed appropriate cellular toxicity up to high concentrations (**Figure 3**) and complement activation studies showed high interaction with the immune system (**Figure 4**). Preliminary ex vivo studies in pig skin have shown an improvement of model antigen transcutaneous administration.
In conclusion, we have successfully developed novel nanosystems with potential for transcutaneous vaccination, which can be considered as an interesting platform for the development of future needle-free vaccines.
Acknowledgment: FONDECYT initial project N° 11140797

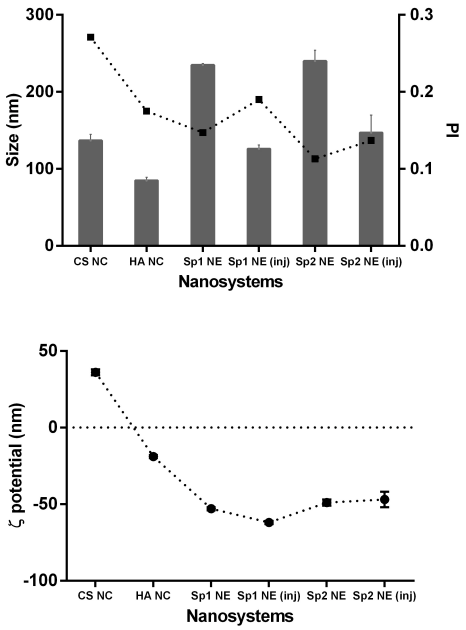


Figure 1 physicochemical characterization of nanosystems mean s.d. n3 .jpg

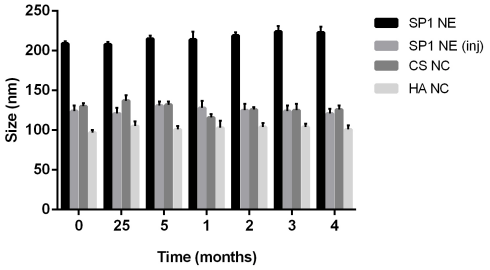


Figure 2 stability of nanosystems in aqueous suspension under storage condition 4 c . mean s.d n 3 .jpg

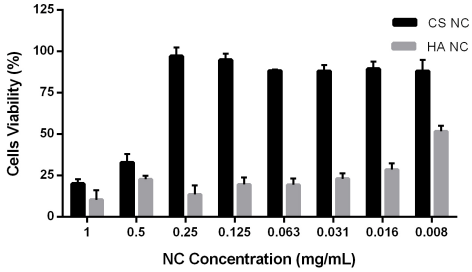


Figure 3 effect of chitosan and hyaluronic acid nanocapsules on the viability of raw 264.7 at 24 h..jpg

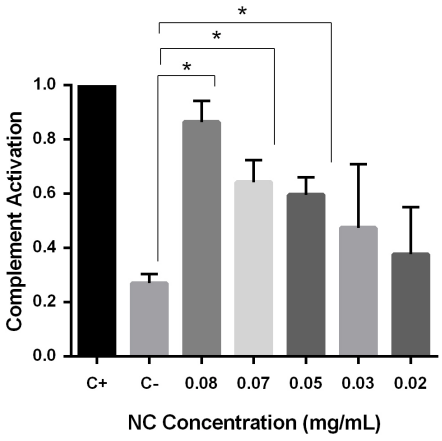


Figure 4 complement activation studies induced by chitosan nanocapsules.jpg

SERS spectroscopy for the detection and identification of microorganisms

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 460

Dr. Dmitry Kopitsyn [1], ***Mr. Maksim Gorbachevskii*** [1], ***Dr. Ekaterina Botchkova*** [1], ***Dr. Pavel Gushchin*** [1], ***Prof. Vladimir Vinokurov*** [1], ***Dr. Andrei Novikov*** [1]

1. *Gubkin University*

Introduction

Detection of microorganisms is an important task in the fields of clinical diagnostics, food safety, and water quality control. The severity of this problem is due to the emergence of clinical infections by multidrug-resistant biofilm-forming bacteria. Traditional methods based on cultivation or PCR are effective, but usually require some preliminary information about the nature of pathogens, take a lot of time and are quite costly, which in most cases makes such studies not applicable. Thus, the problem of a broad-spectrum, feasible and fast way of detecting and identifying microorganisms remains relevant today.

Surface enhanced Raman scattering (SERS) spectroscopy can be efficiently employed for the label-free detection and discrimination of different bacteria. The fingerprint-quality bacterial spectra allow identification of bacteria at the genus, the species, and even at the intraspecies level.

Methods

SERS spectra were acquired with BWS415 spectrometer (BWTEC, Germany). The specimen was put on the XYZ-stage, while the position of laser focus was controlled by USB microscope Mikmed-2000R (Micromed, Russia). For maximum enhancement, SERS substrates were prepared by multilayer deposition of gold nanoparticles without any additional coagulants.

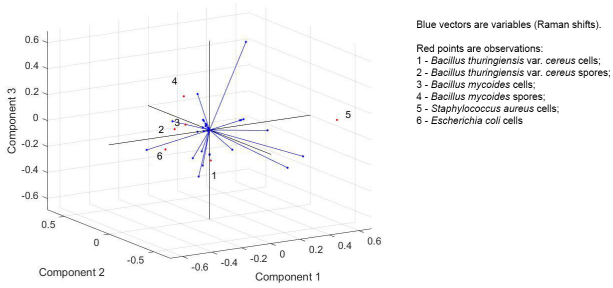
Results

SERS spectra were obtained for the active Gram(+) and Gram(-) bacteria cells and for spores, such as *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus mycoides*, *Bacillus thuringiensis*var.*cereus*, *Desulfovibrio sp*. Spectra of bacteria grown on ^{15}N isotope containing substrate were also registered. Fluorescence background subtraction, filtering, normalizing and automated peak recognition were performed by means of GNU/Octave subroutines developed by us for the processing of spectral data.

Discussion

SERS spectra of bacteria were collected with cheap portable Raman spectrometer. All spectra have distinct differences, including those between spectra of cells and spores of the same strain. Stable isotope labeling revealed the relation between major SERS peaks and N-X bonds oscillations (presumably, in adenine and guanine cycles) – these peaks shifted towards lower Raman shifts. The PCA dimensionality reduction revealed the correlation between certain peaks in bacterial spectra and provided an opportunity to study the changes in cell surface chemistry in the biofilms formation by clinically relevant bacterial species.

This work was funded by the Russian Science Foundation (project 17-79-10489).



Kopitsyn-bacteria-sers-pca.jpg

Design and development of pH-responsive liposomes for targeted delivery of vancomycin against Staphylococcus aureus and methicillin-resistant Staphylococcus aureus

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 122

Mr. Sifiso Makhathini [1]***, Prof. Thirumala Govender*** [1]

1. *University of KwaZulu-Natal*

Sifiso S. Makhathini[1], Rahul Kalhapure[1], Mahantesh Jadhav[1], Ayman Y. Waddad[1], Chunderika Mocktar [1], Thirumala Govender[*,1]

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Introduction: Even though vancomycin is considered as the ultimate drug for the treatment of gram positive bacterial infections, the development of bacterial resistance has become a challenge posing a threat to public health worldwide. Targeted therapy has become a promising strategy to enhance the efficacy of antibiotics. Nano delivery systems with responsiveness to unique conditions at disease sites such as acidic conditions at infection sites can maximize targeting and drug release. The use of pH responsive novel lipids for the preparation of liposomes can improve their performance. The aim of this study is to develop novel pH-responsive liposomes encapsulated with vancomycin hydrochloride for activity against *Staphylococcus aureus(SA) and methicillin-resistant Staphylococcus aureus(MRSA).*

Methods: pH-sensitive lipids (Stearic, Oleic, Linoleic and Linolenic acid derivative) were synthesized and characterized using FTIR, ^{1}H and ^{13}C NMR. Liposomes were formulated using Dehydration-hydration method. Formulated liposomes were characterised for particle size, polydispersity index (PDI), zeta potential (Dynamic Light Scattering), entrapment efficiency (UV Spectrophotometry), *in vitro*drug release studies (Dialysis) and *in vitro* antibacterial activity (Broth dilution)**.**

Result: All derivatives displayed superior antibacterial activity against *SA* and M*RSA* as compared to bare vancomycin. As compared to other derivatives, linoleic acid derivative (LAD) system demonstrated the best antibacterial activity and showed higher activity at pH 6.0 as compared to pH 7.4 against *SA*. The size and the surface charge of LAD system (drug entrapment efficiency of 38.68%) at pH 7.4 and pH 6.0 were 88.52±5.078mn and 158±1.98mn respectively and -11.8±2.99 to 1.023±0.1012 respectively. All derivatives demonstrated a sustained and enhanced release profile at pH 6.0 as compared to pH 7.4. The release behavior from all derivatives was in accordance with Korsmeyer-Peppas model(n=0.658) and release mechanism was non-fikian in both pH.

Conclusion: These results suggest that the novel pH-sensitive liposomes hold a great potential of becoming an alternative targeted intracellular delivery system for antibiotics.

Single-step labeling of microtubule biomolecules using CdTe quantum dots

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 475

Dr. Daniel Oliveira [1], ***Mr. Fernando Menegatti de Melo*** [1], ***Prof. Henrique Toma*** [1]

1. *Instituto de Quimica, Universidade de Sao Paulo*

Introduction

Kinesin is a naturally occurring protein capable of cargo transport upon interaction with a cytoplasmic system of fibers, known as microtubules. Hydrolysis of ATP propels kinesin along microtubules, being therefore a promising tool in the development of synthetic nano transport machines. One of the challenges to study such system is how to observe the motility of either kinesin or microtubule.

Here, we report the use of mercaptopropionic acid (MPA)–capped cadmium telluride (CdTe) quantum dots (QDs) as a fluorescent labeling agent to microtubule biomolecules.

Method

Microtubules were polymerized from commercially available tubulin protein (Cytoskeleton Inc.).

MPA-capped CdTe QDs were synthesized using a fixed molar ratio of 1:7:0.25 (Cd^{+2}:MPA:Te). QDs were characterized microscopically and spectroscopically and the binding affinity of QDs to microtubules was studied via the surface plasmon resonance (SPR) technique.

Results and Discussion

Figure 1 shows the TEM image of synthesized CdTe QDs, with average diameters of 3 nm; while figure 2 shows the fluorescence spectra, as well as the UV-vis spectra (inset) for CdTe QDs in water, exhibiting high fluorescence with emission maxima at 545 nm.

Figure 3 displays obtained SPR data for immobilization of microtubule on the substrate's surface followed by injection of CdTe QDs over the microtubule-coated surface. Insets show only the binding interaction between QDs and the microtubule-bound surface.

The measured SPR angle ($\Delta\theta$) continues to rise throughout the flow of CdTe QDs over immobilized microtubules, suggesting strong binding between biomolecule and QDs.

QDs are believed to adsorb to microtubule's surface through a specific interaction with the histidine amino acids present in the microtubule's surface. The imidazole-nitrogen donor of histidine is an important binding site for transition metal ions in biological system.

Figure 4 shows fluorescence image for a microtubule functionalized with CdTe QDs. As can be seen, QDs are able to not only bind to microtubules *in vitro*, but also to fluorescently label the biomolecule in the green spectral region.

To the best of author's knowledge, no other study to date showed the use of QDs to directly fluorescently label microtubules.

This work was supported by FAPESP grants number 2015/01271-3 and 2013/24725-4

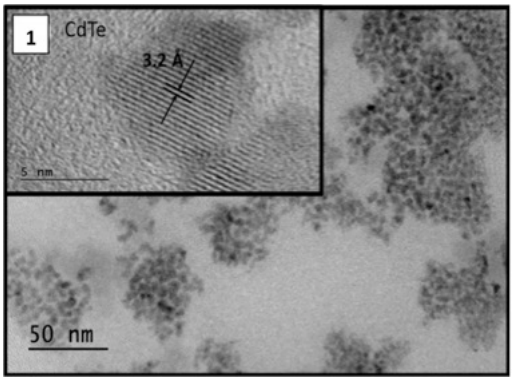


Figure 1 tem image of cdte mpa capped qds .jpg

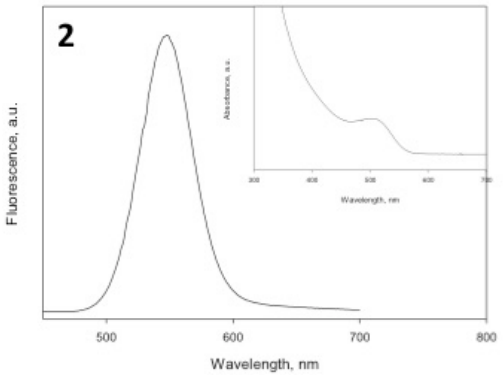


Figure 2 fluorescence spectra of cdte qds uv-vis spectra in inset .jpg

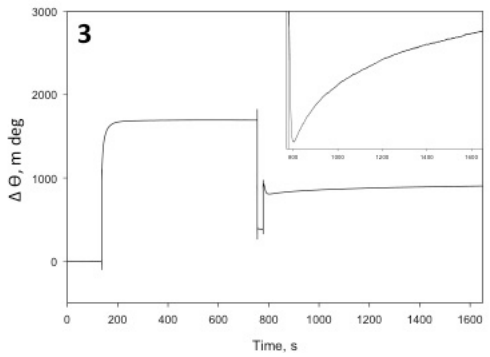


Figure 3 spr sensorgrams for the immobilization of microtubule followed by the interaction between immobilized-microtubules and cdte qds.jpg

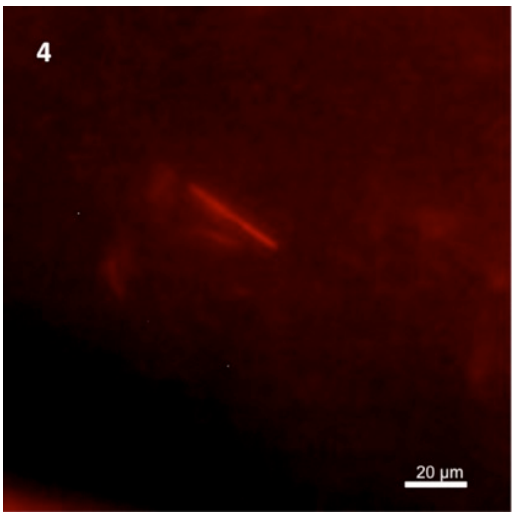


Figure 4 fluorescence image of microtubule labeled with cdte qds.jpg

Specific targeting of microglia using hydroxyl group terminated dendrimer(G4) conjugated with anti-inflammatory drug mitigates neuropathic pain

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 166

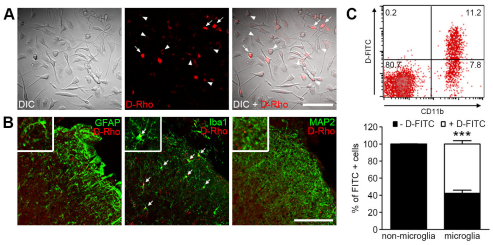
Ms. Boomin Choi [1], ***Dr. Hyunjung Min*** [1], ***Prof. Sung Joong Lee*** [1]

1. *Seoul national university*

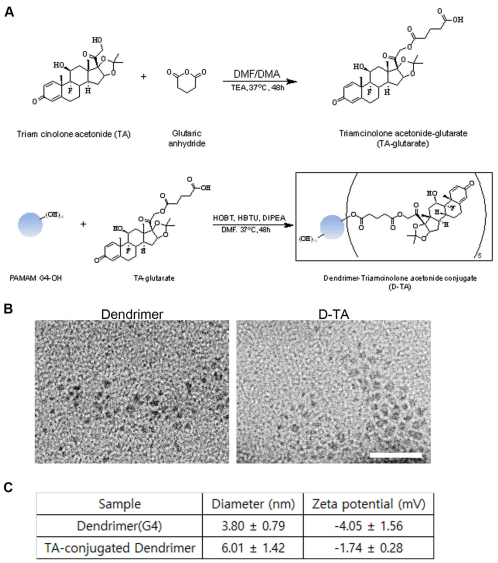
Background: Accumulating evidence on the causal role of spinal cord microglia activation in the development of neuropathic pain after peripheral nerve injury (PNI) suggests that microglial activation inhibitors might be useful analgesics for neuropathic pain. Studies also have shown that polyamidoamine (PAMAM) dendrimer may function as a drug delivery vehicle to microglia in the central nervous system. In this regard, we developed PAMAM dendrimer-conjugated triamcinolone acetonide (D-TA), a previously identified microglial activation inhibitor, and tested its analgesic efficacy in a mouse PNI model.

Result: PAMAM dendrimer was delivered selectively to spinal cord microglia upon intrathecal (i.t.) administration. D-TA inhibited LTA-induced proinflammatory gene expression in primary glial cells. In addition, D-TA administration (i.t.) inhibited PNI-induced spinal cord microglial activation and the expression of pain-related genes in the spinal cord, including Nox2, IL-1β, TNF-α, and IL-6. D-TA administration right after nerve injury almost completely reversed PNI-induced mechanical allodynia for up to 3 days. Meanwhile, D-TA administration 1.5 days post injury (dpi) significantly attenuated mechanical allodynia.

Conclusion: Our data demonstrate that D-TA inhibits spinal cord microglia activation and attenuates neuropathic pain after PNI, which has therapeutic implications for the treatment of neuropathic pain

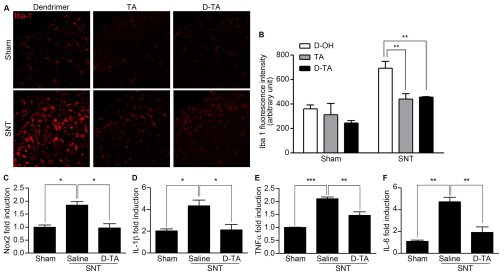


Specificity of d-ta.jpg

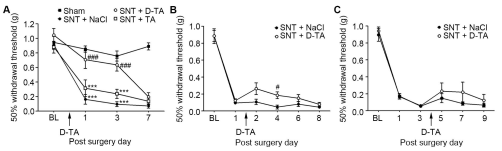


Sample	Diameter (nm)	Zeta potential (mV)
Dendrimer(G4)	3.80 ± 0.79	-4.05 ± 1.56
TA-conjugated Dendrimer	6.01 ± 1.42	-1.74 ± 0.28

Dendrimer-ta.jpg



Tissue analysis.jpg



Behavior test.jpg

Stem cell-based gene delivery mediated by cationic niosomes for bone regeneration purposes

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 400

Dr. Noha Attia [1], ***Dr. Mohamed Mashal*** [2], ***Prof. Gustavo Puras*** [2], ***Prof. Jose Luis Pedraz*** [2]

1. *UPV/EHU & Alexandria faculty of medicine,* ***2.*** *UPV/EHU*

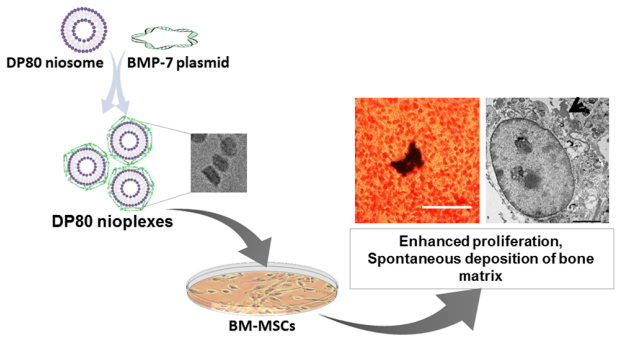
Introduction

BMP-7 protein plays a pivotal role in the transformation of mesenchymal cells into bone. However, its impact is hampered due to its short half-life. Therefore, gene therapy could represent an interesting approach to deliver BMP-7 gene to mouse mesenchymal stem cells (D1-MSCs).

Methods

In this work we prepared and characterized niosomes based on cationic lipid 2,3 di(tetradecyloxy)propan-1-amine, combined with polysorbate 80 for gene delivery purposes. Niosomes were combined and physicochemically characterized initially with pCMS-EGFP reporter plasmid, and later with pUNO1-hBMP-7 plasmid to evaluate osteogenic differentiation. Additionally, specific blockers of most relevant endocytic pathways were used to evaluate the intracellular disposition of complexes.

Results and Discussion D1-MSCs transfected with niosomes showed increased growth rate, enhanced ALP activity and extracellular matrix deposition which suggested the formation of osteoblast-like cells. Such findings could lead to the conclusion that hBMP-7-transfected D1-MSCs could be considered not only as an effective delivery tool of hBMP-7, but also as proliferating and bone forming cells for bone regeneration.



Graphical abstract noha attia.png

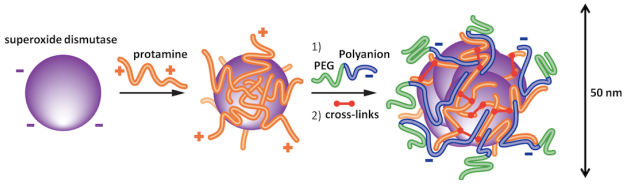
Superoxide dismutase nanoparticles modified by chitosan for ophthalmic applications

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 229

Mr. Anton Aleksashkin [1], ***Mr. Alexander Vaneev*** [1], ***Mrs. Tatiana Abakumova*** [2], ***Dr. Olga Kost*** [1], ***Prof. Alexander Kabanov*** [3], ***Prof. Natalia Klyachko*** [1]

1. *Lomonosov Moscow State University,* ***2.*** *The Serbsky State Scientific Center for Social and Forensic Psichiatry,* ***3.*** *University of North Carolina at Chapell Hill*

Nowadays, an active search for new drugs to treat ocular diseases occurs. The reason is that in the absence of therapy, these diseases lead to blindness. In our work uveitis and burn were observed as therapeutic goals.
Oxidative stress plays an important role in the pathogenesis of uveitis and burn, and injection of antioxidants may be effective. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, are more effective in comparison with small molecular antioxidants, because enzymes react with substrate repeatedly. However, administering native enzymes to the eye in the form of drops is ineffective due to their rapid clearance. Therefore, it is important to create a drug delivery system that will possess long time of circulation and low immunogenicity.
To achieve this goal, SOD nanoparticles covered with chitosan were synthesized. Polymeric shell was used to decrease immunogenicity; chitosan was used to increase time of circulation.
Briefly, SOD nanoparticles were synthesized by mixing of SOD solution with protamine and PLE-PEG solutions sequentially, after that glutaraldehyde was added. Byproducts were removed by centrifugation through centrifugal filters. Nanoparticles of 50 nm in diameter with negative charge were obtained.
The release experiments were conducted using a dialysis container (100 kDa). SOD had being released from nanoparticles more slowly than the native SOD: 90% of SOD had released after 24 hours from the solution with the native enzyme, for the same time 30% of SOD had released from the solution with nanoparticles. This demonstrates that SOD is connected with the polymers strongly and the particles may have a large circulation time as opposed to the native enzyme.
Both nanoparticles and nanoparticles covered with chitosan were internalized by HEK293 cells whereas SOD was not. SOD nanopartices show therapeutic efficacy in preclinical trials on the model of uveitis and alkaline burn in rabbits.
Chitosan solution was added to nanoparticles to cover them. To confirm that particles were covered with chitosan, zeta-potential was measured: it changed from negative to positive values.
Thus, superoxide dismutase nanoparticles covered with chitosan were obtained. They seems to be perspective therapeutic agent due to improved stability and ability to internalize into cells.



2017-05-29 2323.png

Surface charge-dependent intracellular fate of doxorubicin drug delivery systems based on PAMAM dendrimers

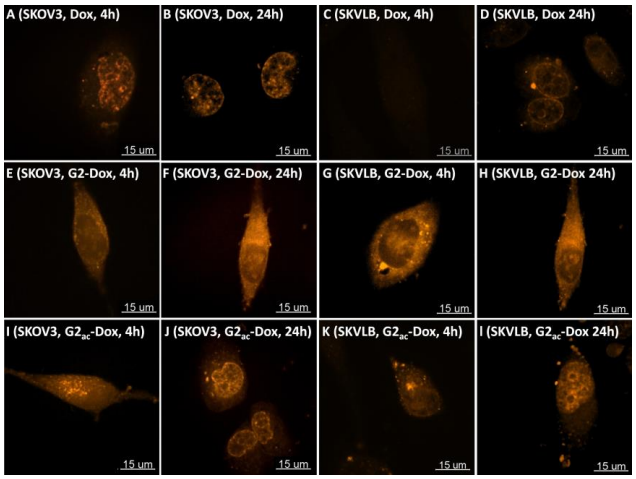
Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 94

Ms. Elena Nikolskaya [1], ***Mr. Nikita Yabbarov*** [1], ***Mrs. Olga Zhunina*** [2], ***Mrs. Oksana Tereshenko*** [2], ***Dr. Evgeniy Severin*** [2], ***Dr. Irina Zamulaeva*** [1]

1. *A. Tsyb Medical Radiological Research Centre – branch of the National Medical Research Radiological Centre of the Ministry of Health of the Russian Federation,* ***2.*** *Institute of Molecular Diagnostics*

Abstract: Dendritic polymers have a huge potential as a molecular cargoes with targeting abilities in diagnosis and treatment of cancer. In our study we synthesized derivates of PAMAM dendrimers using pH-sensitive linkers to promote the absorption or release rate of the drug and to reduce the levels of nonspecific internalization. The amine-terminated (16 primary amino-groups) and acetyl-terminated (14 primary amino-groups out of 16 were blocked) 2nd generation PAMAM dendrimers (G2 and $G2_{ac}$ respectively) were conjugated with doxorubicin (Dox). G2 and $G2_{ac}$ derivates labeled by Dox were absorbed by the cells at 37°C with different efficiency. The fluorescence intensity of the cells (SKVO3 and SKVLB cells) incubated with unmodified G2-Dox during 4 h was a bit higher than the fluorescence of the cells exposed to $G2_{ac}$-Dox and free Dox during the same time intervals. After 24 h of incubation differences in fluorescence levels and pattern of distribution between conjugates revealed – after 24h exposition of both cell lines cells to $G2_{ac}$-Dox fluorescence was observed in the nuclei. Pattern of distribution and fluorescence intensity of each conjugate was specific and did not depend on sensitivity or resistance cell line used to Dox. At the same time SKVLB cells were much more resistant to free Dox and did not show significant levels of internalization. The results obtained indicate cellular internalization pathways dependence on the nature and charge of surface chemical groups of dendrimers used. Simultaneously, $G2_{ac}$-Dox localization in late endosomes and lysosomes allowed Dox to release and migrate into cell nuclei (during longer periods of incubation), where its target – DNA is located. Our study show difference in action pathways on tumor cells of amine terminated conjugate G2-Dox and $G2_{ac}$-Dox, what has been confirmed by studies on the endocytosis, intracellular distribution, and cytotoxicity.

The research was supported by grant of Russian Scientific Foundation # 15-15-10013.



Intracellular localization of dox a b c d g2-dox e f g h and g2ac-dox i j k l in skov3 a b e f i j and skvlb c d g h k l cells after 4h and 24h of incubation. bar 15 m..jpg

Synthesis of aliphatic polycarbonate for siRNA delivery in cancer therapy

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 348

Mrs. Alexandra Baroni [1], ***Dr. Antoine Frère*** [2], ***Dr. Géraldine Piel*** [2], ***Dr. Denis Mottet*** [2], ***Prof. Philippe Dubois*** [3], ***Prof. Bertrand Blankert*** [1], ***Dr. Laetitia Mespouille*** [3]

1. *Université de Mons,* ***2.*** *Université de Liège,* ***3.*** *Universtité de Mons*

INTRODUCTION

Gene therapy has attracted increasing attention worldwide as prominent strategy to treat diseases at the gene level. While therapeutic gene has reached clinical evaluation, their administration as a drug is very challenging because of their rapid clearance from the bloodstream and the lack of tissue selectivity.

Synthetic polymers have become a well-known solution to overcome these issues owing to the ease of synthesis and their ability to drive genetic materials toward targeted cells. Aliphatic poly(carbonate)s (APCs) are biocompatible and biodegradable[1,2] and they constitute an excellent family on this purpose

MATERIALS AND METHODS

In this work, APCs are synthetized by ring-opening polymerization (ROP) using a metal-free and non-toxic catalyst[3]. The tailoring of the polymer is done using monomers carrying the desired function. The obtained block copolymers should over-come biological barriers as the endosomal escape, the gene loading or the cell internalization. PEGylated copolymers are also considered and compared with the hydrophobic one.

Monomers are synthetized from bis-MPA synthon according to well-established procedures[1]. The polymerization is carried out in CH_2Cl_2 in the presence of DBU with an initial monomers concentration of 1 M. The reaction is performed under protected atmosphere (glove box) during 4 hours at ambient temperature using PEO (hydrophilic) or BzOH (lipophilic) (macro-)initiators.

Polymers are characterized by ^{1}H-NMR in $CDCl_3$ and SEC in THF/NEt_3. The association polymer – gene (polyplexe) is evaluated in terms of physico-chemistry (size and zeta potential), siRNA incorporation, cellular transfection and mRNA shut-down.

RESULTS AND DISCUSSION

The results demonstrate that the introduction of guanidinum and morpholino groups in the composition led to a powerful polymer vector for siRNA delivery[4]offering an excellent alternative to the toxic golden standard poly(ethyleneimine). The presence of a hydrophilic tail has a strong impact on gene release and knock down efficiency (PEG dilemma)[5].

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Thermally poly-condensed lysine and lysine-co-histidine for siRNA delivery

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 313

Mr. Ali Alazzo [1], ***Dr. Keith Spriggs*** [1], ***Dr. Snow Stolnik-Trenkic*** [1], ***Prof. Cameron Alexander*** [1]

1. *University of Nottingham*

Introduction

Small interfering RNA (siRNA) has been intensively investigated over the last decades as a promising approach for addressing several diseases. However, the clinical applications of this approach have been hindered due to several biological and technical obstacles that face siRNA delivery. In this context, highly branched and easily functionalized polymers have emerged as attractive materials to develop a safe, efficient and cost-effective delivery system. Here, we aimed to prepare a non-viral vector for siRNA based on hyperbranched polymers due to their advantages in terms of synthesis cost that make them more feasible and applicable for scale-up and manufacturing.

Method

Hyperbranched polylysine was synthesised and modified with histidine during thermal poly-condensation of amino acids. The resultant materials were characterised and their capability to condense and deliver siRNA were evaluated on A549 cells.

Results

The conditions of polymerisation were optimised to afford water-soluble hyperbranched polylysine and hyperbranched polylysine-co-histidine with average molecular weight up to 30 kDa. The physicochemical characterisation indicated that the incorporation of histidine produced polymers of a higher degree of branching, glass transition temperature and buffer capacity (Table 1). The size measurements and protection assay (Figure 1) indicated that the polymers of high molecular weight (~30 kDa) have better ability to condense and protect siRNA into nanoparticles than the lower molecular weights polymers (~15 kDa). Also, a negative impact of histidine incorporation can be seen clearly on the results. Biologically, hb-pK-33kDa achieved the best transfection efficiency (Figure 2) in comparison with other polymers.

Discussion Structurally, the results revealed that the incorporation of histidine modulates the structures of polymers this more likely through occupying the more reactive ε-amine of lysine and directs the polymerisation towards the α-amine of the monomers; consequently, more dendritic polymers were prepared, with a more rigidity as indicated by the DB and *Tg* values. As a result, the high molecular weight polylysine interacted efficiently with siRNA and afforded polyplexes with a higher surface charge and a lower tendency to aggregate in comparison with the other polymers.

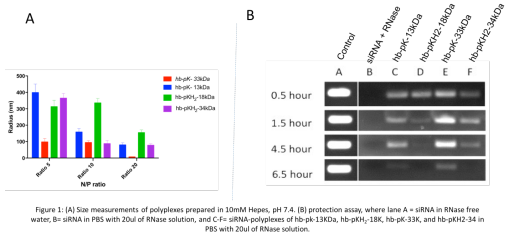


Figure 1: (A) Size measurements of polyplexes prepared in 10mM Hepes, pH 7.4. (B) protection assay, where lane A = siRNA in RNase free water, B= siRNA in PBS with 20ul of RNase solution, and C-F= siRNA-polyplexes of hb-pk-13KDa, hb-pKH$_2$-18K, hb-pK-33K, and hb-pKH2-34 in PBS with 20ul of RNase solution.

Figure 1.png

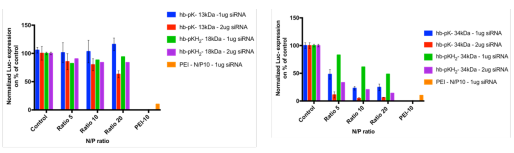


Figure 2: transfection of polyplexes prepared in 10uM hepes buffer, pH7.4, using 1ug and 2ug of siRNA. (A) low molecular weight polymers and (B) high molecular weight polymers.

Figure 2.png

Table 1: Characteristics of thermally polymerised hyperbranched polymers

	K: H Ratio [a]	M_n (KDa) [b]	M_w (KDa) [b]	PDI (Đ)	DB [c]	ANB [d]	T_g (°C)	Buffer capacity [e]	µmol of amine [f]
hb-polyK-13kDa	---	13.4	22.7	1.7	0.34	0.24	56.6	35.2%	5.3
hb-polyK-33kDa	---	33.6	58.8	1.7	0.39	0.27	65.3	37.4%	5.3
hb-polyKH$_2$-18kDa	10:2	18.1	25.3	1.4	0.46	0.39	98.9	44.0 %	4.0
hb-polyKH$_2$-34kDa	10:2	34.5	62.8	1.8	0.40	0.32	108.6	43.9%	3.6

[a]: molar ratios of lysine to histidine, [b]: Malls GPC molecular weights, [c]: degree of branching, [d]: average number of branches, [e]: buffer capacity at pH range of 5-7, [f]: µmol of amine / mg of polymers (fluorescamine assay)

Table 1 characteristics of thermally polymerised hyperbranched polymers.png

Wound dressing membrane consist of β-chitin extracted from cuttlefish bone through electrospinning

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 323

Mr. Jung Hyeongseop [1], ***Mr. Minhee Kim*** [1], ***Prof. Won Ho Park*** [2]

1. *Chungnam National University,* ***2.*** *Chungnam National Unversity*

Wound dressing membrane heals skins faster, protects from bacteria, virus, etc., and also decreases the time to epithelialize by covering the wound, which can interfere with infection. Wound dressing membrane make the wound dehydrated and allows gas permeation between wound and atmosphere.

Chitin is the second abundant polysaccharide after cellulose, has similar structure to cellulose, i.e. poly-(1→4)-β-N-acetyl-D-glucosamine. It is generally extracted from arthropods, crustaceans and insects. Chitin can be classified into α-, β- and γ-form, respectively. Typically, the solubility of α-chitin extracted from shrimp or crab shell is very poor because of its high crystallinity from intermolecular hydrogen bonding between molecular chains. It exists as highly crystalline form in nature. Due to its low solubility, toxic solvent like 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) was usually used. However, *β*-chitin is feasible to be fabricated using a mild solvent like formic acid, and thus can be used in various forms for medical applications, such as wound dressing, suture, artificial skin, and drug delivery system.

Electrospinning is widely used as an excellent method for generating a non-woven nanofibrous membrane. The electrospinning process is simple, but some cases need to figure out the nature on polymer solution and processing unique factors.

Until now, several researches reported that chitin nanofibers were fabricated using *α*-chitin and highly toxic HFIP. In this study, *β*-chitin was extracted from cuttlefish bone, which consists of bundle of nanofibers, and hierarchical structures are wrapped with proteins and cluster, using simple chemical process. Briefly, *β*-chitin was the acid treatment for demineralization, followed by alkali-treated to remove the proteins. Afterwards, *β*-chitin was dissolved in formic acid as a solvent to evaluate the electrospinnability. Structure and physical properties of extracted *β*-chitin were investigated by ATR-IR, ^{1}H-NMR, SEM/EDS, XRD, texturemeter, and viscometer.

αvβ3 nanobody-conjugated PLGA nanoparticles for molecular imaging of angiogenesis

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 489

Dr. Chan Woo Kim [1], ***Mr. Jin-Moo Kim*** [1], ***Ms. Hyo Eun Park*** [1], ***Prof. Kiyuk Chang*** [1]

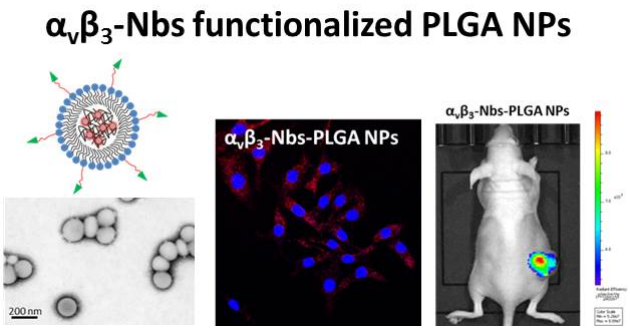
1. *Cardiovascular Center and Cardiology Division, Seoul St Mary's Hospital, The Catholic University of Korea*

[Introduction] For effective diagnosis of pathologic tissues using functionalized nanoparticles, diverse ligands such as antibody, peptide, and aptamer, are being explored as promising recognition moieties that can bind to selective targets with high affinity and specificity. Among them, antibody-based targeting strategies were widely used for imaging and delivering therapeutics. However, there are limitations of conventional antibodies including relatively large size and high Fc-mediated aspecific binding. Nanobodies (Nbs) are single-domain antigen-binding fragments (15 KDa) derived from camelid heavy-chain-only antibodies, showing unique characteristics such as small size, high stability and specificity. We have recently developed $\alpha_v\beta_3$ integrin-specific nanobodies ($\alpha_v\beta_3$-Nbs) as targeting ligand for $\alpha_v\beta_3$integrin receptor, which is overexpressed at sites of angiogenesis in many solid tumors. In this work, we investigate their targeting capabilities functionalized with biocompatible nanoparticles for near-infrared (NIR) imaging of neovascularization related with a variety with tumor malignancy.

[Methods] PLGA nanoparticles loaded with ICG (PLGA NPs) were prepared by single-step nanoprecipitation methods and $\alpha_v\beta_3$-Nbs were subsequently conjugated on the surface of PLGA NPs by EDC/NHS reactions. The obtained $\alpha_v\beta_3$-Nbs functionalized PLGA nanoparticles ($\alpha_v\beta_3$-Nbs-PLGA NPs) were characterized by dynamic light scattering (DLS), zeta potential, transmission electron microscopy (TEM), UV-Vis spectroscopy, and XPS analysis. The targeting capabilities and cytotoxicity of $\alpha_v\beta_3$-Nbs-PLGA NPs were assessed both in vitro/in vivo in U87-MG cancer cells.

[Results and Discussion]

The results showed that PLGA NPs and $\alpha_v\beta_3$-Nbs-PLGA NPs were dispersed as individual nanoparticles with a well-defined spherical shape and size distribution ranged from 180 to 200 nm, showing the effective ICG encapsulation and $\alpha_v\beta_3$-Nbs conjugation. The obtained $\alpha_v\beta_3$-Nbs-PLGA NPs exhibited the significant targeting capabilities compared with PLGA NPs in vitro and in vivo. The results suggest that $\alpha_v\beta_3$-Nbs serve as a great potential to target $\alpha_v\beta_3$ integrin receptor as an alternative ligand, and $\alpha_v\beta_3$-Nbs functionalized PLGA NPs can be used as non-invasive in vivo imaging of tumors and lesions related with $\alpha_v\beta_3$ integrin expression.



Nbs functionalized plga nps.jpg

Design of silaffin-fused ferritin and its silica-coated form as a new drug delivery agent

Monday, 25th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 388

Dr. Mi-Ran Ki [1], ***Ms. Thi Khoa My Nguyen*** [1], ***Mr. Sung Ho Kim*** [1], ***Mr. Ki Baek Yeo*** [1], ***Prof. Seung Pil Pack*** [1]

1. *Korea University*

Ferritin (FT), an iron storage and transport protein found in most living organisms, comprises 24 subunits with interior and exterior diameters of 8 and 12 nm, respectively. FT without iron has the capacity to load many types of metals or small molecules into its interior; thus, FT nanocages have attracted much attention as an ideal drug delivery system (DDS). In order to design FT as a more efficient agent for DDS, FT with silica-forming ability was developed by fusion of the silaffin peptide (Sp) found in diatom with FT at its N-terminus. In such newly-designed fusion protein(Sp-FT), the N-terminal SP is exposed on the surface of the designed FT and can mediate the silica deposition on the surface to generate silica-coated protein (SiO_2/Sp-FT). To investigate the potential of Sp-FT as DDS agent, the release pattern of drug was analyzed. Doxorubicin (Dox), the model drug, was loaded into cage interior by re-assembly of Sp-FT, then was coated additionally with silica matrix by Sp-mediated silicification. The loading amount of Dox by SiO_2/Sp-FT was larger than that of FT. Moreover, the release of loaded Dox was controlled and retarded by the silica-coated matrix. These results showed that the designed SiO_2/Sp-FT is favorable agent for DDS. Moreover, the Sp-FT provides a biocompatible and eco-friendly manufacturing process for the preparation of silica nanoparticles with drug compared to conventional production methods. Therefore, SiO_2/Sp-FT, new silica-protein composite particles developed here is advantageous for applications in the biological and medical fields.

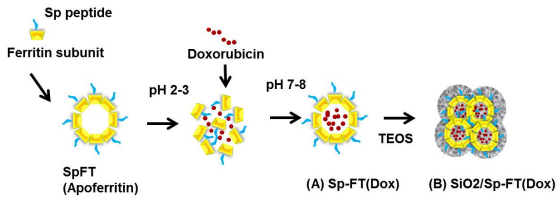


Fig1.jpg

Cationic Ag2S NIR Quantum Dots and Their Theranostic Applications

Monday, 25th September - 15:00 - Multi-Topic - Auditorium - Oral - Abstract ID: 465

Dr. Havva Yagci Acar [1], ***Dr. Fatma Demir Duman*** [1], ***Dr. rouhollah khodadust*** [2], ***Ms. Didar Asik*** [1]

1. *Koc University,* ***2.*** *KOC*

Introduction: Luminescent semiconductor quantum dot research in the field of medicine and biotechnology is moving towards those with emission in the near-infrared in order to improve the penetration depth and reduce scattering and autofluorescence of the natural tissue. QD industry and research is moving towards Cd, Pb and Hg free compositions for both visible and NIR QDs. In such a new era of QD research, Ag2S NIR QDs emerged as a new and one of the most promising compositions. We have developed several cationic Ag2S NIR QDs with strong emission in NIR I region as new theranostic nanoparticles. Synthesis of such particles with PEI containing coating, particle properties and their efficiency in gene transfection, drug delivery and combination therapy coupled with strong luminescence allowing optical detection will be discussed.

Methods: Nanoparticles were synthesized in aqueous solutions of silver salt with a thiol source such as Na2S or TAA in the presence of PEI (branched or linear; 25kDa) and 2-mercaptopropionic acid (2MPA) or L-cysteine (Cys). Reactions were run at different temperatures and pH. Folic acid tagged PEG with a carboxylic acid end group was conjugated with EDC/NHS to cationic QDs in some formulations. GFP and P53 were loaded to QDs at different N/P ratios. Doxorubicin (Dox) was loaded from PBS.

Results and Discussions: Stable colloidal cationic QDs were obtained with PEI/2MPA and with PEI/Cys mixed coating using both linear and branched PEI. QDs with quantum yield as high as 150% (with respect to LDS dye) were obtained. Strong intracellular signal indicated great potential as an imaging tool. Transfection of GFP and P53 to cells demonstrate their efficiency as theranostic nanoparticles. P53/Dox combination was also loaded to some formulations and achieved improved cytotoxicity in P53 knockdown cells. FA-PEG conjugated compositions were loaded with Dox and selectively delivered drug to folate receptor overexpressed HeLa cells in high efficiency.

Overall a portfolio of cationic Ag2S and their theranostic use will be discussed.

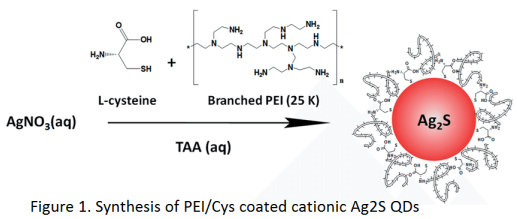


Figure 1. Synthesis of PEI/Cys coated cationic Ag2S QDs

Figure 1 synthesis.png

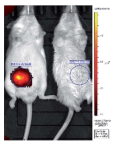


Figure 2. Fluoresence image of Balb/C mice 1h after being injected with PEI/Cys Ag2S QDs. Excitation at 740 nm. Emission filter: 840 longpass

Figure 2. in vivo luminescence.png

Family of bioactive heparins-coated iron oxide nanoparticles with T1-positive magnetic resonance contrast for various biomedical applications

Monday, 25th September - 15:17 - Multi-Topic - Auditorium - Oral - Abstract ID: 41

Dr. Hugo Groult [1], ***Mr. Nicolas Poupard*** [1], ***Dr. Fernando Herranz*** [2], ***Prof. Jésus Ruiz-cabello*** [2], ***Prof. Jean-marie Piot*** [1], ***Dr. Ingrid Fruitier-arnaudin*** [1], ***Prof. Thierry Maugard*** [1]

1. *UMR CNRS 7266, LIENSs,* ***2.*** *CNIC (Centro nacional de investigaciones cardiovasculares)*

Unfractionated heparin (UFH) and low-molecular-weight heparins (LMWH) are well known for their anticoagulant properties with a large clinical use. There is also currently a growing interest in using heparins in targeted cancer therapy. In particular, several types have been described to inhibit heparanase, a key enzyme over-expressed in the tumor microenvironment for angiogenesis progression and metastasis spreading. Here, we propose iron oxide nanoparticles coated with various heparins (HEP-IONP) of distinct anticoagulant and anti-heparanase activity ratios suitable for T1- weighted MRI, a recent contrast obtained with IONP class considered as very promising for sustainable clinical applications. A one-step microwave-based method was adapted to produce small size HEP-IONP stable in physiological media, and importantly, showing relaxivity performances for positive contrast in T1-weighted MRI. As proof of concept, high-resolution magnetic resonance angiography at large magnetic field were succeeded in mice up to 3 hours after intravenous administration of the probes. This unusual IONP-based positive contrast and the long vascular circulating times combined with the different bioactivities of the heparins enable innovative theranostic applications. We showed using advanced *in vitro*tests, how HEP-IONP anticoagulant and anti-heparanase activities were maintained depending on the type of heparin used for the coating. This confirmed that because they are polymeric substrates, specific heparin species can entered in the nanoparticle formulation at the same time as stabilizing surfactant and as particular bioactive group. Overall, the study allowed presenting an IONP coated with a commercial LMWH (Lovenox ®) as a theranostic translational probe for diagnostic magnetic resonance angiography and treatment of thrombosis, and an IONP coated with a specific depolymerized heparin that could be a potential anti-tumor candidate to be used in targeted therapy and diagnostic modalities.

[1] Oduah EI, Linhardt RJ, Sharfstein ST. Pharm Basel Switz. 2016;9(3).

[2] Niers TMH, Klerk CPW, DiNisio M, Van Noorden CJF, Büller HR, Reitsma PH, et al. 2007 Mar;61(3):195–207.

[3] Rivara S, Milazzo FM, Giannini G. Future Med Chem. 2016 Apr;8(6):647–80.

[4] Masola V, Secchi MF, Gambaro G, Onisto M. Curr Cancer Drug Targets. 2014;14(3):286–93.

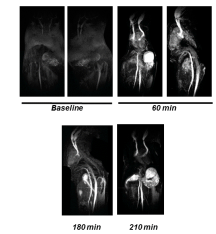


Figure 1. MRI angiography in mice at 60 min, 180 min and 210 min after i.v. administration of usHEP-IONP (2 mg Fe·kg^{-1}).

Figure 1.png

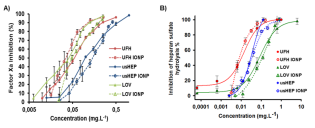


Figure 2. A) IC_{50} values for anti-factors Xa activity of HEP-IONP and their corresponding free heparins (AT III = 0.625 µg.mL^{-1} and factor Xa or IIa = 11.25 nKat.mL^{-1}) B) IC50 of HEP-IONP for inhibition of heparan sulfate hydrolysis by heparanase (100 ng.mL-1) compared to their corresponding free heparins.

Figure 2.png

Synthesis and investigation of magnetite-gold hybrid nanoparticles for visualization and targeted delivery purposes

Monday, 25th September - 15:34 - Multi-Topic - Auditorium - Oral - Abstract ID: 139

Ms. Mariia Efremova [1], ***Ms. Yulia Nalench*** [1], ***Dr. Victor Naumenko*** [1], ***Ms. Anastasia Garanina*** [2], ***Ms. Alexandra Prelovskaya*** [1], ***Mr. Maxim Abakumov*** [3], ***Mrs. Irina Saltykova*** [4], ***Prof. Alexander Savchenko*** [2], ***Prof. Natalia Klyachko*** [4], ***Prof. Alexander Majouga*** [4]

1. *National University of Science and Technology «MISiS»,* ***2.*** *National University of Science and Technology MISiS,* ***3.*** *Pirogov Russian National Research Medical University,* ***4.*** *Lomonosov Moscow State University*

During last decades magnetite and gold nanoparticles (NPs) attract a deep interest of scientists due to their potential application in therapy and diagnostics. Fe_3O_4 NPs have the ability to enhance T2-contrast in magnetic resonance imaging (MRI) and deliver drugs using an external magnetic field. Au NPs are characterized by high stability, biocompatibility, and can be covalently functionalized by a wide spectrum of thiol-containing ligands. The idea of Fe_3O_4-Au hybrid material creation is the combination of magnetite and gold promising properties as well as the presence of two types of surfaces.

Hybrid magnetite-gold NPs were obtained by the decomposition of iron pentacarbonyl on the surface of gold NPs. As a result, so-called dumbbell-like structures were obtained where magnetite with spherical (sample D-1, 13±2 nm) or cubic (sample D-2, 23±2 nm) shape and spherical gold NPs were connected together pairwise.

The samples were transferred into water by means of block-copolymer Pluronic F127. R2-relaxivity rates at the level of 167 and 385 $mM^{-1}s^{-1}$ were obtained for samples D-1 and D-2, respectively; the latter value is a record value for hybrid Fe_3O_4-Au NPs, exceeding the similar characteristics of commercial contrast agents twice.

The sample D-1 was also used for the selective functionalization of Fe_3O_4 NPs surface with anti-cancer drug doxorubicin and Au NPs surface – with the ligand of prostate specific membrane antigen (PSMA). Obtained NPs were found to have dose-related toxicity for human prostate cancer cells (LNCaP cell line) and got into the intracellular space after 45 minutes of incubation (according to fluorescence microscopy data). This can be explained by the affinity of the LNCaP cells to the PSMA ligand.

Thereby, in this work magnetite-gold hybrid NPs, which have a strong potential for biomedical application, particularly in targeted drug delivery to liver and prostate cells, and magnetic resonance imaging, were synthesized and characterized. That paves the way to the development of a new theranostic approach.

The authors gratefully acknowledge the financial support of the Ministry of Education and Science of the Russian Federation in the framework of Increase Competitiveness Program of NUST «MISiS» (№K2-2016-069).

Chlorin-e6 and Paclitaxel loaded on Keratin based nanoparticles synergically induce cell death of osteosarcoma cell lines in vitro.

Monday, 25th September - 16:20 - Nanomedicine for cancer diagnosis & therapy - Room 207 - Oral - Abstract ID: 310

Dr. Elisa Martella [1], ***Dr. Claudia Ferroni*** [1], ***Dr. Chiara Bellotti*** [2], ***Mr. Andrea Guerrini*** [3], ***Dr. Enrico Lucarelli*** [2], ***Prof. Davide Donati*** [2], ***Dr. Greta Varchi*** [1], ***Dr. Serena Duchi*** [1]

1. *Institute of Organic Synthesis and Photoreactivity - Italian National Research Council,* ***2.*** *Rizzoli Orthopaedic Institute,* ***3.*** *Institute of Organic Synthesis and Photoreactivity-Italian National Research Council*

Introduction. The survival rate of osteosarcoma (OS) patients has very poorly improved during the last decades. This is either due to the high doses of toxic chemotherapeutics required for treatments that in turn damage also key organs, and to the development of chemo-resistance. Nano-formulation of chemotherapeutics has improved both low water solubility of cancer drugs and their selective delivery to the tumor sparing healthy tissues. However, chemo-resistance has been scarcely addressed by nanotechnological formulations, so far. In this work, we tested *in vitro* whether the efficacy of keratin nanoparticles loaded with paclitaxel (PTX) could be improved if combined with photodynamic therapy.

Methods. Keratin (Ker) based nanoparticles loaded with PTX and with the second-generation photosensitizer Chlorin-e6 (Ce6) were obtained through desolvation (des) and drug-mediated aggregation (ag) methods (PTX-Ce6@Ker_{des} and PTX-Ce6@Ker_{ag} respectively) and with different PTX/Ce6 loading ratios. *In vitro* metabolic and viability assays were performed on three OS cell lines, e.g. MG63, SaOS-2 e U2-OS. Cells were exposed for 24 h to the nanoparticles and then irradiated with a LED light (λ_{max} = 668 ± 3 nm) for 5 min at RT (fluence 263 J/cm2) and compared to not-irradiated samples.

Results. IC_{50} values were used to measure PTX release and OS cell cycle blockage from the two nanoparticles formulations. PTX-Ce6@Ker_{ag} NPs were able to release PTX and block OS cells with a kinetic similar to that of free PTX, whereas PTX-Ce6@Ker_{des} provided a slower release profile (Fig.1). Cells viability tests show the synergic effect arising from the cytostatic activity of the released PTX and the reactive oxygen species (ROS) produced upon Ce6 irradiation (Fig. 2).

Discussion. Our results prove that our bimodal nanoparticles are able to augment PTX cytotoxicity on different OS cell lines. The proposed multimodal approach once proved to be effective in an *in vivo* model, would significantly enhance the efficacy of drug based treatments, facilitate the surgical removal of the tumor and increase the life expectancy of OS affected patients.

Acknowledgments. This work is supported by Associazione Italiana Ricerca sul Cancro (AIRC MFAG 2015 id16941 to Serena Duchi).

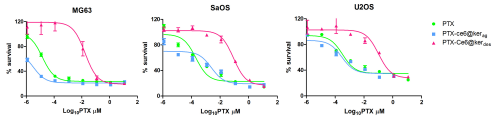


Figure 1.jpg

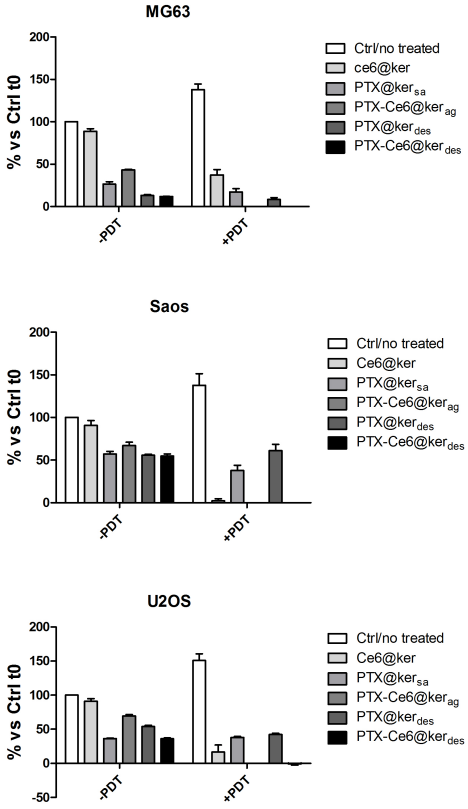


Figure 2.jpg

Interaction of nanoparticles with pulsed light and ultrasound for therapy and drug delivery

Monday, 25th September - 16:37 - Nanomedicine for cancer diagnosis & therapy - Room 207 - Oral - Abstract ID: 334

Prof. Rinat Esenaliev [1]

1. *University of Texas Medical Branch*

We proposed to use interaction of nanoparticles with light or ultrasound that produces direct thermal or mechanical damage to tumors or enhances delivery of anti-cancer macromolecular drugs and genes in tumors. Limited penetration of anti-cancer drugs and genes in tumors substantially reduces efficacy and safety of cancer chemo- and biotherapy. Interaction of light or ultrasound with strongly-absorbing or porous nanoparticles, respectively, may enhance drug and gene delivery or produce damage to tumors without drugs. The nanoparticles incorporating metal (gold, silver, etc.), carbon, or dye that strongly absorb light as well as porous, biodegradable nanoparticles can selectively accumulate in tumor blood vessels using passive delivery based on enhanced permeability and retention (EPR) effect or active delivery using targeting molecules. Our experimental studies were performed with optically- and ultrasound- active nanoparticles in vitro in tumors and in vivo in mice with human breast, colon, and prostate tumors. Strongly-absorbing nanoparticles and biodegradable polymer PLGA (150 – 200 nm sized) were used for optical and ultrasound therapy, respectively. We studied kinetics of the nanoparticles injected in the tail vein of mice bearing human tumors by using high-resolution ultrasound imaging systems (resolution up to 30 microns). Precise, sub-mm damage to tumors was produced by the interaction of the nanoparticles with focused ultrasound. Tumor thermotherapy was performed using interaction of near infra-red laser pulses with the nanoparticles and demonstrated severe damage to the tumors. Moreover, we developed and built an optoacoustic system for monitoring kinetics of the nanoparticles in the tumors in vivo and monitoring of the tumor thermotherapy in real time. Our results demonstrated that the interaction of nanoparticles with pulsed light or ultrasound produce thermal or mechanical damage to tumors and enhances delivery of anti-cancer drugs and genes in tumors.

Nano-seahorse T-cell-recruiting antibody assembly for cancer therapy

Monday, 25th September - 16:54 - Nanomedicine for cancer diagnosis & therapy - Room 207 - Oral - Abstract ID: 382

Mr. Hiroto Fujii [1], ***Dr. Hikaru Nakazawa*** [1], ***Mr. Aruto Sugiyama*** [1], ***Prof. Mitsuo Umetsu*** [1]

1. *Tohoku University*

Structural biology describes hierarchical structure of proteins: functional modules of domains, fragments, and subunits, are clustered as building blocks to create fine machinery molecules with autonomic systematical functions. Antibody is a protein which has been fragmented and rearranged to form recombinant proteins with non-native structure and function, because antibody is a typical module protein that is composed of structurally and functionally independent fragments. In this study, we propose a new structural format of high cytotoxic bispecific antibody assembly recruiting to cancer and lymphocyte cells. Small bispecific T-cell–recruiting antibodies have the potential of low-cost bacterial expression and contributes to low immunogenicity and high penetration into the tumor mass; so that, several bispecific structures are proposed, and some compact antibodies, such as tandem single-chain Fv, have been used in clinical trials. However, most of the compacted structures are monovalency which lead to weaker affinity for target-displaying cells than full-length IgG antibodies. Here, we constructed a compact bispecific and bivalent antibody from antigen-binding modules of single variable domain of the heavy chain of a heavy chain camel antibody (VHH) and single chain Fv (scFv) with two variable domains of heavy chain and light chain joined via a flexible polypeptide linker. The single domain format of VHH is an appropriate building block for generating fusion proteins and the scFv has the potential of intermolecular interaction that induces dimerization: the VHH domains with affinity for the epidermal growth factor receptor (EGFR) overexpressed on cancer cells were fused to the self-dimerized scFv recruiting to CD3 receptors on T-cells, resulting in the formation of bispecific and bivalent antibodies (BiBian) (Fig.1). The VHH-fused scFv fragments were spontaneously assembled to BiBian forms with a unique seahorse conformation in bacterial expression, and affinity increment for both target cells by bispecific and bivalent design of BiBian caused a drastic enhancement of cytotoxicity against tumor spheroids in vitro and in vivo (Fig2, 3). We show a promising sea-horse-shaped high cytotoxic antibody assembly formed from small antibody modules expressed in bacterial expression.

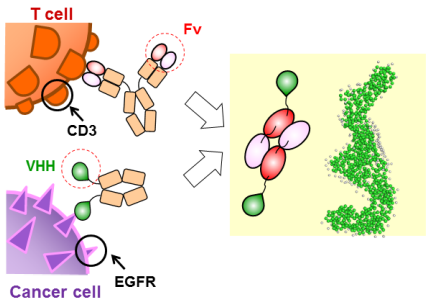


Fig. 1 Bispecific Bivalent antibody format (BiBian)

1.png

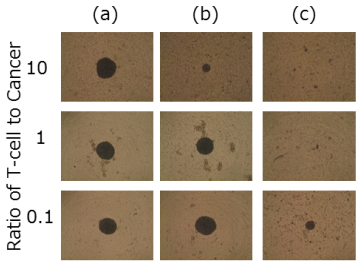


Fig. 2 in vitro cytotoxicity against caner cell spheroid
(a) No antibody
(b) General bispecific antibody (Diabody)
(c) BiBian

2.png

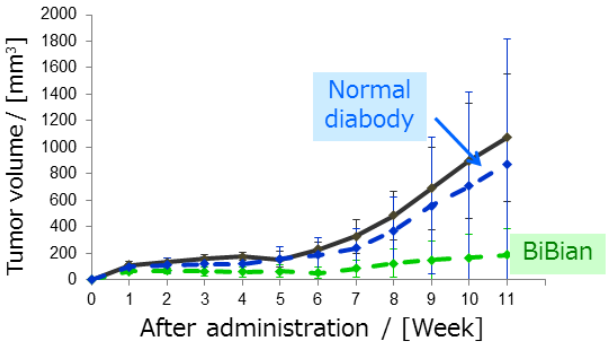


Fig. 3 in vivo cytotoxicity against tumor in mice

3.png

Multifunctional nanocomplex designed for enhanced cell uptake, endosomal escape and improved cancer therapeutic effect

Monday, 25th September - 17:11 - Nanomedicine for cancer diagnosis & therapy - Room 207 - Oral - Abstract ID: 68

Mr. Patrick Almeida [1], ***Dr. Mohammad-Ali Shahbazi*** [2], ***Ms. Alexandra Correia*** [1], ***Mr. Ermei Mäkilä*** [3], ***Dr. Marianna Kemell*** [1], ***Prof. Jarno Salonen*** [3], ***Prof. Jouni Hirvonen*** [1], ***Dr. Hélder Santos*** [1]

1. *University of Helsinki,* ***2.*** *Technical University of Denmark,* ***3.*** *University of Turku*

Introduction

Over the past few decades, breakthroughs in nanotechnology have paved the way for a new era of cancer theranostics.[1]

Porous silicon (PSi) nanoparticles have demonstrated tremendous potential for drug delivery applications, owing to their physicochemical and biological properties, with particular emphasis in cancer nanomedicine.[2] However, its limited cellular internalization and the incapacity for escaping endosomes still stand as deadlocks defying the implementation of these nanocarriers as anticancer drug delivery systems.

An interesting approach for improving the cellular internalization and intracellular trafficking of nanomedicines involves the design of multivalent cationic non-viral vectors.[3] For that purpose, cationic polymers have been used to complex negatively charged encapsulates, generating supramolecular nanostructures known as polyplexes.[4]

Objective

Herein, we envisioned to fabricate a multifunctional nano-in-nanocomplex platform encapsulating both sorafenib (SFB)-loaded PSi and gold (Au) nanoparticles into a polymeric nanocomplex (CPP) (**Figure 1A**). This novel approach aims to enhance the interaction of the PSi nanocarriers with cancer cells and induce their endosomal escape, ultimately improving the cytoplasmatic delivery and, consequently, the chemotherapeutic efficacy of the loaded anticancer agents.

Materials and Methods

The nanocomposites were physicochemically characterized and evaluated *in vitro* for cyto- and hemocompatibility, cellular association and internalization, endosomolytic properties, cytoplasmatic drug delivery and chemotherapeutic effect.

Results and Discussion

The nanocomposites were successfully produced and exhibited adequate physicochemical properties (**Figure 1B, 1C**), as well as superior *in vitro* cyto- and hemocompatibilities. The encapsulation of PSi nanoparticles in the nanocomplexes significantly enhanced their cellular internalization and enabled their endosomal escape (**Figure 2**), resulting in the efficient cytoplasmic delivery of these nanosystems. Sorafenib-loaded nanocomposites showed a potent *in vitro* anti-proliferative effect on MDA-MB-231 breast cancer cells (**Figure 3**).

Conclusion

The multifunctional nanocomposites showed great promise for the cytoplasmatic delivery of chemotherapeutics, as well as for the further development as nanoplatforms for cancer theranostic applications.

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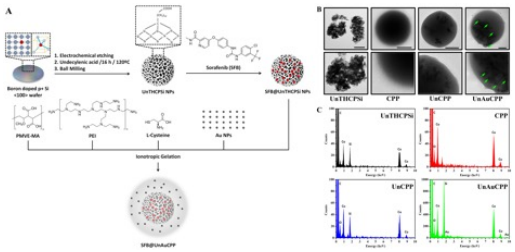

Figure 1.jpg

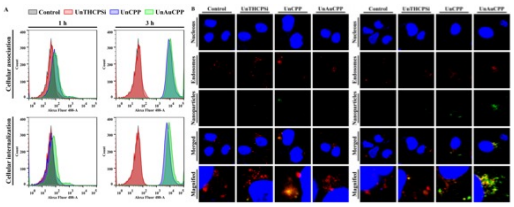

Figure 2.jpg

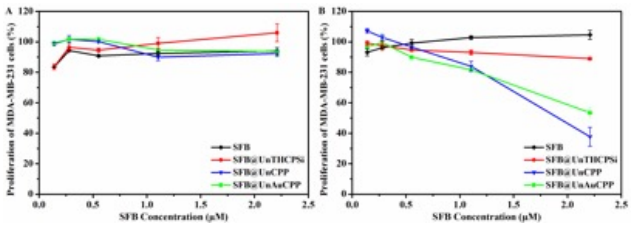

Figure 3.jpg

pH-responsive keratin nanoparticles for the controlled delivery of doxorubicin: a thorough physicochemical and in vitro study

Monday, 25th September - 17:28 - Nanomedicine for cancer diagnosis & therapy - Room 207 - Oral - Abstract ID: 112

Dr. Greta Varchi [1], ***Prof. Marzia Gariboldi*** [2], ***Dr. Claudia Ferroni*** [1], ***Dr. Giovanna Sotgiu*** [1], ***Mr. Marco Ballestri*** [3], ***Mr. Andrea Guerrini*** [3], ***Dr. Annalisa Aluigi*** [3], ***Prof. Elena Monti*** [2]

1. *Institute of Organic Synthesis and Photoreactivity - Italian National Research Council,* ***2.*** *University of Insubria,* ***3.*** *Institute of Organic Synthesis and Photoreactivity-Italian National Research Council*

Introduction

Doxorubicin (DOX) is one of the most effective cytotoxic drug used for the treatment of a wide range of tumors. However, the use of DOX is limited by serious drawbacks, which might be partially overcome by means of drug delivery systems based on nanotechnology. Keratin possesses excellent biocompatibility and low toxicity to cells;[1]moreover, specific aminoacidic sequences are present on the protein backbone, e.g. “Arg-Gly-Asp” (RGD) and “Leu-Aps-Val” (LDV), which are known to specifically bind to integrins receptors over-expressed in several cancer cells.[2] Therefore, in the present work we describe the synthesis, physicochemical and in vitro biological characterization of DOX-loaded keratin nanoparticles (DOX-KNPs) against breast cancer cells.

Methods

Stable and monodisperse DOX-KNPs were prepared through two diverse in-water methods,e.g. ionic gelation and aggregation, and characterized in terms of size, zeta-potential, morphology, stability in physiological media and drug release profiles. Mathematical models were applied to experimental data with the aim of understanding the release mechanism of DOX. Finally, antiproliferative activity against MCF7 and MDA MB 231 cells was evaluated in 2D models and compared with those of free DOX.

Results

KNPs-DOX1 and KNPs-DOX2 are obtained in quantitative yield by ionic gelation and aggregation method, respectively and with a drug loading ratio up to 30% wt. They are spherical in shape with hydrodynamic diameters of about 180 nm and a DOX release profile dependent on pH conditions (Fig. 1). In vitro biological evaluation highlighted the ability of KNPs-DOX to enter cancer cells more efficiently of free DOX and to inhibit their proliferation with IC_{50} values comparable to those of the free drug (Fig. 2).

Discussion

In the present work, the ability of keratin extracted from raw wool to function as carrier of doxorubicin was explored by exploiting two diverse yet quantitative methods. Overall, data demonstrate the effectiveness or our newly synthesized keratin nanoparticles in delivering the drug, paving the way for a thorough investigation on the use of keratin nanoparticles as carriers of hydrophilic and toxic anticancer drugs.

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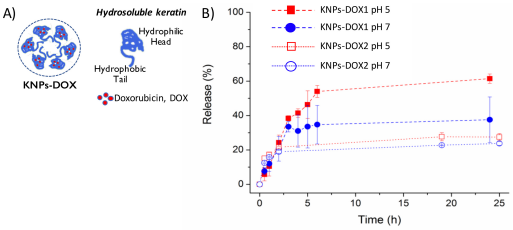

Figure 1. A) Schematic representation of KNPs-DOX nanoparticles; B) Effect of pH on DOX release from keratin nanoparticles prepared by two different methodologies (KNPs-DOX1 & KNPs-DOX2).

Figure 1bis.jpg

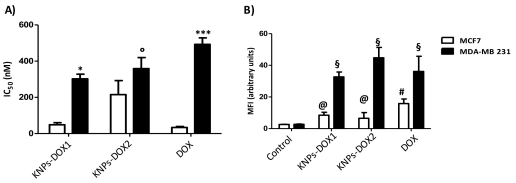

Figure 2. IC_{50} values(A) and DOX accumulation levels (B) obtained on MCF7 and MDA-MB 231 cells following 72h exposure to KNPs-DOX1, KNPs-DOX2 and DOX. (*p<0.05 vs MCF7 and DOX same cell line; *** p<0.0001 vs MCF7; ° p<0.01 vs DOX and KNPs-DOX1 same cell line, @ p<0.05 vs control; # p<0.05 vs all the others same cell line; § p< 0.001 vs MCF7 and control).

Figure 2bis.jpg

Evaluation of the dermal health risk of silver nanowires as prospective medicine tool

Monday, 25th September - 16:20 - Toxicology and risk assessment of nanomedicine systems - Room 412 - Oral - Abstract ID: 448

Dr. Sylvia Lehmann [1], ***Dr. Benjamin Gilbert*** [2], ***Dr. Thierry Maffeis*** [3], ***Prof. Laurent Charlet*** [1]

1. *University Grenoble Alps,* ***2.*** *Lawrence Berkeley National Laboratory,* ***3.*** *Swansea University*

Silver nanowires (Ag NWs) have a particularly large range of anticipated nanotechnology applications because of their intriguing optical, conductive, antimicrobial, chemical and thermal properties. They have been proposed to develop some diagnosis and monitoring tool as patches and electrodes applied on the skin or incorporated into clothes.

However, despite numerous studies of the environmental and human health impacts of silver nanoparticles, there are currently very few studies of the potential hazards of silver nanowires It is likely that some Ag NW-enabled diagnosis technologies will be in contact with human skin but to date the potential impact of such exposure has not been assessed. To address this knowledge gap, we have performed Ag NW uptake and cytotoxicity studies on human primary keratinocytes and reconstructed epidermis.

Briefly, we studied Ag NWs of two length coated with PVP. Cytotoxicity was investigated in primary keratinocytes by MTT and Neutral Red uptake assays. Penetration of Ag NW in cells was investigated by SEM and confocal microscopy. Data obtained showed cytotoxicity of Ag NW, which was lower than silver ion but not due to silver ion release in the medium. Ag NW were efficiently internalized by cells and found to be persistent. Monolayer cultures of cells do not fully represent the real (3D) architecture of skin. To overcome these limitations and to be closer to physiological conditions, we used reconstructed human epidermis that were exposed to Ag NW. Cytotoxicity was assessed by MTT assay and skin section were performed and analyzed by TEM. No cytotoxicity was observed even after 72h of exposure and penetration inside the deeper layers of the epidermis was not observed.

These data strongly suggest an efficient protection of the stratum corneum in 3D epidermis against Ag NW exposure and provide preliminary evidences on the future potential of AgNW as diagnosis and monitoring tool.

Lateral Size of Thin Graphene Oxide Sheets is a Critical Factor for Induction of Oxidative-Stress Mediated Cellular Responses

Monday, 25th September - 16:37 - Toxicology and risk assessment of nanomedicine systems - Room 412 - Oral - Abstract ID: 378

Dr. Sandra Vranic [1], ***Mr. Artur Filipe Rodrigues*** [1], ***Dr. Maurizio Buggio*** [1], ***Dr. Cyrill Bussy*** [1], ***Prof. Kostas Kostarelos*** [1]

1. *University of Manchester*

The interest for graphene and its translation into commercial products have been expanding at high pace during the last few years. In consideration of the previously described pulmonary health and safety concerns for carbon nanomaterials, there is a great need to understand the critical parameters impacting interactions between graphene based materials (GBMs) and the cells of the pulmonary system. With this regard, the aim of present study was to determine the importance of two key parameters: lateral dimensions of the material and coating with proteins from the serum in relation to each other and their impact on human lung epithelial cell line BEAS-2B. Secondly, we interrogated whether the cellular response to graphene oxide (GO) could be explained and predicted using oxidative stress paradigm.

In order to address these questions, we produced and thoroughly characterized endotoxin-free material with two distinct lateral dimensions – large, micrometer-sized GO (5 – 15 μm) and small, nanometer-sized GO (50 – 200 nm). The role of protein adsorption in the initial phase of the interaction between the GO flakes and cells was addressed by controlling the presence of serum during the first 4 h of interaction. We exploited the intrinsic fluorescence of GO to image the material without introducing additional surface modifications or attaching fluorescent dyes. Using confocal live cell imaging, we were able to show for the first time the behavior of the cells in response to the material exposure. Toxicity was confirmed to be time and dose dependent, with large material inducing higher levels of cellular death correlated with elevated ROS production and increased expression of pro-inflammatory genes, which is in agreement with oxidative stress paradigm. In addition, toxicity of small material was completely alleviated at 24 h in the presence of FBS, while only mitigated for large material, confirming the hypothesis that lateral dimensions are a predominant factor of toxicity and inflammation *in vitro*.

Protein coating determines the cellular responses in the abdominal cavity after graphene oxide administration

Monday, 25th September - 16:54 - Toxicology and risk assessment of nanomedicine systems - Room 412 - Oral - Abstract ID: 403

Mr. Artur Filipe Rodrigues [1], ***Mr. Leon Newman*** [2], ***Dr. Dhifaf Jasim*** [2], ***Prof. Kostas Kostarelos*** [1], ***Dr. cyrill bussy*** [2]

1. *University of Manchester,* ***2.*** *The University of Manchester*

Graphene oxide (GO) is a graphene-based material that has attracted commercial interest in a wide range of applications, from inks and spray coatings to drug delivery. Understanding the toxicological profile of GO is crucial for this implementation to occur. A number of studies have discussed the toxicity of GO flakes, but the *in vivo* impact of their lateral dimensions is still not clear. Our group has previously demonstrated that GO flakes of small lateral dimensions (s-GO, < 1 μm) do not trigger significant inflammation after intraperitoneal injection. Here, we investigated the impact of GO flakes of large, micrometre-sized lateral dimensions (l-GO, 1 to 20 μm) after i.p. injection, to test whether high aspect ratio nanomaterials such as l-GO have a more deleterious impact than their smaller counterparts. We also aimed to address whether a protein corona would alter the biological response by testing different dispersing modalities (0.5% BSA in saline solution *vs* 5% dextrose in water).

Using SPECT/CT imaging, we observed that both GOs were able to travel towards the peritoneal mesothelium of the diaphragm. The presence of GO was confirmed by Raman mapping of diaphragm histological sections obtained 1 and 7 days after injection. Histology and SEM analyses showed that GO did not induce significant recruitment of granulocytes to the mesothelium, irrespective of their size and dispersion.

We then characterised the potential of these materials to induce inflammation by differential staining of cells extracted from the peritoneal cavity. We observed that s-GO pre-dispersed in 5% dextrose elicited a greater recruitment of monocytic cells in the peritoneal cavity 24 h after injection, which coincided with their greater ability to be internalised. However, when it was pre-coated with BSA proteins, the recruitment of immune cells by s-GO was reduced.

In conclusion, GO flakes did not induce mesothelial granuloma irrespective of their lateral dimensions and dispersion. But s-GO triggered monocytic cell recruitment in the peritoneal cavity in the absence of protein pre-coating. These results highlight the importance of a bio-corona in the surface reactivity of GO flakes towards biological systems.

Biological Recognition of Graphene Nanoflakes

Monday, 25th September - 17:11 - Toxicology and risk assessment of nanomedicine systems - Room 412 - Oral - Abstract ID: 358

Dr. Valentina Castagnola [1], ***Dr. Luca Boselli*** [2], ***Ms. Maria Cristina Lo Giudice*** [1], ***Dr. Ester Polo*** [1], ***Ms. Fatima Alnasser*** [1], ***Dr. Margarita Esquivel Gaon*** [1], ***Prof. Kenneth A. Dawson*** [2]

1. *University College Dublin,* ***2.*** *University College of Dublin*

Graphene has recently attracted tremendous interest in several biomedical fields such as drug delivery, cancer therapies and biosensing. Opportunities in this emerging field therefore will require the systematic study of the biological interactions of graphene when exposed to biofluids and cells. We now recognize that the early stages of biological impacts, governed for example by receptor interactions, require surface presentations that are representative of real exposure conditions in blood.

In this work we developed a facile, biocompatible long-term colloidally stable water dispersion of few-layered graphene nanoflakes directly in the biological media of interest, by mean of ultrasound assisted liquid exfoliation.[1] We applied recently developed immunoprobing protocols[2, 3] in order to assess the functionality and availability of specific epitopes of interest situated on the periphery of the graphene nanoflakes biological surface (Figure 1). These key motifs are likely to mediate most of the early biological interactions. Finally, by mean of cell transfection protocols, we studied the interactions between graphene nanoflakes and specific overexpressed cell receptors able to engage with the key motifs identified on the surface.[4]

The evidence accumulated from experiments and controls suggests that, in sharp contrast to many other nanomaterials, graphene flakes present a negligible proportion of apolipoprotein B100 type recognition motifs, but are rich in effective apolipoprotein A-I presentation. This is confirmed by the receptor driven uptake that resulted much higher when SRB1 receptors (able to recognise apolipoprotein A-I) were overexpressed on the surface of the cell (Figure 2).
The basis set here using graphene and serum proteins opens the way for detailed and mechanistic biological studies based on meaningful biomolecular surface presentations.

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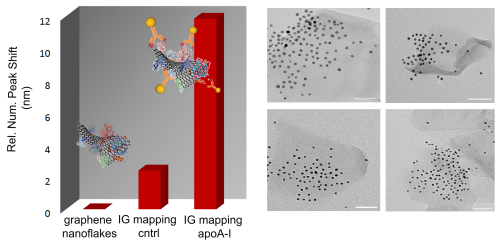
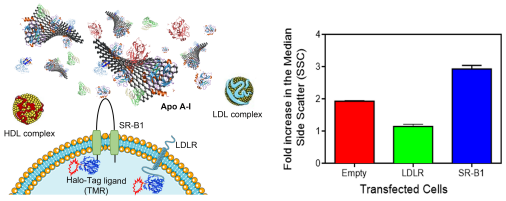

Rel. Num. Peak Shift (nm)
12
10
8
6
4
2
0
graphene nanoflakes
IG mapping cntrl
IG mapping apoA-I

F1.png


HDL complex
Apo A-I
LDL complex
SR-B1
LDLR
Halo-Tag ligand (TMR)
Fold increase in the Median Side Scatter (SSC)
4
3
2
1
0
Empty
LDLR
SR-B1
Transfected Cells

F2.png

Toxicogenomic Profiles of Titania Nanotube Arrays on a Panel of Human Cell Lines

Monday, 25th September - 17:28 - Toxicology and risk assessment of nanomedicine systems - Room 412 - Oral - Abstract ID: 164

Dr. Rabiatul Basria S M N Mydin [1], ***Prof. Ishak Mat*** [1], ***Prof. Mustafa Fadzil Farid Wajidi*** [2], ***Dr. Roshasnorlyza Hazan*** [3], ***Prof. Srimala Sreekantan*** [4]

1. *Oncological and Radiological Sciences Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia,* ***2.*** *School of Distance Education, Universiti Sains Malaysia,* ***3.*** *Materials Technology Group, Industrial Technology Division, Nuclear Malaysia Agency,* ***4.*** *School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia*

Titania nanotube arrays (TNA) have a great potential to be applied in nanomedicine due to their distinctive structure. Note that TNA nanometric topography plays a critical role in cellular stability and cell survival. Recently, the key concern is whether nanotubes array with titanium dioxide could present any cellular or molecular threat. Due to larger surface area properties, TNA may allow possible integration between cells and its biological components such as lipids, proteins and nucleic acids. Therefore, the present cell–TNA study focus on molecular risk assessments in various *in vitro* systems including epithelial, fibroblast and osteoblast cells. Data were collected and interpreted from Scanning Electron Microscopy with Energy Dispersive X-ray spectroscopy (SEM-EDX), real-time PCR gene expression profiling, enzyme-linked immunosorbent assay (ELISA), immunofluorescence staining, southern blot and western blot analyses. Initially, the SEM-EDX results showed cell's adaptation response on TNA surface. Further analyses on genes and proteins predicts that cell-TNA cytoskeleton remodeling mechanisms may involve cell's extracellular matrix tensile alterations, shear stiffness response, plasma membrane modulation, cell polarity and locomotor behaviors. Moreover, the nano-architecture structures of TNA showed could be beneficial to cells as a supply or storage route for nutrients and mediator growth signals. Furthermore, cell-TNA interaction also indicates the expression of genes and proteins involved in positive growth regulation via homeostatic proliferation response. Interestingly, the data from this findings stipulate cell-TNA molecular sensitivity in senescence-associated secretory phenotype could also reduce the inflammatory response. Thus, the intricate molecular mechanisms behind cell–TNA cellular response are crucial for positive cell growth with mechanosensitive activities. Molecular understanding of this nanomaterial is beneficial for further nanomaterial characterization in advanced medical applications.

Stimuli-responsive delivery nanosystems: A versatile technological platform

Monday, 25th September - 16:20 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 89

Mr. Eloy Pena [1], ***Mr. Antoni Ruiz Bayarri*** [1], ***Dr. Gemma Vilar Palos*** [1], ***Ms. Jessica Romero*** [1], ***Dr. Lorena García-fernández*** [1], ***Dr. Socorro Vázquez-campos*** [1]

1. *Leitat Technological Center*

Several studies have demonstrated the potential use of nanocapsules to improve efficacy/toxicity profile of drugs. In particular, for dermatological applications, they offer many advantages including controlled and sustained release of active ingredients (AI) and protection of the encapsulated AI from its environmental degradation. All these features lead to an improvement of bioavailability and therefore, improving its biological effectiveness. Stimuli-responsive polymer nanocapsules (PNCs) are smart nanocarriers that encapsulate active ingredients and release them on demand upon external triggers. Stimuli-responsive PNCs are of interest because the AI can be released via different stimuli avoiding unwanted and unspecific release.

Different biodegradable core-shell nanocapsules containing different AI were synthesized and studied for their potential as drug-delivery systems for dermatological applications. The core of these drug delivery systems consists of a polyester polyester polymer which is at the front line of attention because of their attractive safety profile. Since their degradation products are easily metabolized by the Krebs cycle and therefore easily eliminated. On the other hand, the shell was added via layer by layer assembly of different polymer electrolytes. Dynamic light scattering and zeta potential measurements were used to analyze the layer by layer assembly process and microscopy analysis to determine the thickness of the layer. These polyelectrolytes (shell) confer control over the AI release specifically in the presence of proteases (slowing down the degradation process). Therefore, the release studies were performed in different media: Different behavior was observed when the release was done in conditioned medium from Human Dermal Fibroblast (HDF), indicating that AI release exhibits a protease dependent behavior. A combination of analytical techniques was used to monitor the AI release.

In conclusion, a set of stimuli responsive safe and biodegradable core-shell nanocapsules have been developed and these nanosystems provide a versatile technological platform for topical applications.

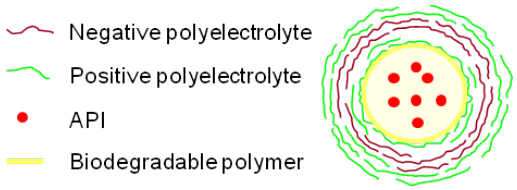


Figure 1. Scheme of core-shell nanocapsule

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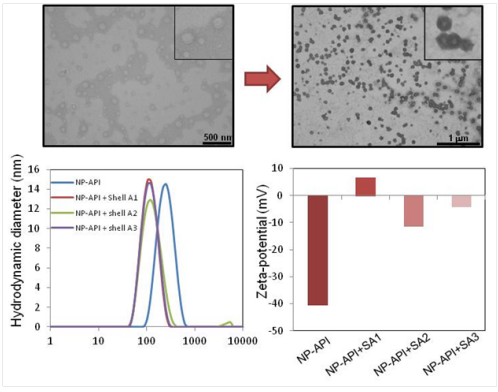

Figure 2. Monitoring of the addition of polyelectrolites

Imagen2.png

Synthesis, characterization and antimicrobial activity of PLGA nanoparticles loaded with P19 peptide against two pathogenic bacteria

Monday, 25th September - 16:37 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 480

Mr. Nicolas Sebastian Gomez Sequeda [1], ***Ms. Jennifer Ruiz*** [1], ***Dr. Claudia Cristina Ortiz López*** [1]

1. *Universidad Industrial de Santander*

Antimicrobial Peptides (AMPs) are macromolecules formed by less than 100 aminoacids and sizes <10kDa. These are very attractive because of fast action and wide spectrum activity against bacteria, fungi, protozoan, parasites and viruses. For this reason, peptides are generating great interest to affront health issues such as resistant pathogenic microorganisms. However, despite all the advantages offered by AMPs, these are limited in comparison to conventional therapeutic molecules due to its high susceptibility to proteolysis and denaturation. In this sense, one alternative to avoid these interferences is the nanoencapsulation with polymeric nanoparticles. This represents a viable and highly versatile way for the protection of active principles, allowing that its biological activity and structural identity be conserved. This work focuses on the synthesis, characterization and antimicrobial activity of poly (lactic-co-glycolic) acid (PLGA) nanoparticles loaded with the AMP P19 against two pathogenic bacteria, Escherichia coli O157:H7 and Methicillin Resistant Staphyloccocus aureus (MRSA), which are priority pathogens for research and development of new antibiotics according to World Health Organization (WHO). AMP P19 was designed and synthesized in our lab by Fmoc solid phase peptide synthesis. The AMP obtained was encapsulated in colloidal solution using Double Emulsion Solvent Diffusion (DES-D) method. Different concentrations of P19 were encapsulated (NP-P19) with the aim of determining the best ratio peptide/polymer, spherical and monodispersed polymeric nanoparticles of 257±2.84 nm and zeta potential of 12.90±0.80 mV were obtained (figure 1), suggesting high thermodynamic stability. This methodology allowed us to obtain an encapsulation efficiency of 80.48±1.75% (figure 2), NP-P19 showed a rapid cumulative release (>51%) in the first 20 min and then slow and controlled release up to 2880 min (>95%) (figure 3). Finally, the antimicrobial activity of free P19 and NP-P19 gainst E. coli O157: H7 and MRSA was evaluated, obtaining a Minimal Inhibitory Concentration (MIC50) for NP-P19 of 3.13 µg/mL and 0.7 µg/mL, respectively. This represents an increase in the activity of the peptide compared to the free P19 (Table 1). Our results suggest that nanoencapsulation favors the activity of antimicrobial peptides that eventually could be applied in the development of alternative therapies for infection of this pathogenic bacteria.

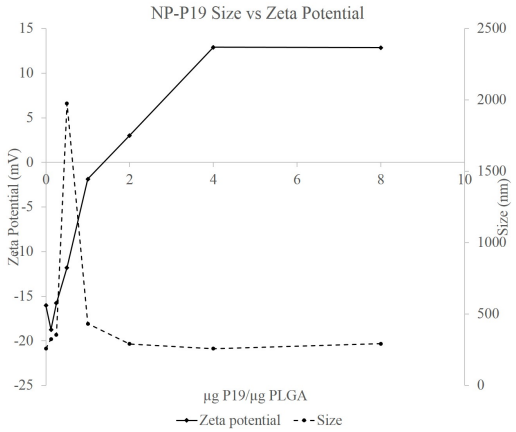

Figure 1. effect of the peptide-polymer ratio over size and zeta potential.jpg

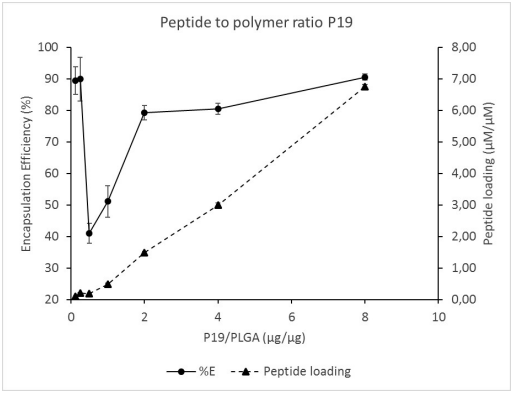

Figure 2. effect of the peptide-polymer ratio over peptide encapsulation and encapsulation efficiency.jpg

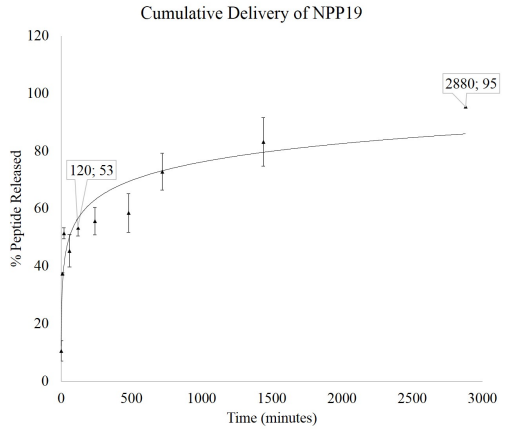

Figure 3. percentage cumulative release from np-p19.jpg

Table 1. MIC_{50} and MBC (µM) of P19 and NP-P19 against *E. coli* O157:H7 and MRSA

	E. Coli O157:H7			MRSA		
	MIC_{50}	MIC_{99}	MBC	MIC_{50}	MIC_{99}	MBC
P19	12.5	20	70	1.5	6.5	70
NP-P19	3.13	20	100	0.7	20	100
NP	>100	>100	>100	>100	>100	>100

Table 1. mic50 and mbc of p19 and np-p19 against e. coli o157h7 and mrsa.jpg

Dynamic combinatorial approach as synthetic strategy for the formation of non-viral vectors for gene therapy

Monday, 25th September - 16:54 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 186

Dr. Lilia Clima [1]

1. *"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania*

The next level in **Drug Discovery** is the easy building and self-generation of multifunctional nanostructures from commercially available or "easy to prepare" units, which will further self-assemble in a complex, tunable and multifunctional materials, suitable for specific targeted drug/gene delivery. DNA and target cells are highly variable and therefore, rational design is limited to a relatively small number of components and a high number of synthetic steps. One possible solution to this problem is to employ the constitutional dynamic chemistry (CDC) as a new evolutionary approach to produce chemical diversity. A specific advantage with constitutionally generated systems addresses the possibility to self-adjust to biological target species at a given time, in a certain environment at nanoscale dimensions. The key concept is exploring the multivalent molecular recognition and self-assembly by using adaptive platforms interacting with biological targets. The use of reversible interactions as dynamic interfaces between the target and Dynamical Constitutional Frameworks (DCF) components will allow one to self-adjust the system's tridimensional geometry and functional properties. In presented work adaptive dynamic vectors based on polyethylene glycol, cationic moiety components and in some cases squalene derivative, which are reversibly connected to core centers are prepared and tested as vectors for DNA transfection. Depending on their tuneable composition, these modular vectors dynamically self-adapt to their DNA targets, allowing the rapid screening of most effective vectors, optimally matched to DNA 3D surrounding space. Our strategy allows easy and efficient identification of adaptive vectors with high DNA complexation ability, good transfection efficiency, and well tolerated by mammalian cells.

This work was supported by Horizon 2020 WIDESPREAD 2-2014: ERA Chairs Project no 667387 and 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.

The best of both worlds: spatially-resolved hybrid hydrogels for controlled release

Monday, 25th September - 17:11 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 328

Mr. Phillip Chivers [1], ***Prof. David Smith*** [1]

1. *University of York*

Introduction

Interpenetrating polymer network (IPN) gels comprising two orthogonally assembled polymer networks are of great interest in the pharmaceutical industry for active pharmaceutical ingredient (API) delivery because they can demonstrate desirable properties of the individual networks whilst mitigating the drawbacks of each.[1,2] Reports of IPNs comprising two polymer gelator (PG) networks have become increasingly common,[3] however reports where low-molecular-weight gelator (LMWG) and PG networks are combined are surprisingly limited. LMWG hydrogels are of significant interest in applications as diverse as tissue engineering, drug release and environmental remediation because of changes in material structure in response to external stimuli including pH, light and temperature.[4,5] By harnessing the pH-dependent API release of a LMWG (DBS-$CONHNH_2$)[6] in combination with the mechanical robustness of a photopatternable PG (PEGDM), we are able to demonstrate the 'best of both' gels in one material (Figure 1).

Methods

DBS-$CONHNH_2$/PEGDM networks incorporating non-steroidal anti-inflammatory drug naproxen (NPX) were formed sequentially to yield hybrid materials. LMWGs were assembled by heat/cool cycle followed by UV-patterning of the PG network. PG formation was spatially controlled using a photomask. These materials have been fully characterised by NMR spectroscopy, rheology and thermal analysis.

Results

The mechanical properties of the material were tuned by varying PG content. At 10% wt/v PEGDM, material stiffness increased dramatically and the gels became self-supporting. Breaking strain also increased much beyond that of the LMWG. These robust materials released NPX selectively into buffers above the drug pK_a with similar preference to the LMWG alone. By photopatterning a NPX-loaded hybrid gel band, we generated a self-supporting material which demonstrated unidirectional release into a compartment of pH 7 buffer rather than into pH 2.8 buffer (Figure 2)

Discussion

We have developed for the first time a photopatternable LMWG/PG hybrid material which demonstrates great potential for targeted delivery and tissue engineering by selective release of drugs/growth factors into a suitable environment. Crucially, each gelator plays an important role in determining the material properties. The PG is necessary to enable photopatterned shaping of the material and provide mechanical robustness, whilst spatial resolution and pH-dependent release are not possible in the absence of the LMWG.

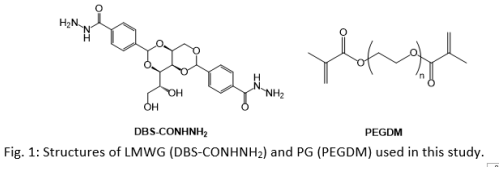

Fig. 1: Structures of LMWG (DBS-$CONHNH_2$) and PG (PEGDM) used in this study.

Figure 1.png

Fig. 2: Spatially controlled formation of a NPX-containing gel allows for sophisticated control of release.

Figure 2.png

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Hyaluronic Acid and CD44: A Not So Straightforward Relationship

Monday, 25th September - 17:28 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 52

Mr. Julio Manuel Rios de la Rosa [1], ***Dr. Annalisa Tirella*** [1], ***Dr. Maria Pelliccia*** [1], ***Ms. Alice Spadea*** [1], ***Dr. Enrique Lallana-Ozores*** [1], ***Ms. Ponpawee Pingrajai*** [1], ***Prof. Ian J. Stratford*** [1], ***Prof. Nicola Tirelli*** [1]

1. *University of Manchester*

Introduction: CD44 is the major receptor of hyaluronic acid (HA) on cell membranes, where it fulfils anchoring, signalling and endocytic functions. This multi-faceted role renders CD44 a key mediator in cellular responses to their microenvironment both in homeostasis and pathological processes. In particular, the (over)expression of CD44 variant isoforms (CD44v) in solid tumours has attracted interest in the design of HA-based targeting therapies. However, the caveats of such strategies are a poor understanding of the target itself (in terms of HA interactions) and its ubiquitous expression in healthy tissue.

Methods: In order to predict the targeting behaviour of HA already *in vitro*, we have first evaluated the expression of CD44 in relevant cancer and normal human cell lines (via flow cytometry) and then cross-correlated it with: the uptake kinetics of fluorescently-labeled HA-exposing chitosan nanoparticles on cell lysates or live cells, respectively discriminating between overall uptake and internalisation, and the functional delivery of siRNA, examined by RT-PCR.

Results: We found a high CD44 expression in colorectal cancer cell lines and a moderate-to-high expression in pancreatic ones, the latter comparable to that of fibroblasts and endothelial cells. Macrophages expressed polarisation-dependent low levels of CD44. As expected, the distinct expression pattern in cancer and normal cells was reflected in a different nanoparticle uptake, hence in their overall silencing efficiency. In most cases the relationship between CD44 expression and targetability of HA-exposing nanoparticles was straightforward; for instance, CD44-overexpressing HCT-116 cells showed a significantly higher nanoparticle internalisation compared to normal cells and achieved >70% knockdown. However, we found an inverse correlation between nanoparticle internalisation and CD44 expression for some cell lines (e.g. THP-1 M1, HT-29).

Discussion: Despite the preferential uptake of HA-exposing nanoparticles by cancer cells, the relationship between CD44 expression and HA internalisation is not always as simplistic as 'higher CD44 = better targetability'. Paradoxically, our results suggest that cells expressing high levels of CD44, and potentially best HA binders, may turn out to be the most difficult to treat because of the slower/more difficult internalization of HA-based materials.

Response-triggering stimuli in drug release

Tuesday, 26th September - 09:00 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 441

Prof. Maria Vallet Regí [1]

1. *Complutense University of Madrid*

This lecture will describe the production of smart nanosystems capable of: (1) carrying antitumor agents selectively to a tumor tissue, and (2) releasing them there thanks to the application of an external stimulus. We use the term *smart* because those nanocarriers are able to release the drugs when and where they are needed. The surface of our nanosystems can be decorated with molecules able to recognize specifically tumor cells and to trigger the penetration of nanocarriers into them, like a Trojan horse. The main advantage of developing selective nanocarriers able to accumulate only in tumor tissues are: (1) increased selectivity of the therapy, which allows reducing the cytotoxic dosage; (2) higher control over the administered doses; and (3) the reduction of side effects, because the drugs will not be distributed throughout the whole body. Taking into account that most anticancer drugs are cytotoxic, their release must take place only inside tumor cells. This can be achieved using Chemistry, which provides the necessary tools to prepare stimuli-responsive nanocarriers in which the release of the drug can be controlled and triggered from the outside. In this talk the different stimuli, both internal and external, (4,5,6) that we are using in our research group will be described. We would detail the whole process from the nanomedicines design to their evaluation using different models and their potential translation to the clinic.

Keywords: nanomedicine, drug carriers, stimuli-responsive system, smart nanosystems.

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MVR acknowledges funding from the European Research Council (Advanced Grant VERDI; ERC-2015-AdG Proposal No. 694160)

Ligand-free metal nanoparticle colloids: benefits in standardized toxicity assays and functional bioconjugates

Tuesday, 26th September - 09:40 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 7

Prof. Stephan Barcikowski [1]

1. *Technical Chemistry I and Center for Nanointegration Duisburg-Essen (CENIDE), University of Duisburg-Essen*

The interactions between metal nanoparticles and biological systems are known to be highly complex as effects occurring at the nano-bio interface are ruled by multiple, often interdependent factors like particle size, particle dose, surface charge, surface functionalization[1] as well as the protein corona formed upon contact with body fluids[2]. This complexity makes systematic studies on the biocompatibility as well as *in vitro* and *in vivo* functionality of nanoparticles and their bioconjugates highly challenging. One drawback, in this context, is that nanoparticles from chemical synthesis always contain large quantities of artificial ligands, which cannot be quantitatively removed or exchanged[3] and may interfere with biomedical applications.

An alternative approach is the use of ultrapure totally ligand-free metal nanoparticles available via a modern physical synthesis route called pulsed laser ablation in liquids (PLAL)[4]. These nanoparticles can be easily generated on a gram/hour scale[5] and exhibit completely “naked” partially oxidized surfaces which allow their good stability in diluted aqueous media[6]. These ligand-free ultrapure nanoparticles constitute an ideal platform for systematic studies in biology and medicine. One example is the risk assessment of colloidal nanoparticles, which still suffers from a lack of standardization. In this context, ligand-free nanoparticles could serve as ideal reference materials in order to differentiate toxic effects originating from the nanoparticle core and the ligand shell[7]. Extensive studies on the cytotoxic effects of ligand-free laser-generated gold, silver and gold-silver alloy nanoparticles on spermatozoa, oocytes and embryo development verify the suitability of these materials for systematic toxicological trials[8]. Another example is the use of laser-generated nanoparticles in functional bioconjugates. In this case the ligand-free surfaces of laser-generated metal nanoparticles allows their facile and defined functionalization with biomolecules like peptides[9] and oligonucleotides[10] via thiol-based chemistry. PLAL-fabricated bioconjugates were successfully utilized for cellular imaging[11] as well as transfection experiments e.g. with regulatory T-cells[12]. In a recent series it was furthermore demonstrated that multivalent peptide conjugates based on laser-generated gold nanoparticles can be used to prevent pathological protein aggregation and were even more efficient than the free functional ligand due to avidity effects[13].

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Structure-Inherent Targeting for Bioimaging and Nanomedicine

Tuesday, 26th September - 10:45 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 3

. Hak Soo Choi [1]

1. *Harvard Medical School*

Two fundamental and unsolved problems facing bioimaging and nanomedicine are nonspecific uptake of intravenously administered therapeutic agents by normal tissues and organs, and incomplete elimination of unbound targeted agents from the body. To solve these problems, we have developed a new concept of "structure-inherent targeting" using near-infrared (NIR) fluorophores that combines imaging and tissue-specific targeting components into a single molecule. The compact design enables the contrast agent to be easily cleared by the body, which reduces background signal and makes visualizing signal emitted from the targeted tissue easier.
On the basis of this "structure-inherent targeting" concept, we have generated a series of targeted contrast agents with systematically varying net charge, conformational shape, hydrophilicity/lipophilicity, and charge distribution that dictate biodistribution and targeting such as thyroid and parathyroid glands (*Nat Med.* 2015), bone (*Angew Chem.* 2014), and cartilage (*Angew Chem.* 2015). Within the new type of contrast agents, the targeting component is incorporated directly into the chemical structure of the fluorophore to reduce the overall size and encourage clearance; thus our study solves two fundamental problems associated with fluorescence image-guided surgery and lays the foundation for additional targeted contrast agents development with optimal optical and *in vivo* performance.

Nanosafety and industry: needs, expectations and challenges

Tuesday, 26th September - 11:25 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 487

Prof. damjana drobne [1]

1. *University of Ljubljana*

Nanosafety was recognised as a strategically important issue for the successful development of nanotechnology as soon as nanomaterials started to be used in commercial applications.In May 2004, the European Commission (EC) adopted the document which proposes an integrated and responsible strategy for Europe (Towards European Strategy for Nanotechnology; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2009:0607:FIN:EN:PDF). In June 2005, the EC published an action plan for implementation of this strategy. In 2006, OECD launched a programme of work to ensure responsible development of nanotechnologies. In February 2008, the EC adopted the recommendation for a “Code of Conduct for responsible nanosciences and nanotechnologies research”. http://ec.europa.eu/research/science-society/document_library/pdf_06/nanocode-apr09_en.pdf. All these documents stress health, safety and environmental aspects in the development of nanotechnology, with an emphasis on effective dialogue with all stakeholders. While this area has received extensive funding in the EU, the US and other countries, the resulting range of research findings is broad, but it remains unclear how well these current research efforts have answered detailed industrial needs and supported industrial innovation. In living systems, nanomaterials become immediately coated with biological molecules and form a corona. The corona may then influence the outcome of the biological response. In this context, it is important to realise that only certain aspects can make nanomaterials risky. It is now clear that each nanomaterial may pose specific challenges, but in most instances, they can be addressed with existing test methods and assessment approaches. In some cases, it might be necessary to develop new methods of sample preparation and dosimetry for safety testing. Adaptations may be needed also for certain Test Guidelines. But it will not be necessary to develop completely new approaches for nanomaterials. A fundamental cornerstone in the context of risk assessment of nanomaterials, is lack of guidance for industry to consistently determine if a certain material falls within or outside “nano” and a lack of synergies via different international activities. In the future, it will be necessary to help industry to ensure transparency about nanomaterials on the market and thus increase public trust. Trust has been shown as a significant factor when it comes to nanotechnologies.

A Family of Highly Efficient Ru(II) Photosensitizers with Enhanced DNA Intercalation

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 329

Mrs. ANNA PANTELIA [1], ***Mr. Theodoros Mikroulis*** [1], ***Dr. Georgios Rotas*** [1], ***Dr. Eleftherios K. Pefkianakis*** [1], ***Dr. Dimitra K. Toubanaki*** [2], ***Dr. Evdokia Karagouni*** [2], ***Prof. Theodossis A. Theodossiou*** [3], ***Prof. Georgios C. Vougioukalakis*** [1]

1. *Laboratory of Organic Chemistry, Department of Chemistry, National and Kapodistrian University of Athens,* ***2.*** *Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute,* ***3.*** *Institute of Cancer Research, Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital*

Transition metal photosensitizers are very promising for photobiological and photomedicinal applications, as they possess tunable and rationally altered photophysical, photochemical, and photobiological properties.
In our present study, a family of tailor-designed Ru(II) based photosensitizers was synthesized and characterized. This family of photosensitizers, bearing a new bipyridine-type ligand of extended conjugation and an attached anthracene moiety, exhibit, upon light activation, enhanced singlet oxygen generation ability even at excitation wavelengths above 600 nm. The complexes obtained carry either PF_6^- or Cl^- counterions, which determine their hydrophobic or hydrophilic character and, therefore, dictate their solubility in biologically-related media.
All photosensitizers exhibit high efficiency in generating cytotoxic singlet oxygen (ΦΔ -0,8). Additionally, the interaction of these compounds with double-stranded DNA was studied fluoro- and photo-spectroscopically and their bonding affinities were found to be of the order of $3x10^{-7}$ M^{-1}.
All complexes are photocytotoxic to DU145 human prostate cancer cells. The highest light-induced toxicity was conferred by the photosensitizers bearing Cl^- counterions, probably due to the looser ionic “chaperoning” of Cl^-, in comparison to PF_6^-, leading to higher cell internalization[1].

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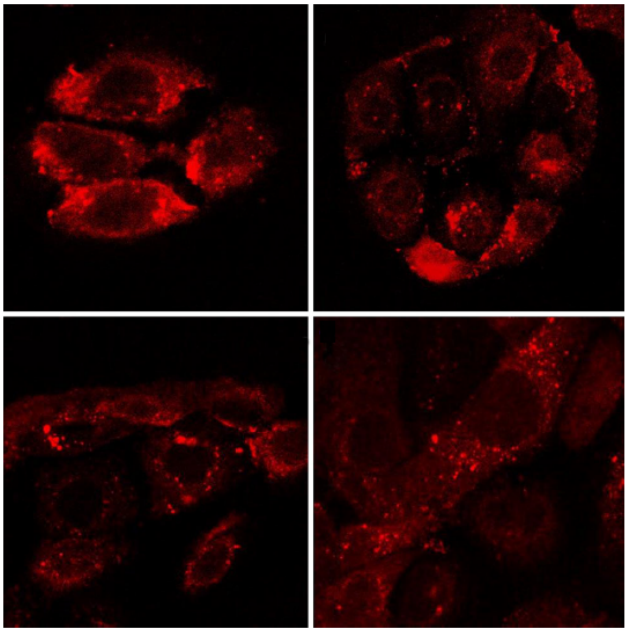

Figure 2.png

Adhesion properties of catechol functionalized poly-r-glutamic acid (r-PGA)

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 324

Mr. Minhee Kim [1], ***Mr. Jung Hyeongseop*** [1], ***Prof. Won Ho Park*** [1]

1. *Chungnam National Unversity*

For a long period of time, sutures and staples were used as a mechanical binder. However, this mechanical binder may cause disadvantages such as surrounding tissue site damage, and low oxygen permeability etc. In addition, the accuracy is decreased when applied to the poor accessibility tissue site. Therefore, the tissue adhesive is challenging to develop a material that can replace the mechanical binder such as sutures and staples. Tissue adhesive is generally a substance that can be adhere the surface of the tissue and/or non-tissue and these materials have been widely studied due to their excellent advantage such as convenience, excellent hemostatic effect, and air leakage prevention effect, and reduced surgery time and bleeding. However, commercially used tissue adhesives have low adhesion strength in wet condition and cytotoxicity. Accordingly, it has become a big issue to develop a new high performance tissue adhesive which can be used in wet condition and various tissue sites. Poly-r-glutamic acid (r-PGA) is anionic, naturally occurring polyamide that is consist of D-and L-glutamic acid units linked by amide bond between a-amino and g-carboxylic acid groups. r-PGA is a microorganism metabolite produced by some *Bacillus* species such as *licheniformis* and *subtilis*. Another naturally occurring source of r-PGA is mucilage of natto (fermented soybean). With excellent biodegradability and biocompatibility, r-PGA and its derivatives have been widely used in the fields of drug delivery, wound dressing, and tissue engineering. Mussels are able to anchor to underwater surface by secreting an adhesive plaque composed of mussel adhesive proteins (MAPs). Especially, the catechol moiety in MAPs tethering to surfaces through formation of insoluble poly(DOPA) derivatives by cross-linking. Mussel-inspired adhesives are expected as biological adhesives for wet condition under the clinical environments. In this study, to improve the adhesive properties in wet condition and biocompatibility of tissue adhesives, functional catechol group was introduced into r-PGA, and the adhesive properties was evaluated.

Cellular uptake and trafficking of nanoparticles

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 195

Dr. Natalia Vtyurina [1], ***Dr. Anna Salvati*** [1]

1. *Groningen Research Institute of Pharmacy*

Nanosized materials have received increased interest as potential drug carriers for targeted therapies in nanomedicine. They have unique capacity of interacting with the cellular machinery by entering the cells using cellular pathways, as opposed to many common small drugs that simply diffuse and partition inside cells according to their solubility. Although research on the use of nanocarriers for drug delivery has increased exponentially in the last decades, in many cases still little is known about the molecular details of the interactions of nanosized materials, such as nanoparticles (NPs), with cells and their trafficking into subcellular structures. Thus, in this study we focus on the mechanisms by which different cells internalize and process NPs. We start from NP physico-chemical characterization and flow cytometry to quantify uptake and understand the basic mechanisms by which cells process nanomaterials. Further, we employ live-cell fluorescence microscopy to track NPs passing through endocytic compartments, and determine their colocalization with labelled proteins or organelles and final distribution within the cells. Overall, by tracking of NPs in live cells to characterize the mechanisms cells use to internalize and process them will greatly enhance the understanding of the mechanism of drug delivery at the molecular level and accelerate the clinical translation of nanomedicine.

Chemical reactivity of the domain of union of the hormonal receptors with the tamoxifen and its different metabolites

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 19

Ms. Linda Landeros [1], ***Dr. Daniel Glossman*** [1], ***Dr. Erasmo Orrantia*** [1], ***Dr. Norma Flores*** [1]

1. *Advanced Materials Research Center (CIMAV)*

According with the World Health Organization, breast cancer it represents 16 % of female cancers worldwide. Cancer can be called **estrogen-receptor-positive**or**progesterone-receptor-positive**, it depends of the signals received by the cancer cells from estrogen or progesterone to promote their growth. The study of the hormone receptor is important to define the right treatment.

One of the treatments used for hormone receptors is tamoxifen (TAM) known as a selective estrogen receptor modulator (SERMs) [1, 2]. It is used for the treatment of hormone receptors that express breast cancer [3]. This drug is metabolized in the liver producing three different metabolites: 4-hydroxy-TAM (4OHTAM), N-dismethyl-TAM (NDTAM) and 4-hydroxy-N-dismethyl-TAM also known as endoxifene (END) [4, 5]. Both the TAM molecule and its three metabolites are considered SERMs, due to their ability to bind to hormone receptors.

The present work reports the characterization and molecular docking of TAM and its metabolites with the macromolecules estrogen receptor (ER) and progesterone receptor (PR) to obtain an active site of the hormone receptors. A charge transfer analysis was also performed using the amino acids of the active site of the hormone receptor. Also, the chemical reactivity of the amino acids of the active sites of each ligand (TAM, 4OHTAM, NDTAM and END) was determined. The results were obtained within the framework of the Density Functional Theory (DFT) with the theory level M06 / 6-31G (d) and the continuous model of solvation CPCM using water with solvent. The use of the molecular docking technique allowed us to find that TAM has a highest more effectiveness in estrogen receptors than in progesterone receptors. The highest charge transfer is in the Leu 346-Thr 347 residue with -0.1000 in the ER and -0.076 in the residue Leu 718-Asn 719 in the PR.

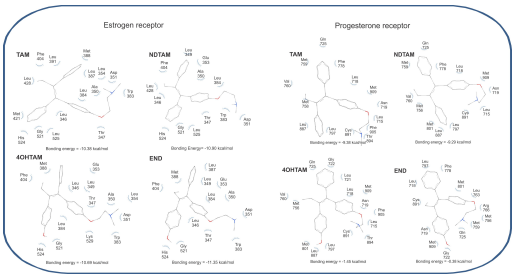

Fig 1.jpg

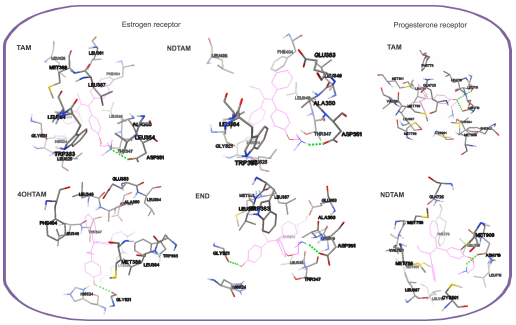

Fig 2.jpg

Chitosan Nanocarrier for Anticancer Drug delivery

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 498

Ms. Srirupa Bhattacharyya [1], ***Prof. Siddhartha Sankar Ghosh*** [1]

1. *IIT guwahati*

With the escalating numbers of world-wide morbidity due to cancer , it has become crucial to explore efficient alternatives to chemo and radiotherapy, which might render poor prognosis, critical side-effects and drug resistance. Major drawbacks of chemotherapeutic drugs include systemic toxicity and poor water solubility. Nanocarriers have emerged as a potential drug delivery vehicle to modulate the pharmacokinetic characteristics of chemotherapeutic agents and to also reduce their systemic toxicity by accumulating at tumour site through Enhanced Permeability and Retention (EPR) effect. Chitosan- a naturally occurring biopolymer, has been reported to develop polymeric nanodrug delivery systems. It is endowed with unique features like biodegradibility, biocompatibility, high stability and low toxicity. Herein, we have synthesised chitosan nanoparticles by ionic gelation method for co-delivery of the anticancer hydrophobic drugs paclitaxel and pyrrolidinedithiocarbamate (PDTC). Characterisations of the drug loaded nanoparticles were carried out using Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) measurements. Antiproliferative effect of the dual drug loaded nanocarrier was studied by cell vialibility assay in both cancerous and normal cell lines.

Conjugation of Antibodies and Nanobodies to Nanoparticles for Targeted Cancer Immunotherapy

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 396

Ms. Valentina Lykhopiy [1]

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Valentina Lykhopiy [1]***, Juan C.Mareque-Rivas*** [1,2,3]

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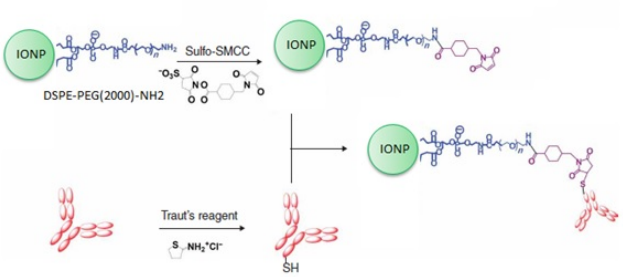
Antibody-conjugated iron oxide nanoparticles (Ab-IONPs) have shown great potential in theranostic applications due to their ability to target specific cells and tissue more accurately and with high specificity. An exciting new application of these constructs could be in cancer immunotherapy –an area currently generating tremendous interest and promising results. In one of our approaches, hybrid NPs are coated with two different antibodies to simultaneously target receptors on the T cells and cancer cells. In this study, we describe the first step in developing one such dual-targeting hybrid NP system, to be constructed with an anti-CD3 antibody and an anti-HER2 nanobody.

We engineered IONPs conjugated with a defined number of antibodies or nanobodies. Anti-CD3 antibody that induces the activation of T cells or a tumour-targeting anti-HER2 nanobody were conjugated to the surface of IONPs using thiol-maleimide chemistry. IONPs coated with DSPE-PEG(2000)-amino were reacted with sulfo-SMCC, while the antibodies/nanobodies were activated with Traut's reagent through lysines. The resulting constructs were purified and characterised by different techniques, including gel filtration, size-exclusion chromatography, DLS and TEM. We assayed *in vitro* the functionality of the IONP-attached antibodies and nanobodies by flow cytometry and ELISA.

Thiol-maleimide "click" reactions provided successful bioconjugation of an anti-CD3 antibody and an anti-HER2 nanobody to the IONP-filled micelles. The number of conjugated antibodies per nanoparticle can be adjusted. In addition, we show that the covalently attached proteins on the surface of the IONPs are functional and recognise their target receptors. The chemistry and constructs offer a highly flexible and customizable system which may be tailored for cancer immunotherapy and other applications.

We gratefully acknowledge funding from the European Union through the PET3D project (H2020-MSCA-ITN-2015, Grant No. 675417). We also thank Catarina Xavier (In vivo Cellular and Molecular Imaging Lab, Vrije Universiteit Brussel) for the anti-HER2 nanobody supply.



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Construction of small antibody-drug complexes to pass nanoporous structure

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 116

Mr. Shuhei Hattori [1], ***Dr. Hikaru Nakazawa*** [1], ***Dr. Teppei Niide*** [1], ***Prof. Mitsuo Umetsu*** [1]

1. *Tohoku University*

The antibody with cytotoxic molecule conjugated (antibody-drug complex, ADC) can critically make damages on the solid tumors which are hardly damaged only by normal antibody functions. For chemical conjugation on proteins, the use of the thiol group in cysteine residue is a simple approach for site-specific conjugation; however, in the case of antibody, the activation of thiol groups lead to instabilities of antibody structures, because the disulfide linkages in each domain of antibody are critical for the structural stability of antibody. Recently, recombinant small antibodies constructed only from antigen-binding domain are studies, because they can penetrate deep into the tumor mass. However, their structures are seriously dependent on the formation of the disulfide linkages. In this study, we applied the amino group in lysine residue for the site-specific chemical conjugation of organic molecule on recombinant small antibody and we tried to identify the prior conjugated lysine residues by mass spectrometry and extract rules characters the small antibody's lysine residues conjugated organic molecule (Fig. 1).

In this study, biotin molecules are applied instead of drug and small antibody is the bivalent antibody fragment (diabody). NHS-bound biotin molecules were mixed with recombinant small antibody fragments at 5 times moles in the solution at several pH values. We analyzed the small antibody-biotin conjugate with the object of chemical reaction rate and bound positions by HABA assay and LC/ESI-MS/MS.

The chemical reaction rate at pH6.5, 7.4 and 8.5 became gradually higher. We identified the modified lysine residues and analyzed the modified rate of each lysine residue from mass spectrometry intensity (Fig.2). The most high modified lysine residue (VL107) was bound at 46.87% where is C-terminal of small antibody. We investigated homo diabody Crystal structures on Protein Data Bank (PDB): consequently we found the same orientation homo diabody 5GRW. The Kabat numbering residues of 5GRW same as modified lysine residues have a highest side chain solvent accessibility and average isotropic displacement in the other residues.

In conclusion, we succeeded that identified the prior modified lysine residues in small antibody and characterized the modified lysine rule from the same orientation homo diabody.

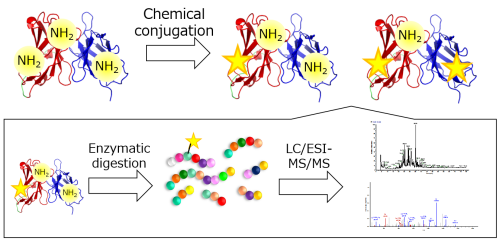

Fig. 1 schematic of the modified lysine residues analysis.png

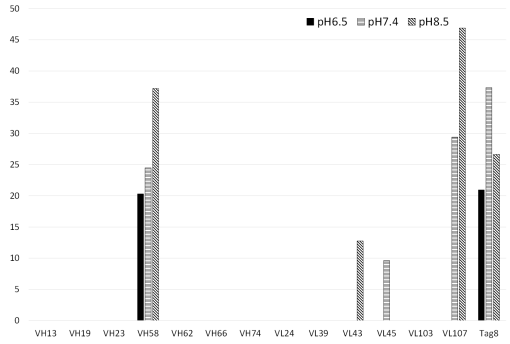

Fig. 2 the rate of the lysine residue conjugated biotin in each ph condition.png

Contrast agents based on switchable near-infrared fluorescent nanoprobes for highly sensitive optical imaging

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 132

Mrs. mi young cho [1], ***Dr. Hye Sun Park*** [1], ***Dr. Kwan Soo Hong*** [1]

1. *Korea Basic Science Institute, Korea Research Institute of Bioscience & Biotechnology*

We have synthesized and characterized photophysical properties of a novel contrast agent based on switchable near-infrared (NIR) fluorescent nanoprobes that enables optical imaging with a higher sensitivity than conventional contrast agents allow. The high sensitivity was a result not only of the intrinsically high signal-to-background ratio that could be obtained in the NIR spectral region, but also from the increased signal intensity that was provided by using activatable nanoprobes. The NIR fluorescent nanoprobes were synthesized by encapsulating indocyanine green (ICG) into pH-responsive polymer nanoparticles. The intensity of the NIR fluorescence signal from the nanoparticles increased about 11-fold in acidic solution, due to the release of ICG molecules from the nanoparticles. The efficacy of fluorescence recovery from the quenched state to the dequenched state could be modulated by controlling the relative concentrations between pH-responsive polymer and ICG. The fluorescence recovery properties of switchable NIR nanoprobes were also evaluated by cellular uptake experiments. Our
experimental results support the use of pH-responsive nanoparticles with incorporated contrast agents as activatable molecular imaging nanoprobes.

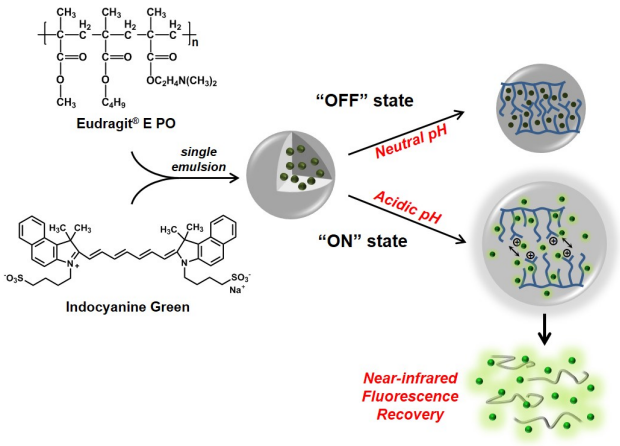

Figure1 iconan2017 mycho.jpg

Dendritic polyglycerols as carriers for the delivery of highly potent drugs

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 92

Ms. Nadine Rades [1], ***Dr. Katharina Achazi*** [1], ***Dr. Carlo Fasting*** [1], ***Dr. Kai Licha*** [1], ***Prof. Rainer Haag*** [1]

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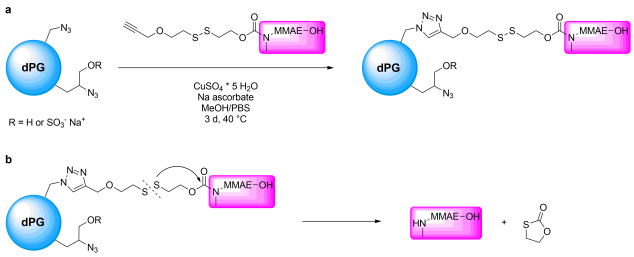
Introduction: In the past decades, polymer therapeutics have emerged as a promising technology in the area of drug design and delivery. In this work, we synthesized and characterized polymer-drug conjugates consisting of dendritic polyglycerol (dPG) as carrier, either sulfated or non-sulfated, and the highly potent drug monomethyl auristatin E (MMAE). Dendritic polyglycerols are based on a spherical polyether polyol scaffold with many hydroxy groups on the surface that results in good water solubility. Furthermore, they are known to be highly biocompatible and therefore studied extensively for biomedical applications. It was demonstrated recently that sulfation leads to polyanionic derivatives which exhibit high binding affinities to positively charged protein motifs resulting in strong anti-inflammatory properties due to interaction with P- and L-selectin in particular. In connection with these findings we currently study the utility for tumor targeting. Monomethyl auristatin E is a highly potent antiproliferative drug, which hitherto is only known from receptor-mediated antibody-drug conjugates, while polymer-MMAE conjugates have not been reported so far. Therefore, we suggest that by coupling MMAE to dendritic polyglycerol using a similar linker strategy, we obtain potent polymer-drug conjugates which can be used even more generally against several tumor cell lines.

Methods: We determined drug-to-polymer ratios by HPLC and examined the conjugates' cell viability by cell proliferation cytotoxicity assay. In addition, after dye labeling, we performed cellular uptake studies using confocal microscopy and flow cytometry.

Results: We successfully synthesized sulfated and non-sulfated dendritic polyglycerol monomethyl auristatin E conjugates using a self-immolative disulfide linker (dPG-SS-MMAE and dPGS-SS-MMAE) which has already been used in a similar manner for reductively cleavable antibody-auristatin conjugates. Accordingly, our polymer-drug conjugates should be cleaved in tumor cells and release the drug.

Discussion: Our results show that the non-sulfated conjugates are less taken up by cells, hence are less toxic in comparison to the sulfated conjugates, which are cytotoxic with nanomolar efficacy.



Drug conjugation a and drug release b .png

Design and characterization of curcumin loaded initial or modified mesoporous silica carriers

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 83

Prof. Denitsa Momekova [1], ***Dr. Ivalina Trendafilova*** [2], ***Prof. Ágnes Szegedi*** [3], ***Prof. Pavletta Shestakova*** [2], ***Prof. Spiro Konstantinov*** [4], ***Prof. Neli Koseva*** [5], ***Prof. Margarita Popova*** [2]

1. *Faculty of Pharmacy, Medical University of Sofia,* ***2.*** *Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, Bulgaria,* ***3.*** *Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, HAS, 1025 Budapest, Hungary,* ***4.*** *Faculty of Pharmacy, Medical University-Sofia,* ***5.*** *Institute of Polymers, BAS, 1113 Sofia, Bulgaria*

Recently mesoporous silica materials attracted interest as drug carriers due to their advantages, such as tunable pore size and morphology, and dual-functional surface (external and internal). The preparation of appropriate drug delivery systems based on these materials can solve some problems associated with low stability and poor bioavailability of bioactive molecules. Curcumin is a natural polyphenol compound with pleiotropic pharmacological activity (anticancer, antiviral,and antioxidant activity) whose exceptionally low aqueous solubility and poor pharmacokinetic properties have hampered its development beyond the preclinical level. A possible approach to overcome these limitations is the encapsulation of curcumin into nano-carriers, incl. mesoporous silica materials.

In the present study we have investigated curcumin loaded initial or NH_2-modified mesoporous silicates (KIT-6 and KIL-2).

Fig. 1. Structural formula of curcumin and TEM image of NH_2-modified mesopurous silica carrier.

The obtained systems were further modified by surface coating with an oppositely charged couple of polyelectrolytes, i.e. carrageenan and chitosan. KIL-2 and KIT-6 were synthesized and modified by post-synthesis method with amino groups. Curcumin was loaded by incipient wetness impregnation method on the parent and NH_2-modified mesoporous supports. The prepared systems were characterized by powder XRD, N_2 physisorption, TEM, TG analysis, UV-Vis and ATR- FT-IR and solid state NMR spectroscopies. The formulated NH_2 – modified mesoporous silica carriers were caracterized with higher curcumin loading capacity as compared to initial materials. Spectroscopic data suggested the formation of curcumin complexes on the NH_2-modified supports. The *in vitro* release profiles of curcumin from the tested silica carriers were investigated as a function of time at physiological pH. Additionaly, a comperative study of citotoxic potential of free and formulated curcumin was further performed on a panel of human tumor cell lines. The results show that curcumin encapsulated into polymer coated, modified silica carriers proved to exert superior antineoplastic potential as compared to free drug. Thus, it can be concluded that modified silica particles are promising carriers for delivery of curcumin.

Acknowledgements: Financial support from National Science Fund of Bulgaria (grant DH 09/18) is greatly acknowledged.

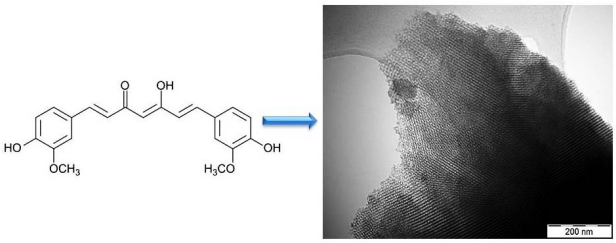


Fig structural formula of curcumin and tem image of nh2 modified mesoporous silica.jpg

Dual functionalized nanoparticles for personalized breast cancer theragnosis

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 383

Ms. María Dolores Cayero [1], ***Dr. Marco Perez*** [2], ***Ms. Rocio Iglesias-Jerez*** [3], ***Mrs. Roque Salazar-Cabrera*** [4], ***Dr. Amancio Carnero-Moya*** [2], ***Ms. Isabel Borrego-Dorado*** [3], ***Dr. Lucía Martín-Banderas*** [1]

1. *Pharmacy and Pharmaceutical Technology, University of Seville,* ***2.*** *Institute of Biomedicine of Seville,* ***3.*** *Servicio de Medicina Nuclear. Hospital Universitario Virgen del Rocío,* ***4.*** *IBA Molecular*

1. INTRODUCTION Dual functionalized nanoparticles have gained a widespread application in the diagnosis and therapy of several diseases. Surface nanoparticles functionalization with: (i) biomolecules as monoclonal antibodies and (ii) radiolabel compounds as ^{99m} Tc offers an exciting new paradigm change for real medicine due to the possibility of combination treatment and diagnostic, known as *theragnosis*.

2. OBJECTIVE. The purpose of our work is to study the suface functionalization process of taxol-loaded PLGA nanoparticles by radiolabelling with ^{99m} Tc and by bioconjugation process with a MAb specific for HER-2+ breast cancer cells.

3. MATERIALS AND METHODS PLGA nanoparticles were prepared using the nanoprecipitation method. Nanoparticles were surface modified with (i) a MAb against HER2+ cancer cells using the carbodiimide strategy. The conjugated amount of MAb was determined by an HPLC-SEC method and (ii) Different amounts of stannous fluoride in aqueous solution were added to a suspension of 5mg of nanoparticles. Then, ≈74MBq (2 mCi) of ^{99m}Tc was added and incubated for 10 min. ^{99m}Tc-NPs suspensions were analyzed by TLC with silica gel strips.

For citotoxicity assay, SK-BR-3 cell line was seeded in 96-well plates and the treatment was assayed after the application of decreasing concentrations in a 1:3 fixed ratio to exponential phase growing cells. Proliferation was determined by crystal violet assay after 96 hours. Cytotoxicity was measured by absorbance at 595 nm using a microplate reader (BIORAD iMark™ Microplate Reader); then, the IC50 was estimated using GraphPad Prism 4 software.

4. RESULTS AND DISCUSSION. We obtained PLGA nanoparticles 190 nm in diameter with a zeta potential around -20 mV. After MAb-conjugation particles diameter increases slightly up to 250-300 nm. ZP values were nearly neutrality which indicates the presence of MAb on particle surface. Antibody conjugation efficiency was around 90%. In addition, using 1 µg of SnF_2 as a reducing agent, PLGA nanoparticles were labelled with ^{99m}Tc with a yield ≥ 90%. Cytotoxicity assay, demonstrated a superior antitumoral activity of encapsulated vs. free taxol with improved IC50 values against SK-BR-3 cells.

5.CONCLUSIONS Results obtained, indicated that nanoparticles are suitable as future platform for in vivo therganosis of breast cancer.

Functionalization of second harmonic generation nanoparticles for theranostic applications

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 85

Mr. Jérémy Vuilleumier [1], ***Mr. Raphaël de Matos*** [1], ***Dr. Solène Passemard*** [1], ***Dr. Luigi Bonacina*** [2], ***Prof. Sandrine Gerber-Lemaire*** [1]

1. *EPFL Lausanne,* ***2.*** *Université de Genève*

Nowadays, cancer is the leading cause of death in developed countries. The emergence of new multimodal nanodevices for *in vivo* imaging offers the perspective of cancer detection at a very early stage.[1] The recent progress in nanotechnologies has generated high expectation that nanomaterials could provide unprecedented contrast agents in imaging set-ups and multifunctional platforms for drug delivery.[2] In this context, harmonic nanoparticles (HNPs), which are composed by non-centrosymmetric materials, can be easily imaged by their second harmonic generation signal in multiphoton imaging platforms.[3]

We recently disclosed efficient protocols for the biocompatible coating [4] and post-functionalization of bismuth ferrite ($BiFeO_3$, BFO) and $LiNbO_3$ HNPs as well as their favorable properties for targeted imaging of human cancer cells and tissue.[5] We report therein the conjugation of BFO HNPs to caged molecular cargos through a photocleavable linker based on coumarinyl and *o*-nitrobenzyl derivatives. Excitation of these functionalized HNPs in the visible or near IR region generated second harmonic UV emission [6] and subsequent selective release of the conjugated drug models.

These multifunctional HNPs offer the possibility for decoupled imaging modality and photo-activation process by tuning the wavelength of the excitation beam.

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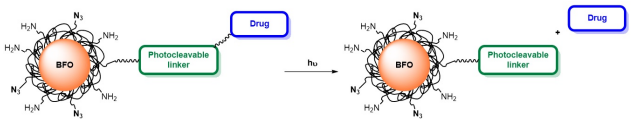


Drug release from hnps via ir light irradiation.jpg

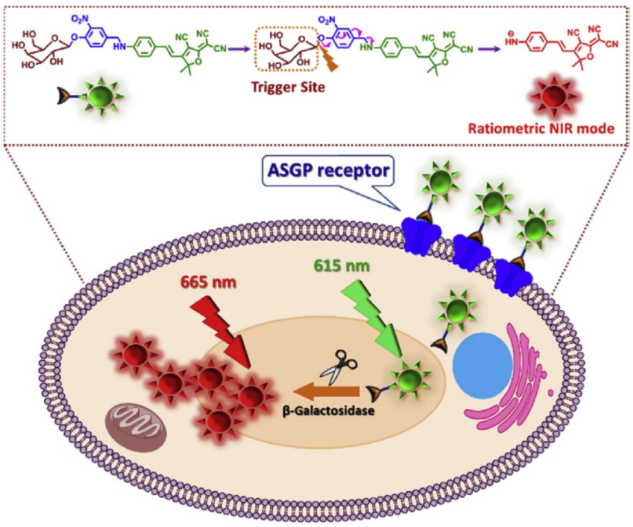
Hepatocellular carcinoma targeted near-Infrared in vivo imaging by β-galactosidase stimulated fluorescent probe

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 110

Ms. Hyun Min Kim [1], ***Mr. Hyunseung Lee*** [1], ***Dr. Kwan Soo Hong*** [1]

1. *Korea Basic Science Institute, Korea Research Institute of Bioscience & Biotechnology*

Development of targeted, selective, and noninvasive fluorescent probes for *in vivo* visualization of tumor-associated over-expressed enzymes are highly anticipated for cancer diagnosis and therapy. Herein, we developed a noninvasive fluorescent probe (DCDHF-βgal) for the sensitive detection, and in vivo visualization of β-galactosidase in hepatocyte HepG2 cells and its xenograft model. As a model system for *in vivo*targeted imaging, DCDHF-βgal possessing galactose unit selectively target hepatocyte and monitor the β-galactosidase activity with deep tissue penetration, and low background interference. DCDHF-βgal was activated by intracellular β-galactosidases as the driving force for the release of NIR fluorophore, thereby exhibiting ratiometric optical response. Initial fluorescence emission measured at 615 nm was changed to fluorescence at 665 nm upon activation of DCDHF-βgal with β-galactosidase. Ratiometric fluorescence detection of β-galactosidase was also observed in hepatocellular carcinoma cells and tumor xenograft. The noninvasive *in vivo* optical imaging facilitated by targeted and enzymeactivated imaging agent would be useful in various biomedical and diagnostic applications.



Khm fig 1.jpg

Influence of structural features of porphyrins on their ability to effect on DNA

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 33

Prof. Vigen Barkhudaryan [1], ***Mrs. Gayane Ananyan*** [1]

1. *Yerevan State University*

In order to clarify the effect of influence of porphyrin molecules configuration and chemical structure of their side radicals on hydrodynamic and spectral behaviors of DNA water soluble cationic *meso*-tetra-(3N-, 4N-hidroxyethylpyridyl [1-4], 3N-, 4N-alylpyridyl [5]) porphyrins (H_2THOEPyP4, H_2THOEPyP3, H_2TALPyP4, H_2TALPyP3) and their metallocomplexes using viscometry and spectroscopy were investigated. The planar porphyrins H_2THOEPyP4, H_2THOEPyP3,[1-4] H_2TALPyP4, H_2TALPyP3[5] and their metallocomplexes with Ni- and Cu- leads to an increase of DNA solutions viscosity at relatively small concentrations, and then decrease of stable values. Such behavior corresponds to intercalation of these porphyrins in DNA structure, which results a decrease of helical twist and lengthening of the DNA molecules. When all intercalation sites are occupied, external binding occurs also. In case of porphyrins with axial ligands (Zn, Co metallocomplexes) external binding mode occurs only. Change in position of peripheral radicals on pyridylic ring has absolutely no effect on interaction of outside binding porphyrins with DNA. In presence of planar porphyrins, the structural changes of DNA are similar to previous one, but there are noticeable behavioral differences. Comparison of different locations of peripheral radicals on pyridylic rings leads to the conclusion that porphyrins with side radicals at 3N-position and its metallocomplexes are favorably located relative to the DNA helix axis than at 4N-position. It was shown that among the studied porphyrins, H_2TAlPyP3 [5] and its metallocomplexes interact with DNA most intensively. The data obtained allows us to conclude that viscometry method provides the most definitive means of inferring the binding mode of porphyrins to DNA in solution even in the absence of other structural data.

KEYWORDS: Porphyrin, ct-DNA,Viscometry, Intercalation, Outside binding

Liposomal Texaphyrin-Doxorubicin Delivery System for Early Diagnosis and Treatment of Metastatic Liver Cancer

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 108

Mr. Hyunseung Lee [1], ***Ms. Hyun Min Kim*** [1], ***Dr. Kwan Soo Hong*** [1]

1. *Korea Basic Science Institute, Korea Research Institute of Bioscience & Biotechnology*

Reported here is a new theranostic agent, 1, which consists of a Gd3+-texaphyrin core conjugated to a doxorubicin prodrug via a disulfide bond. Conjugate 1 was designed to undergo cleavage in the presence of glutathione (GSH), a species typically upregulated in cancer cells. As prepared, conjugate 1 displays no appreciable fluorescence. However, when exposed to excess GSH an increase in the fluorescence intensity at 592 nm is observed that is ascribed to release of free doxorubicin. To improve the solubility and enhance the tumor targeting of 1, it was loaded into folate-receptor-targeted liposomes to produce FL-1 (for folate liposome loaded with 1). As inferred from both fluorescence turn on studies and independent HPLC analyses, FL-1 was found to undergo selective uptake and cleavage to release free Dox in the KB and CT26 cell lines, which express folate receptors on the cell surface, relative to the HepG2 and NIH3T3 cell lines, which show low expression of those receptors. FL-1 was found to produce a greater antiproliferative effect in the case of the KB and CT26 cell lines as compared to that in the HepG2 and NIH3T3 cell lines. FL-1 was also found to provide enhanced magnetic resonance imaging in vivo under conditions of T1 contrast in the early stage of metastatic cancer progression. Finally, time-dependent tumor regrowth studies involving both subcutaneous and metastatic liver cancer mouse models revealed that FL-1 is capable of reducing the tumor burden in vivo.

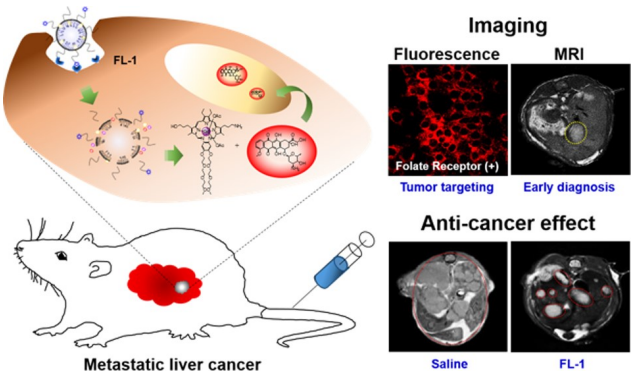


Fig.jpg

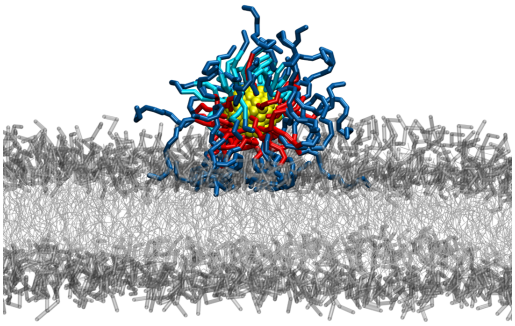
Membranes & Proteins Vs Engineered Nanoparticles: A Computational and Experimental Investigation at the Bio-Nanointerface

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 201

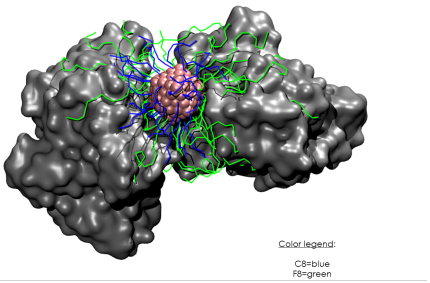
Dr. Silvia Boccardo [1], ***Dr. Filomena Guida*** [1], ***Dr. Maria Sologan*** [1], ***Dr. Domenico Marson*** [1], ***Dr. Paolo Pengo*** [1], ***Prof. Sabrina Pricl*** [1], ***Prof. Alessandro Tossi*** [1], ***Prof. Lucia Pasquato*** [1], ***Prof. Sabrina Pacor*** [1], ***Dr. Paola Posocco*** [1]

1. *University of Trieste*

Self-assembled monolayer (SAM)-protected inorganic nanoparticles (NPs) are emerging as a promising tool for diagnostic devices and drug delivery systems. Tuning their surfaces properties with mixture of immiscible ligands that can self-assemble in specific nanoscale patterned (e.g., striped, patched and Janus) monolayers allows to tailor the functionality of such engineered NPs. A key aspect for the delivery of NPs into cells is their interaction with both biological membranes and proteins. Employing computational techniques, we investigate the effects of the monolayer composition and morphology on the adhesion to lipid bilayer, the first important step for NP internalization. Evidences emerging from the theoretical predictions are discussed in light of experimental data obtained by Surface Plasmon resonance (SPR) on model membranes and by cytometry assays (in vitro) human cell lines. When nanomaterials are exposed to biological fluids, they immediately interact with proteins and other biomolecules to form a dynamic “corona”. Thus, in addition to membrane/cell interaction evaluation, in this contribution, we explore by molecular dynamics and free energy calculations how these NPs interact with common proteins and how the SAM morphology affects the properties of this bio-nanointerface.



Np binds membrane.png



Np binds proteins.png

Mesoporous silica nanoparticles in biomedical applications

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 435

Dr. Xinyue Huang [1], ***Dr. Helen Townley*** [1]

1. *University of Oxford*

Mesoporous silica nanoparticles (MSNPs) are promising biomaterials for advanced nano-carriers applicable to a large number of biomedical applications. Cargo can include dyes, peptides, nucleic acids and other therapeutics. Compared to most commercially available options, MSNPs have very large surface areas, functionalisible surfaces, tuneable size and porosity, various architectures, low cytotoxicity and low cost.

In our study, we produced a number of MSNPs with different architecture. Figure 1 shows a selection of MSNPs synthesised in the laboratory. In order to demonstrate the physical and biological properties of the MSNPs in depth, a number of techniques have been used to characterise the particles to give parameters such as size, surface potential, porosity, surface area and cytotoxicity of the MSNPs.

After extensive characterisation, we demonstrated that MSNPs can be taken up by different cancer cells, and therefore can be used as intracellular anti-cancer agent carriers. The cellular uptake efficacy was largely dependent on the cell type and particle physical properties. MSNPs can be customised with controlled size and modified surface to provide an ideal candidate designed for maximum cellular uptake of a specific cell type.

Furthermore, we determined that MSNPs were able to load many small cargo molecules *via* adsorption and chemical conjugation, including molecules with different electric potential and/or different hydrophobicity. We found that the drug loading efficiency is dependent upon the porosity and surface area of the MSNP and the electric potential difference between the cargo and the surface of MSNPs. In *in vitro* study, we found that MSNPs can deliver drugs which have been shown to be difficult to deliver in conventional ways to cancer cells efficiently.

We also showed that MSNPs can be used as *in vitro* siRNA carrier. Several MSNPs candidates were shown to be more efficient than commercially available vectors and to lead to much higher transfection and knock-down efficiency.

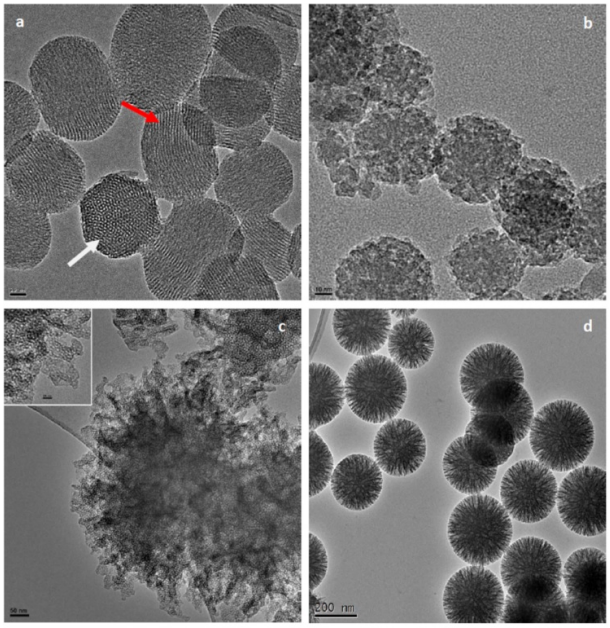


Figure 1. Typical TEM images of a selection of different MSNPs as drug delivery carrier candidates: a) HMSNP (scale bar: 20 nm, arrows point out two views of HMSNP); b) BMSNP (scale bar: 10 nm); c) CMSNP (scale bar: 50 nm; higher magnification inset [scale bar: 10 nm] details the pores on the 'petal' structure) and d) WMSNP (scale bar: 200 nm)

Figure 1.jpg

Microbubble mediated delivery of hydrophobic drugs using lipid oil nanodroplets

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 234

Ms. Antonia Charalambous [1], ***Dr. Victoria Mico*** [2], ***Dr. Sally Peyman*** [2], ***Prof. Stephen Evans*** [2], ***Prof. Alexander Markham*** [1], ***Dr. P. Louise Coletta*** [1]

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Introduction:
Drug delivery to tumours is fundamental for effective treatment, however systemic delivery leads to a number of off-site toxicities. The pharmaceutical industry produces a substantial number of compounds that are hydrophobic and therefore intravenous delivery is difficult. Some of these compounds show excellent potential *in vitro*, however their hydrophobicity compromises their clinical use. There is therefore an unmet clinical need for the production of vehicles that will aid the delivery of hydrophobic drugs. We have produced Lipid Oil Nanodroplets (LONDs)[1], for encapsulating hydrophobic drugs. Through on-chip assembly LONDs were attached to lipid shelled, gas filled microbubbles (MBs). The MB-LOND construct exhibits the ultrasound imaging properties characteristic of MBs and the hydrophobic drug encapsulation potential of an oil-based nanodroplet.
Methods:
LONDs were produced in a two-step high pressure homogenisation process, in the size range of 100-300nm. The vascular disrupting agent Combretastatin A4 (CA4) was encapsulated in tripropionin LONDs. *In vitro*immunofluorescence for cell morphology, flow cytometry for cell cycle analysis and mass spectrometry to detect drug in tumour xenografts were all used to evaluate LONDs. VEGFR2 targeted MB-LOND constructs were injected intravenously, a low intensity ultrasound was used to burst the MBs and release LONDs near the tumour region. Following treatment the perfusion marker Hoechst 33342 was injected to assess tumour vascularisation.
Results & Discussion:
In vitro CA4 released and/or uptaken in endothelial cells leads to extensive cytoskeleton rearrangement, cell cycle arrest in mitosis and cell death. CA4 was detected by mass-spectrometry in human colorectal cancer xenografts following treatment with LONDs. Using Hoechst 33342 it was observed that treated tumours had significantly less perfused vessels compared to untreated. Our results show that LONDs enable hydrophobic drug encapsulation and effective *in vitro*and *in vivo* delivery, paving the way for other hydrophobic compounds. The further development and use of MB-LOND constructs has the potential to improve and enhance delivery, as well as the therapeutic index of previously undeliverable compounds.
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Modification of Fe3O4-Au dumbbell nanoparticles with fluorescent dyes and drugs for new opportunities in biomedical application.

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 120

Ms. Alexandra Prelovskaya [1], ***Ms. Mariia Efremova*** [1], ***Ms. Anastasia Garanina*** [1], ***Ms. Nurgustaana Neustroeva*** [1], ***Mrs. Tatiana Abakumova*** [2], ***Mr. Pavel Melnikov*** [3], ***Mr. Maxim Abakumov*** [2], ***Prof. Alexander Savchenko*** [1], ***Prof. Alexander Majouga*** [4]

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Recent developments in nanotechnology and molecular biology have helped to translate multifunctional magnetic nanoparticles into medical application. From a number of diseases, cancer is one of the major cause of death due to difficulty in accurate diagnosis and treatment of this. Early detection and treatment of cancer are critical factors for a favorable prognosis.

Thus, new possibilities in nanotechnology area are related with so called theranostics, which are defined as a material for the combination of therapy and imaging within a single platform.

Hybrid materials based on nanoparticles with different surface nature as well as different chemical properties represent particularly especial interest. Such materials can be controlled modified in various ways simultaneously, in particular with drugs and targeted molecules [1]. Surface modification of nanoparticles with fluorescence dyes opens horizons for visualization capabilities, also allows us to trace the behavior of particles in a biological environment, which is essential for their future biomedical applications [2].

In this work we developed synthetic procedure for magnetite-gold dumbbell nanoparticles modified with different copolymers containing: fluorescein and sulfo-Cy5 and investigated of the similar system with a drug (doxorubicin) instead of the dye. Next, in vitro biodistribution of nanoparticles was studied on LNCaP cell line (PSMA-positive prostate cancer cell line). More detailed information for the synthesis, characterization and biological testing will be discussed in report.

The authors gratefully acknowledge the support of the Ministry of Education and Science of the Russian Federation (grant № 14.578.21.0201 (RFMEFI57816X0201)).

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P-selectin targeted cationic liposomes efficiently deliver siRNA to endothelial cells

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 66

Ms. Cristina Ana Constantinescu [1], ***Dr. Daniela Rebleanu*** [1], ***Ms. Geanina Voicu*** [1], ***Mrs. Mariana Deleanu*** [2], ***Dr. Monica Tucureanu*** [1], ***Dr. Elena Butoi*** [1], ***Dr. Ileana Manduteanu*** [1], ***Dr. Virginie Escriou*** [3], ***Dr. Manuela Calin*** [1]

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Introduction. P-selectin is specifically expressed by endothelial cells (EC) in inflammatory pathologies (such as atherosclerosis) and therefore a potential target for nanotherapy. The aim of this study was to obtain P-selectin targeted cationic liposomes to function as efficient vectors for siRNA delivery to EC.

Methods. A peptide with high affinity for P-selectin was coupled to the surface of PEGylated liposomes containing the cationic lipid 2-{3-[Bis-(3-amino-propyl)-amino]-propylamino}-N-ditetradecyl carbamoyl methyl-acetamide (DMAPAP) combined with 1,2-Dioleoyl-*sn*-glycero- 3-phosphoethanolamine (DOPE). The lipoplexes obtained after complexation of cationic liposomes (P-sel_Lipo) with siRNA were characterized for size (DLS) and the amount of peptide coupled to the surface (HPLC). The cytotoxicity studies were performed by MTT assay after exposing bEnd.3 endothelial cells to various charge ratio (+/-) of lipoplexes P-sel_Lipo/siRNA for 48 hours. The binding and internalization of fluorescently labeled P-sel_Lipo/siRNA lipoplexes were determined in static and dynamic conditions, using bEnd.3 cells (that constitutively express P-selectin) by flow cytometry and fluorescence microscopy. As control, scrambled peptide coupled liposomes complexed with siRNA (Scram_Lipo/siRNA) was used.

Results and Discussions. 1) the size of lipoplexes was around 300 nm; 2) the cellular viability was not significantly affected for charge ratios (+/-) up to 4 and a siRNA concentration of 20 nM; 3) under dynamic conditions, the binding of P-sel_Lipo/siRNA to EC was higher in comparison to Scram_Lipo/siRNA, suggesting a specific adhesion; 4) at 10 minutes of static incubation, the internalization of P-sel_Lipo/siRNA was significantly higher as compared to Scram_Lipo/siRNA; 5) P-selectin targeted lipoplexes deliver intracellularly siRNA with higher efficiency than commercial transfection vectors.

Conclusion. P-selectin-targeted lipoplexes bind specifically and efficiently deliver siRNA to P-selectin expressing endothelial cells.

Acknowledgements. The work was supported by UEFISCDI: PN-II-RU-TE-2014-4-1837 project.

Polymeric nanoparticles assisted delivery of nucleic acid for targeted gene therapy

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 239

Ms. GEETA ARYA [1], ***Dr. Surendra Nimesh*** [1]

1. *CENTRAL UNIVERSITY OF RAJASTHAN*

Gene therapy refers to the introduction of a therapeutic gene or nucleic acid to modify and regulate the expression of a target gene. This is emerging as a promising and alternative strategy against drug treatment, surgical approach and enzyme/protein therapy for the treatment of various deadly diseases. According to a fact sheet of World Health Organization (WHO), cardiovascular diseases are causing the highest percent of death rate all over the world. Despite of implementation of many diagnostic techniques and remedies, the success rate against diseases is very low. Gene therapy is come up as a better way to surmount this problem. Further, the therapeutic potential of a gene relies upon its safe and targeted site delivery. For this, several delivery systems have been explored, however limited success have achieved. In current study, we have designed chitosan nanoparticles, a bio-compatible and biodegradable polymer based delivery vectors for siRNA delivery to target cardiovascular diseases. Chitosan would be modified with suitable ligands for targeted delivery of siRNA to mammalian cells, HepG2. The modification would be evaluated by Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR). Nanoparticles thus prepared would be characterized for size, shape, surface morphology by DLS, SEM and TEM, respectively. Entrapment efficiency and in vitro gene expression was evaluated by fluorescence microscopy. Further, quantitative analysis of gene expression would be analyzed and this study could be used for the development of an efficient delivery system.

Polymeric nanoparticles conjugated with engineered albumin for enhanced FcRn-mediated delivery across mucosal barriers

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 374

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To maintain patient compliance, oral administration is the preferred drug delivery route. Yet, many drugs, including biopharmaceuticals do not endure the harsh environment of the gastrointestinal tract and cross epithelial barriers inefficiently. Functionalization of nanoparticles (NPs) with ligands that can bind specifically to surface receptors may be one strategy to overcome these obstacles and to improve the delivery of pharmaceuticals through biological barriers. The neonatal Fc receptor (FcRn) is a cellular receptor that binds and rescues albumin and IgG antibodies from intracellular degradation. In addition, FcRn mediates transport of its ligands across polarized epithelial cells. We aimed to explore whether polymeric nanoparticles (NPs) conjugated with engineered human albumin for enhanced FcRn binding could be an attractive strategy for delivery of encapsulated drugs across mucosal barriers. To address this, we have designed polymeric NPs using double emulsion/evaporation technology where engineered human albumin was site-specifically conjugated to the polymers. This was done by utilizing a free cysteine residue within albumin domain I, which is located distally from the core interaction site for FcRn. By the use of maleimide chemistry, engineered albumin was conjugated to the surface of the polymer, and the designed NPs were shown to bind human FcRn with expected binding hierarchy. Next, we will explore the use of the NPs using an *in vitro* transcytosis assay as well as *state-of-the-art* human FcRn transgenic mouse model. The FcRn-targeted approach may pave the way for more efficient delivery of NP-encapsulated drugs.

Size and shape-dependent effects of the iron oxide nanoparticles in biomedical applications: T2-relaxation, hyperthermia, magnetic properties and cytotoxicity

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 142

Dr. Timur Nizamov [1], ***Ms. Irina Kharisova*** [1], ***Mr. Maxim Abakumov*** [2], ***Mr. Ivan Grebennikov*** [1], ***Mr. Thanh Luan Nguyen*** [1], ***Ms. Regina Salakhova*** [3], ***Ms. Anastasia Garanina*** [4], ***Prof. Alexander Majouga*** [3], ***Prof. Alexander Savchenko*** [4]

1. *NUST MISIS,* ***2.*** *Pirogov Russian National Research Medical University,* ***3.*** *Lomonosov Moscow State University,* ***4.*** *National University of Science and Technology MISiS*

Magnetic nanoparticles (MNP) attract particular interest for usage in different biomedical branchs, such as targeted drug delivery, magnetic resonance imaging and magnetic hyperthermia. Widespread use of magnetite nanoparticles in biomedicine became possible due to their magnetic properties, low toxicity and biodegradability. It is also known that the shape and size of the MNP can affect their magnetic and structural properties. Thus the aim of the study is to reveal the effect of particles shape on magnetic and contrast properties and cytotoxicity towards the PC3 and LNCaP prostate cancer cell lines.

The following forms of particles were obtained by method of high-temperature thermal decomposition: spherical, cubic, octahedral, hexagonal and tetragonal prisms, plates. X-ray phase analysis confirmed the presence of the inverse spinel phase in all samples.

The cytotoxicity of nanoparticles has been tested on human PC3 and LNCaP cell lines. Cell survival with the addition of MNP suspensions in concentration varying from 2.5 to 50 μg/ml was 80-100%, except octahedral and cubic particles, which demonstrated slightly higher cytotoxicity on LNCaP culture.

Temperature change measurements of MNP suspensions from the exposition time under an external magnetic field (H = 95 Oe, f = 100 kHz) resulted in the following values of the temperature: nanoplates - 99° C, hexagonal prisms - 60° C, spheres - 41° C, octahedrons - 40° C.

It was shown that the hexagonal prisms possess the greatest coercive force and saturation magnetization. Nanoplates of the smallest size had low toxicity and the highest T2-contrast, which makes them promising MNP for targeted drug delivery and MRI. They also showed higher heating temperature under magnetic field and can find application in hyperthermia.

The authors gratefully acknowledge the support of the Ministry of Education and Science of the Russian Federation (grant № 14.578.21.0201 (RFMEFI57816X0201)).

Sample (shape / characteristic)	Size, nm	Crystallite size, nm	H_c, kA/m (E)	$4\pi I_s$, $A \cdot m^2/kg$	T_2-relaxivity, $mM^{-1} \cdot s^{-1}$
Spheres	20	16	2,3 (29)	34,2	240,6
Cubes	18	21	4,7 (59)	44,7	262
Hexagonal prisms	55	47	8,3 (104)	88,3	15,1
Octahedrons	19	17	2,2 (28)	73,8	236,5
Tetragonal prisms	19	24	3,6 (45)	87,4	241,3
Nanoplates	12x12x2,5	5	4,8 (60)	52,1	280,3

Table.jpg

Synthesis and characterization of various glycopeptide polymers for targeted/controlled drug delivery in cancertheraphy

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 352

Mrs. Pinar Sinem Omurtag [1], ***Dr. Aydan Dag*** [2]

1. *İstanbul Medipol University,* ***2.*** *Bezmiâlem Vakif University*

Polymers are widely investigated as carrier system for drugs in order to maintain the concentration of the drug in the body within a therapeutic window while avoiding frequent drug administrations.

Glycopolymers, synthetic polymers carrying pendant sugar units, have been shown to target carbohydrate receptors on cancer cells. They have attracted extensive attention in the fields of polymer chemistry, material science and biomedicine due to their properties, which include biocompatibility and bioactivity. Particles consisting of self-assembled, amphiphilic, galactosylated block glycopolymers revealed an enhanced uptake by human hepatocellular carcinoma cell line (HepG2), as compared to the water-soluble homopolymers.

In this work, since platinum drugs are highly efficient in the treatment of cancer despite of their adverse side effects, a range of triblock copolymers have been studied with three different sugars able to self-assemble into nanoparticles when conjugated with cis-platin (cis-Pt).

Trimethylsilyl-protected propargyl methacrylate (TMSpMA) monomer was polymerized in the presence of a furan protected maleimide functionalized 4-cyanopentanoic acid dithiobenzoate (CPADB-MI). P(TMSpMA) Macro-RAFT agent was then chain extented with the monomer of 1,1-Di-tert-butyl 3-(2-(Methacryloyloxy)ethyl)-butane-1,1,3-tricarboxylate (MAETC). Following this polymerization, γ-Benzyl-L-glutamate N-carboxyanhydride was polymerizaized by using the ring-opening polymerization to obtain poly(β-benzyl L-glutamate) (PBLG) and PBLG was coupled with P(TMSpMA-*b*-MAETC) via click reaction to give corresponding triblok glycopeptide polymer. After deprotection of trimethylsilyl units, a range of glucosylated triblock copolymer were synthesized by reacting a mixture of 2-azideoethyl β-D-glucopyranoside ($GlcEtN_3$), 2-azideoethyl β-D-mannopyranoside ($ManEtN_3$) and 2-azidoethyl β-L-fucopyranoside ($FucEtN_3$) with P(PMA-b-MAETC-b-PLBG) using copper-catalyzed azide−alkyne cycloaddition (CuAAC). The resulting polymer was used as a macromolecular ligand for the conjugation with platinum drug. Thermogravimetric analysis showed full conjugation. The resulting drug loaded nanoparticles had hydrodynamic diameters of around 100 nm. For chacterization of organic synthesis and polymerizations ^{1}H-NMR, ^{13}C-NMR, FT-IR and GPC analysis are succesfully done.

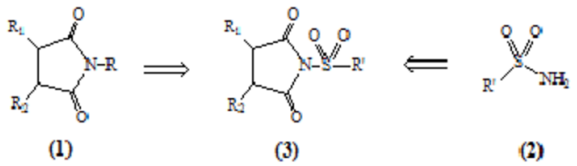
Synthesis of new cyclic imides containing sulfonamide moiety for possible application as carbonic anhydrase inhibitors

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 314

Dr. BOUGHELOUM CHAFIKA [1], ***Prof. Messalhi Abdelrani*** [1]

1. *Badji Mokhtar Annaba University*

Cyclic imides **1** (fig.1) represent an important class of bioactive molecules that show a wide range of pharmacological activities [1,3] such as anti-inflammatory, antibacterial, antidepressive, antiviral, antitumor.
On the other hand sulfonamide **2** (fig.1), is considered as a pharmacophore, which is present in a number of biologically active molecules [4,5]. Because of their common properties it was decided to prepare compound **3** having structural features of both 1 and 2 with a convenient, clean and environmentally friendly procedure by using a catalytic amount of heteropolyacids including $H_6P_2W_{18}O_{62}$, $H_{6P}2W_{12}Mo_6O_{62}$, $H_3PW_{12}O_{40}$.



(1) (3) (2)

Picture.png

Synthesis, characterisation and cytotoxicity profiling of chitosan-coated MgFe2O4 and Mg0.5Co0.5Fe2O4 nanoferrites for biotherapeutic application.

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 461

Ms. Seipati Mokhosi [1], ***Ms. Wendy Mdlalose*** [1], ***Ms. Sanele Mngadi*** [1], ***Prof. Moganavelli Singh*** [2], ***Dr. Thomas Moyo*** [2]

1. *University of KwaZulu-Natal,* ***2.*** *University of Kwa*

In recent years, there has been increased use of magnetic nanoparticles (NPs) geared towards biotherapeutic application including MRI, hyperthermia treatments, magneto-targeted therapy and drug delivery. Nanoferrites comprising CoFe2O4 core, have been shown to present unique properties such as their facile synthesis, large surface-to-volume ratio and high magnetisation saturation. With precise engineering by conjugation with various molecules, specificity, optical detectability, longer circulation times and therapeutic delivery are made possible. Targeted therapy using an external magnetic field presents for an attractive feature in biotherapeutic applications. Chitosan is a natural polymer, which is non-toxic, biodegradable and biocompatible. It has been extensively explored for use as a polymer of choice, and recently in combination with magnetic NPs.

In this study, the glycol-thermal method was employed to synthesise MgFe2O4 and Mg0.5Co0.5Fe2O4 NPs. Comparative analysis was conducted, following coating of the synthesised NPs with chitosan. Analytical characterisations were performed using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), High Resolution TEM (HR-TEM) and Scanning Electron Microscopy (SEM). Magnetisation properties were analysed using the Vibrating Sample Magnetometer (VSM). Nanoparticle Tracking Analysis (NTA) was employed to measure stability of the NPs.

Synthesis of NPs was confirmed to be single phase formation. XRD revealed spinel structure of MgFe2O4 and Mg0.5Co0.5Fe2O4 with the average crystallite size diameter of 13 nm. The chitosan layer on the surface of NPs was confirmed by FTIR with additional peaks characteristic of chitosan and ferrites. TEM, SEM and HR-TEM results for both coated and uncoated NPs showed spherical morphology and sizes not more than 20nm in diameter. VSM indicated superparamagnetic characteristics of the nanoparticles with magnetisation saturation of up to 70 emu/g. These were marginally reduced in coated NPs, also attributed to chitosan. NTA results revealed relatively stable NPs where agglomeration was reduced in coated NPs. The naked and coated NPs were better tolerated in some cell lines at varying concentrations. In conclusion, all NPs hold much potential as nanocarriers of choice for targeted gene and drug delivery. By enhancing biodegradability and biocompatility of these NPs through chitosan-coating, this allows for a more attractive and feasible bio-application for these nanoferrites.

Ultrasound cavitation of microbubbles for an in vitro study of drug delivery

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 214

Mrs. giulia silvani [1], ***Dr. Giorgia Sinibaldi*** [1], ***Mr. Davide Caprini*** [1], ***Dr. Giovanna Peruzzi*** [2], ***Prof. Luca Marino*** [1], ***Prof. Mohamed Kiani*** [3], ***Prof. Carlo Massimo Casciola*** [1]

1. *Department of Mechanical and Aerospace Engineering, La Sapienza, Rome,* ***2.*** *Istituto Italiano di Tecnologia; Center for Life Nanoscience@Sapienza,* ***3.*** *Temple University, Department of Mechanical Engineering, Philadelphia, United States*

In traditional drug delivery system, the uptake of molecules suffers ofpoor efficiency due to the low permeability of the endothelial barrier. Methods combining focused ultrasound and micro bubbles (MBs) offer the unique capability of non-invasively, locally and transiently open the endothelial barrier (*Hernot S. and Klibanov A.L. 2008*). MBs injected into the bloodstream undergo volume oscillations under ultrasound irradiation and possibly collapse. The resulting mechanical action induces a transient increase of barrier permeability due to the increase of inter-cellular spaces facilitating drug extravasation into the site of interest. The goal of this project is to induce cavitation of MBs (SonoVue®) in a microfluidic device (Fig.1) specially designed (*DeosarkarP.S. et All. 2015*) to investigate the effects of MBs behaviour on the endothelial barrier. The device is previously cultured with HUVECs cells with a reliable and reproducible protocol (Fig.2). Quantification of membrane permeability is evaluated through measurements of fluorescent dye diffusion towards a pores membrane with a confocal microscope operated in epi-fluorescence mode (Fig.3). The diffusion of the dye for a free-cell device was first evaluated to optimize the method. Afterwards, the permeability of cell-free device was compared to the permeability of the HUVECs cultured device. As expected, a decrease of the permeability value in presence of the biological barrier was observed (Fig.4).

The *in vitro*acoustic setup consists of an ultrasound transducer driven by a function generator through a power amplifier. It is designed and adapted to host the bio-chip, the piezoelectric transducers within a water-filled, temperature-controlled chamber located on the microscope stage. In order to investigate the effects of ultrasound MBs cavitation on the barrier permeability, the same experimental methodology will be adopted in the HUVECs cultured device with MBs with diameter range of 2-10 μm injected in the vascular channel and irradiated with ultrasounds.

The combination of the proposed high fidelity biomimetic device with the possibility to control the response of MBs to ultrasound has the great potential to allow the study of cavitation-enhanced permeability of the endothelium improving the efficiency in targeted drug delivery and giving a tool suitable for clinical trial of new medicines.

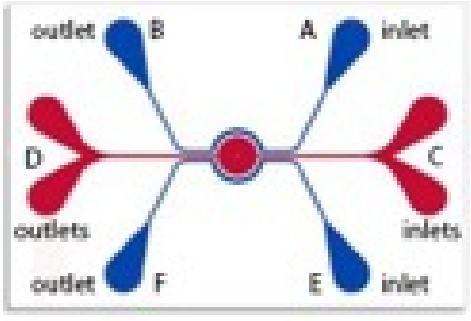


Fig1.sketch of the microfluidic device.jpg

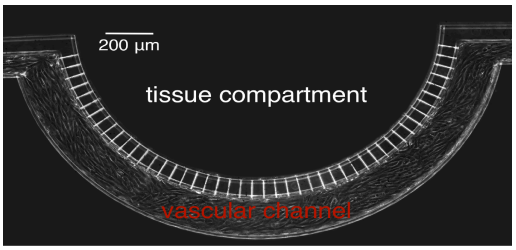


Fig2. bright-field image of the device cultured with huvecs.jpg

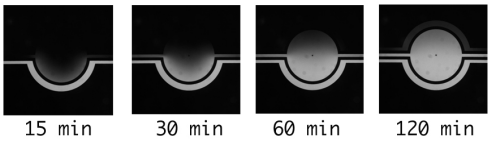


Fig3. timelapse freecell device with confocal microscope..jpg

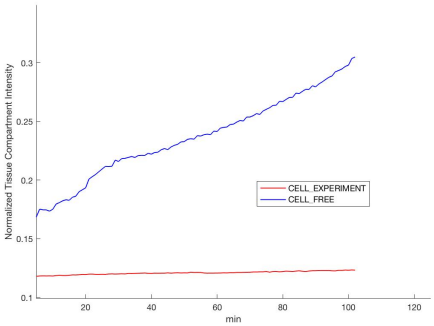


Fig4. comparison of cell-free and a cell-cultured device permeability.jpg

VEGF-targeted magnetite nanoparticles for breast cancer chemotherapy and MRI-visualization

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 95

Ms. Alevtina Semkina [1], ***Mr. Maxim Abakumov*** [1], ***Prof. Vladimir Chekhonin*** [1]

1. *Pirogov Russian National Research Medical University*

Breast cancer treatment strategies often include chemotherapy, which may cause serious side effects because of nonspecific drug action on tumor cells. To solve this problem it is possible to use different nanoparticles for loading of these drugs and provide targeted delivery to the deseased tissues. The goal of this research is to obtain the complex of Doxorubicin (Dox) with iron oxide magnetic nanoparticles (MNP), modified by monoclonal antibody to vascular endothelial growth factor (VEGF), to investigate its biochemical properties and anti-tumor efficiency (therapy and diagnostics) during *in vivo* experiments.

MNP were synthesized by thermal decomposition of iron acetyl acetonate (III). The coating of these nanoparticles was carried out by bovine serum albumin and polyethylene glycol. The morphology was analyzed by TEM. Dox loading was carried out by adsorption of drug molecules on MNP surface. The sizes of MNP, MNP-Dox and zeta-potential values were analyzed by method of DLS. Cytotoxic activity of samples was determined with MTS-assay (4T1 cell line). Internalization of Dox-loaded nanoparticles in living 4T1 cells was analyzed by confocal microscopy on Nikon A1R MP+. Nanoparticles cellular uptake was measured by flow cytometry (FACS). In vivo experiments were carried out with Balb/c female mice, which have experimental tumor 4T1. Nanoparticles accumulation in tumor *in vivo* was detected by MRI.

We obtained biocompatible, stable under physiological conditions MNP with core-shell strucrure and Dox-loaded by 8% by weight. MNP-Dox zeta-potential increased with increase of Dox-loading, therefore we proposed electrostatic interactions as driving force of complexation process. Dox-release under acidic condition was more intensive compared to physiological conditions, however Dox-loaded MNP showed quite similar cytotoxicity compared to free Dox. It was shown, that MNP-VEGF-Dox can provide more effective Dox accumulation in 4T1 cells compared with MNP-IgG-Dox due to specific interactions between antibodies and VEGF-receptor. MNP-VEGF-Dox intravenous administration resulted in increased of breast adenocarcinoma mice survivability compared to Dox. In addition MNP-VEGF were able to visualize breast adenocarcinoma during MRI of mice.

Thus, MNP-VEGF seem to be promising tool for tumor MRI-visualization and targeted Dox delivery in breast adenocarcinoma.

Work was supported by by grant of President of Russian Federation MK-6371.2016.7.

Combining nanocomposite scaffolds and human circulating multipotent cells as a self standing and autologous system for neural regenerative medicine

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 508

Ms. yuriko suemi hernandez gomez [1], ***Ms. Giorgia Scapin*** [1], ***Mrs. Mattia Bellon*** [1], ***Mr. Nicola Vicentini*** [1], ***Dr. Teresa Gatti*** [1], ***Prof. Enzo Menna*** [1], ***Dr. Thomas Bertalot*** [1], ***Prof. Rosa Di Liddo*** [1], ***Prof. Francesco Filippini*** [1]

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INTRODUCTION

We recently set up nanocomposite, self standing scaffolds recapitulating nanotopographical features of the neural environment by combining the biocompatibility of a poly-L-lactic (PLLA) matrix with the electrical, mechanical and chemical properties of carbon nanostructures. In the prototype system, dispersed carbon nanotubes (CNTs) in PLLA proved to support differentiation of human SH-SY5Y neuronal precursors. Then, we used human circulating multipotent cells (hCMCs), which are free from ethical restrictions, not genetically transformed and easily accessible, as a source for autologous and safe transplant. In order to further optimize scaffold features, we tested varying nanostructure concentrations and types comparing as nanofillers carbon nanohorns (CNHs) and reduced graphene oxide (rGO) to CNT-PLLA scaffolds.

MATERIAL AND METHODS

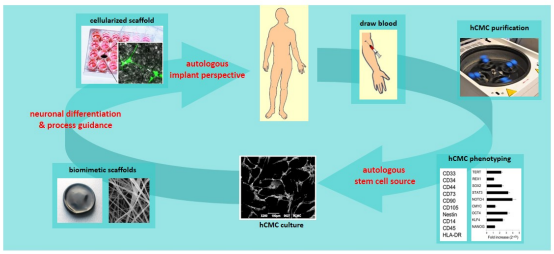
Nanocomposite scaffolds were prepared using CNTs or CNHs or rGO dispersed at 0.25% or 5% in the PLLA matrix. Biocompatibility and cell proliferation were assayed by LDH release and resazurin methods. hCMCs were isolated from volunteer healthy donors by Ficoll density gradient separation and characterized by flow cytometry (FCM) and qPCR analysis. Neuronal commitment was followed up by both fluorescence microscopy and evaluation of the expression of neural marker genes (Nestin, βIII tubulin/TUBβ3, Microtubule-associated protein 2/MAP2 and L1 CAM).

RESULTS

In presence of proper growth factors and media, hCMCs can be alternatively committed toward adipocyte, osteoblast, myocyte or neuronal lineages. Intriguingly however, even in the absence of neurotrophins, hCMCs are committed towards neuronal lineage when cultured onto CNT-PLLA scaffold (see figure), as shown by both microscopy and qPCR evidences. Comparison among CNTs, CNHs and rGO at varying concentrations is on-going and preliminary evidence with SH-SY5Y is encouraging as all tested conditions proved to be fully biocompatible.

DISCUSSION

This work suggests that hCMCs grown and differentiated onto our biomimetic and conductive scaffolds may represent a self standing prototype for reparation and repopulation of neural tissue injuries as well as, in general, a safe and autologous source for regenerative medicine and tissue engineering applications. Further variation of the scaffold composition will hopefully help us to boost neuronal differentiation and to shed light on contributions of peculiar scaffold features (shape, stiffness, conductivity) to this path.



Combining nanocomposite scaffolds and human circulating multipotent cells as a self standing and autologous system for neural regenerative medicine.jpg

Role of the fisetin on allergenic profilins: A theoretical approach

Tuesday, 26th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 503

Ms. Haruna L. Barazorda-Ccahuana [1], ***Mr. Diego Valencia*** [1], ***Ms. Cristal Mixcan*** [1], ***Ms. Rosa Villanueva*** [1], ***Dr. Badhin Gomez*** [1]

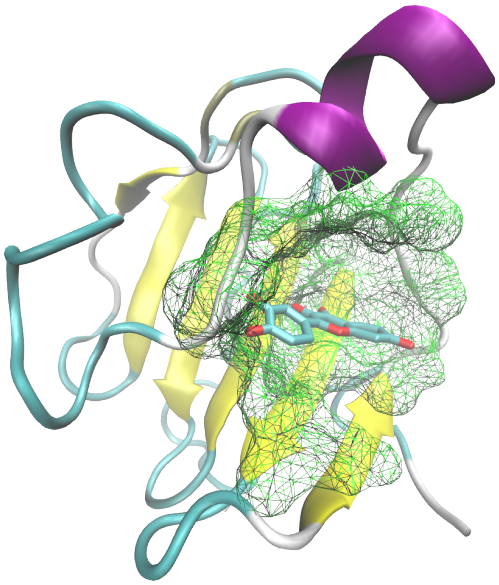
1. *Universidad Católica de Santa María*

Introduction. The profilins are proteins of molecular weights between 12 to 15 kd that are found in all eukaryotic cells, it has been reported crossreactivity between species of homologous profilins. Polyphenols have been the capacity to interfere with the allergic response, being able to bind to proteins in a reversible and irreversible ways. We found a polyphenol called fisetin with high bind at proteins.

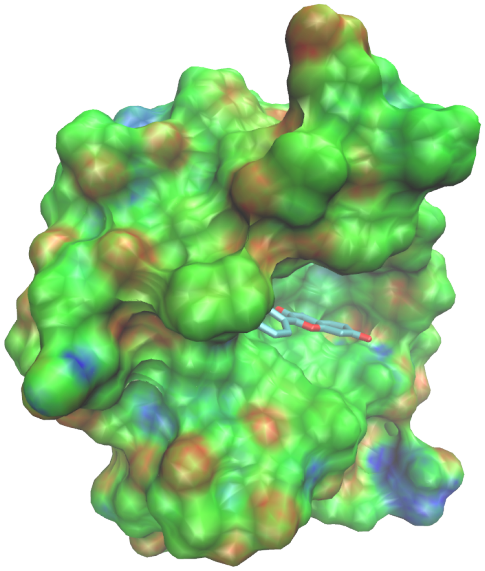
Objective. The aim of present work was to evaluate the interactions between fisetin and allergic proteins like profilin, in search of binding to epitopes recognized by the immune system and result a cross reactions currently occurring with this type of proteins.

Method. We have used computational biology and chemistry, template-based modeling in the Modeller software, molecular dynamic (MD) simulations in the canonical ensemble, a simulation mode implemented in GROMACS. Graphic visualization in VMD, analysis of structural couplings by protein pocket's prediction with ability to bind drug-like molecules with high affinity in PockDrug-Server and free-binding energy calculation with GROMACS.

Results and Discussion. Five profilins were studied: Ana c 1 (*Ananas comosus*), Cit s 2 (*Citrus sinensis*), Fra a 4 (*Fragaria ananassa*), Mal d 4 (*Malus domestica*) and Mus a 1 (*Musa acuminata*). The percent of homology been acceptable (>80%) in comparison to the template Art v 4 (*Artemisa vulgaris*). We have applied molecular dynamics simulations considering a canonical ensemble NVT (the number of particles N, the volume V, and the temperature T of the system are kept constant) by 100ns, in the OPLSAA force field using GROMACS. Molecular docking has a high score of druggability (0.9 - 1) and free-binding energy in the range of -62.31Kcal.mol^{-1} to -57.81Kcal.mol^{-1}. We concluded that fisetin could be an effective ligand to block allergy reactions induced by profilins, an promising alternative in nanomedicine.



Sitio activo altamente drogable de mal d 4mal2.png



Potencial electrost tico del acoplamiento mal d 4 - fisetina.png

Sistema	DeltaG Kcal.mol^{-1}
Ana c 1 - Fisetina	-57.81
Cit s 2 - Fisetina	-59.85
Fra a 4 - Fisetina	-58.45
Mal d 4 - Fisetina	-60.83
Mus a 1 - Fisetina	-62.31

Tabla n 1. energ as de interacci n.png

Tirapazamine-copper complexes for selective hypoxia cancer therapy

Tuesday, 26th September - 15:00 - Multi-Topic - Auditorium - Oral - Abstract ID: 63

Ms. Vera Silva [1], ***Dr. Wafa Al-Jamal*** [2]

1. *University of East Anglia/ School of Pharmacy,* ***2.*** *Queen's University Belfast*

Introduction: Hypoxia pro-drugs have emerged as novel alternative cancer therapies. Tirapazamine (TPZ) is the most advanced hypoxia-activated prodrug and has shown great specificity and potency in inhibiting tumour growth at moderate to severe hypoxic conditions. It is currently in phase III clinical trials to treat cervical cancer, but its clinical efficacy has been limited due to rapid metabolism and consequently, poor diffusion in the tumour mass. The coordination of pro-drugs to metal centres has shown potential modulation in the physicochemical properties of pro-drugs, while maintaining their hypoxia selectivity. In this study, we report the preparation of copper-tirapazamine $Cu(TPZ)_2$ complexes and their potential use as a selective hypoxia therapy for prostate cancer (PC).

Methods:$Cu(TPZ)_2$ were prepared and characterised using different techniques, such as FTIR and MALDI-TOF, HPLC, UV/Vis, spectrofluorometry and TEM. TPZ and $Cu(TPZ)_2$ *in vitro* cytotoxicity was assessed in different prostate cancer cell monolayers, cultured under normaxia and 1% hypoxia. The cytotoxicity was evaluated using resazurin cell viability assay. The potency and selectivity of $Cu(TPZ)_2$and TPZ were compared by calculating the HCR (hypoxia cytotoxicity ratio).

Results:$Cu(TPZ)_2$ complexes were successfully prepared with a high yield (>70%). FTIR and MALDI, confirmed the complexation. Further analytical data, showed that both TPZ and $Cu(TPZ)_2$ were stable over a wide range of solvents, buffers, and pH values. Furthermore, these complexes showed interesting properties that could have applications in theranostics and image-guided drug delivery. $Cu(TPZ)_2$ complexes maintained their hypoxia selectivity *in vitro* and demonstrated a statistically significant potency at 1% hypoxia, compared to normaxic conditions. More interestingly, a high HCR ratio (>50) was observed in some PC cells, suggesting an enhanced therapeutic activity of $Cu(TPZ)_2$ compared to TPZ alone.

Conclusions: This is the first study reporting the preparation and the characterisation of $Cu(TPZ)_2$complexes, as well as their enhanced toxicity in prostate cancer cells. Our hypoxia-selective complexes could be used in combination with chemo- or radio-therapy to enhance their therapeutic efficacy in advanced prostate cancer patients.

Acknowledgements: This work was supported by Prostate Cancer UK (Grant CDF-12-002), the Engineering and Physical Sciences Research Council (EPSRC) (EP/M008657/1), and University of East Anglia.

DNA chips and related sensors based on dendrimers. Towards rapid and accurate diagnosis of pathogens

Tuesday, 26th September - 15:17 - Multi-Topic - Auditorium - Plenary Speech - Abstract ID: 505

Dr. Anne-Marie Caminade [1]

1. *LCC Toulouse - CNRS*

Dendrimers [1] are perfectly defined hyperbranched nanomolecules, which possess many biological properties. [2] They can be used as 3-dimensional linkers between a solid surface and a bioprobe, for improving the sensitivity of sensors. [3] Three main advantages are expected when using dendrimers instead of classical (linear) linkers for the elaboration of microarrays: *i)* an increase of the density of probes per unit surface, *ii)* a greater accessibility of the target to the probe, and *iii)* a larger spacer from the glass surface avoiding unspecific interactions.

From fundamental researches [4] to commercial kits for determining the best treatment to prescribe in the context of personalized medicine, [5] the advantages of using dendrimers will be emphasized.

[1] Dendrimers. Towards Catalytic, Material and Biomedical Uses. Caminade A.M., Turrin C.O., Laurent R., Ouali A., Delavaux-Nicot B, Eds. 21 chapitres, 528 pages, John Wiley & Sons, Chichester (UK), September 2011. ISBN: 978-0-470-74881-7

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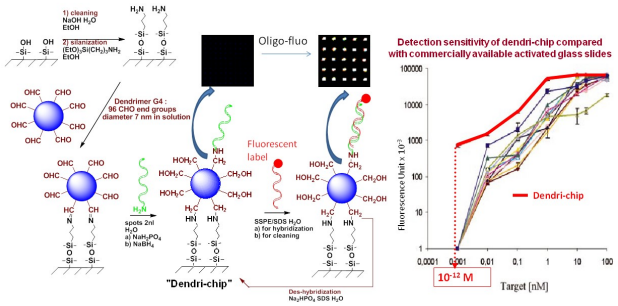
[3] Feng C.L., Zhong X.H., Steinhart M., Caminade A.M., Majoral J.P., Knoll W. *Small***2008**, *4*, 566-571.

[4] a) Le Berre V., Trevisiol E., Dagkessamanskaia A., Sokol S., Caminade A.M., Majoral J.P., Meunier B., François J. *Nucleic Acids Res.* **2003**, *31*, e88.1-e88.8; b) Trévisiol E., Leberre-Anton V., Leclaire J., Pratviel G., Caminade A.M., Majoral J.P., François J.M., Meunier B. *New J. Chem.* **2003**, *27*, 1713-1719.

[5] Dendris: http://www.dendris.fr/



Dendrichip.jpg

Assessment of atherosclerotic lesions permeability in ApoE(-/-) mice using fluorescent blood pool agent

Tuesday, 26th September - 16:30 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 346

Ms. Alessia Cordaro [1], ***Dr. Paolo Oliva*** [2], ***Dr. Chiara Brioschi*** [2], ***Dr. Erika Reitano*** [2], ***Dr. Luigi Miragoli*** [2], ***Dr. Giovanni Valbusa*** [2], ***Dr. Claudia Cabella*** [2]

1. *University of Torino,* ***2.*** *Bracco Imaging SpA*

Introduction. Atherosclerosis, the major cause of death in Western society, is an artery degenerative disease resulting in plaques leading to stenosis, embolization and thrombosis.
The FP7 project "NanoAthero" was funded to develop novel nanosystems for imaging and therapy of atherosclerotic diseases, with the contribution of several European research labs.
Currently, most of imaging techniques are not able to predict acute event based on plaque composition. A diagnostic tool aimed to stratify plaques with respect to different permeability could help clinicians to evaluate the response to nanosystem-based therapies.
The aim of this study was the investigation by Optical Imaging of the permeability of lesions in ApoE(-/-) mice, that develop hypercholesterolemia and atherosclerosis.To this purpose, the fluorescence signals of two blood-pool agents, Human Serum Albumin Cyanine5-conjugated (HSA-Cy5) or the albumin binder B26170, containing an aminodeoxycholic acid conjugated with IrDye800CW, were analysed on plaques of ApoE(-/-) mice at different stage of development.
Methods. ApoE(-/-)mice, under high-cholesterol diet, were administered with HSA-Cy5 or B26170. OI images were acquired *ex vivo* on excised aortic trees and the fluorescent signal was analysed on lesions compared to surrounding tissue. Hematoxylin/eosin staining and immunostaining for macrophages and endogenous Mouse Serum Albumin (MSA) were performed on cryosections of plaques.
Results. The fluorescence signal of both HSA-Cy5 and B26170, decreases with the progression of pathology, thus with ageing and loss of plaque permeability. Moreover, the highest signals were found in lesions at early development with widespread presence of macrophages, characterizing inflamed plaques, and with large MSA content, reflecting the endothelial permeability to serum protein. Conversely, fibro-calcific plaques, with rare macrophages and low MSA content, displayed the lowest signal.
Discussion. The permeability of an atherosclerotic lesion to albumin, using HSA-Cy5 or the albumin-binder B26170, reflects plaque morphology and composition, with respect to different staging. The proof of concept obtained with OI, could be proposed for a clinical tool in MRI, to establish which plaques are suitable for an anti-inflammatory nanoparticles-based therapy.
In this sense, the gadolinium-based contrast agent B22956/1, a MRI albumin binder analogue to B26170, could enable clinicians to define plaques features and to establish a proper therapeutic regimen.

Standardization of DMSA ligand exchange reaction to achieve uniformly coated iron oxide nanoparticles and their application for cancer imaging

Tuesday, 26th September - 16:47 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 106

Dr. Marco Filice [1], ***Mr. Ehsan Yazdanparast*** [2], ***Dr. Ines Martin Padura*** [2], ***Prof. Miguel Angel Del Pozo*** [2], ***Dr. Puerto Morales*** [3], ***Prof. Jesus Ruiz-Cabello*** [4], ***Dr. Marzia Marciello*** [3]

1. *National Research Centre for Cardiovascular Disease (CNIC); Biomedical Research Networking Center for Respiratory Diseases (CIBERES),* ***2.*** *National Research Centre for Cardiovascular Disease (CNIC),* ***3.*** *Institute of Material Science (ICMM-CSIC),* ***4.*** *Complutense University of Madrid*

Introduction:

Since the interactions of iron oxide nanoparticles (IONPs) with physiological environments are mediated by their surface coating, the quality and reproducibility of the IONPs' surface is a crucial parameter for their successful biomedical application and clinical potential translatability. Within the surface coating reactions, the oleic acid /dimercaptosuccinic acid (DMSA) ligand exchange is one of the most widely used. However, this surface coating reaction has not been yet fully characterized and optimized. As consequence, the potential biological activity of DMSA-coated IONPs further modified with bioactive compounds will be highly unpredictable.

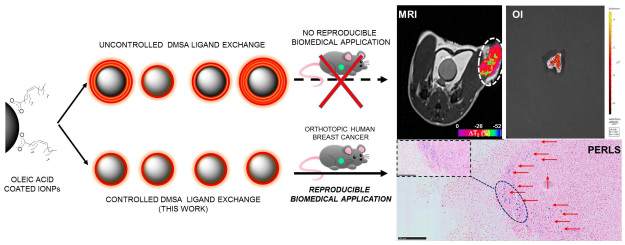
Methods:

We produced and characterized DMSA-coated IONPs of 10 nm core . By means of chemical strategies, we elucidated the mechanistic aspects hampering the reproducibility of OA/DMSA ligand exchange reaction. Hence, we designed a novel protocol accessing monodisperse IONPs with controlled and reproducible DMSA-surrounding shell (n=4). Then, we fully characterized the produced lots and demonstrated the viability of our strategy compared with untreated DMSA-IONPs. To assess their biomedical potential, we PEGylated (via EDC chemistry) and labeled with a fluorophore (via thiol-maleimide 'click' reaction) the treated DMSA-IONPs, finally accessing a dual contrast agent promoting optical and magnetic resonance imaging (OI/MRI). As control, the same procedure was repeated using untreated DMSA-IONPs. Both set of particles were compared by confocal laser scanner microscopy (CLSM). Finally, the ability of treated IONPs as dual contrast agent has been assessed in the diagnostic OI/MRI imaging of an orthotopic mice model of human breast cancer (n=4). The satisfactory imaging results were also confirmed by immunohistochemistry.

Results and Discussion:

Herein, we elucidate the mechanistic aspects of OA/DMSA ligand exchange on IONPs and present a standardized protocol that enables controlled and reproducible DMSA shell grafting. The physical-chemical characterizations confirmed our hypothesis. The as-prepared DMSA-IONPs were successfully PEGylated and labeled, showing by CLSM a fluorescence intensity almost one order of magnitud higher than the untreated ones. As contrast agent, these extravasate into the tumor's interstitium by enhanced permeability and retention effect (EPR) enabling a T_2 signal decrease around 20% (n=4) and optical tracking.

In sum, the standardization of IONPs multifunctional coating as theranostic agents for biomedical application has been achieved.



Abstract.jpg

Sythesis of Glycopeptide Nanoparticles and Investigation of Their Efficacy in Cancer Therapy

Tuesday, 26th September - 17:04 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 315

Dr. Aydan Dag [1], ***Mrs. Pinar Sinem Omurtag*** [2], ***Ms. Sezen Atasoy*** [3]

1. *Bezmiâlem Vakif University,* ***2.*** *İstanbul Medipol University,* ***3.*** *İstanbul University*

Platinum drugs are highly efficient in the treatment of cancer despite of their adverse side effects. To reduce side-effects, development of nanocarriers with precise biological functions is a critical requirement. Platinum drugs are most commonly delivered by conjugating the drug directly to the drug carrier, less via physical encapsulation. The therapeutic potency of platinum-based anticancer drugs can be substantially improved through the use of polymeric nanocarrier systems to target cancer cells efficiently.

In this work, we synthesised a series of glycopeptide triblock copolymers with three different sugars able to self-assemble into nanoparticles when conjugated with cis-platin (cis-Pt). The monomer trimethylsilyl-protected propargyl methacrylate (TMSpMA) was polymerized in the presence of a furan protected maleimide functionalized reversible addition-fragmentation chain transfer (RAFT) agent. P(TMSpMA) Macro-RAFT agent was then chain extented with the monomer of 1,1-Di-tert-butyl 3-(2-(Methacryloyloxy)ethyl)-butane-1,1,3-tricarboxylate (MAETC). On the other hand, poly(β-benzyl L-glutamate) (PBLG) was prepared through the ring-opening polymerization of BLG-NCA and PBLG was coupled with P(TMSpMA-b-MAETC) via click reaction to give corresponding triblok glycopeptide polymer. After deprotection of trimethylsilyl units, a range of glucosylated triblock copolymer were synthesized by reacting a mixture of 2-azideoethyl β-D-glucopyranoside ($GlcEtN_3$), 2-azideoethyl β-D-mannopyranoside ($ManEtN_3$) and 2-azidoethyl β-L-fucopyranoside ($FucEtN_3$) with P(PMA-b-MAETC-b-PLBG) using copper-catalyzed azide−alkyne cycloaddition (CuAAC). The resulting polymer was used as a macromolecular ligand for the conjugation with platinum drug. Thermogravimetric analysis revealed full conjugation. The resulting drug loaded nanoparticles had hydrodynamic diameters of around 100 nm.

They were all readily taken up intracellularly by the breast cancer cell lines MCF-7 and MDA-MB-231, prostat cancer cell line PC3 and the renal cancer cell line 769-P. All cell lines expressed a high preference for the fucosylated nanoparticles. The nanocarriers were themselves nontoxic, but exhibited high cytotoxicity and increased efficacy when conjugated with the cis-Pt drug. This finding suggests that these glycopeptide based nanoparticles can be used for targeted drug delivery toward cancer cells.

Design and insilico modeling of Dunaliella bardawil biomass encapsulated N-succinyl chitosan nanoparticles for effective anticancer activity

Tuesday, 26th September - 17:21 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 161

Prof. Balasubramanian Somasundaram [1], ***Dr. Selvaraj Kunjiappan*** [2]

1. *Kalasalingam University, Krishnankoil-626126, Near Madurai,TamilNadu,* ***2.*** *Kalasalingam University*

Introduction

The drugs with enhanced effectiveness and least side effects is one of the major tasks in current drug discovery. Microalgae have shown promising interest in food and pharmaceutical industries as well as it has shown vast potential as a sustainable energy carrier. To achieve this immense target, the N-succinyl chitosan (NSC) and bioactive compounds from *Dunaliella bardawil* biomass were chosen to formulate nanoparticles (NSC-NPs).

Methods

Dunaliella bardawil biomass obtained through microwave assisted extraction and the resultant bioactive compounds were analyzed using LC-MS. LC-MS chromatogram identified the individual active compounds through molecular mass and structural formula of the compounds.

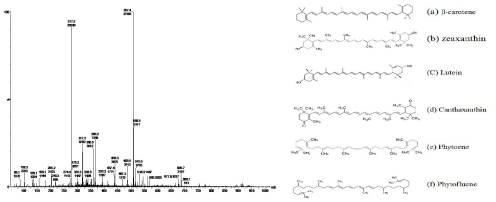
Bioactive compounds encapsulated NSC-NPs were characterized to study the molecular shape, particle size, stability and polydispersity index by FTIR, XRD, SEM, TEM and Zetasize Nano analyzer. *Insilico* and *in vitro* anticancer activity studies were performed choosing 721P and liver cancer cell lines (HepG2) respectively. Further, apoptotic cell cycle analysis was carried out using Annexin V-FITC and Propidium Iodide by Flow cytometry.

Results

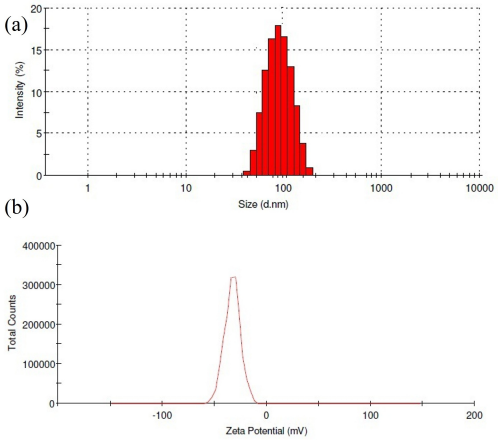
The obtained chromatogram revealed 6 compounds. Two major bioactive compounds β-carotene and lutein and other compounds such as carotenoids (cis and trans), phytoene, canthaxanthin, zeaxanthin and phytofluene were identified. The prepared NPs showed an average particle size of 80±5.6 nm size, spherical in shape, crystalline nature, zeta potential of -32±2.7 mV and polydispersity index of 0.51±0.02. Interestingly, the observed *insilico* study reports showed strong interaction of NSC-NPs and binding pockets of H-Ras P^{21}protooncogene. At 50 µg/mL concentration NPs displayed 95.60% cytotoxicity in HepG2 cell line. The apoptotic cell cycle analysis showed the cell death for 24hr and 48 hr representing 13.13% and 47.04% respectively.

Discussion

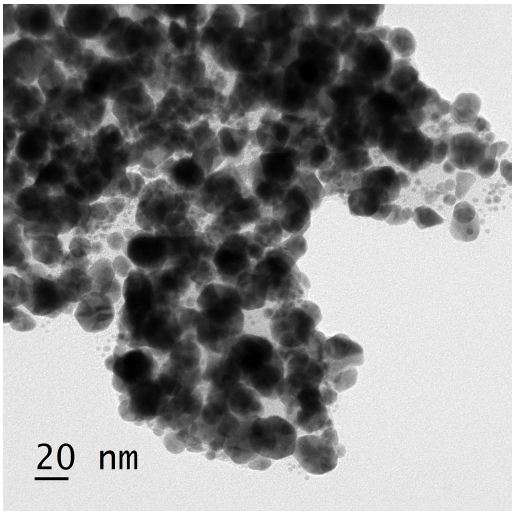
The highly cross-linked, biocompatible, biodegradable, nontoxic NSC-NPs were actively involved in destruction of targeted cancer cells. The obtained results showed that active NPs could enhance the controlled; site specific drug delivery and it can serve as a novel nanodrug in the management of cancer.



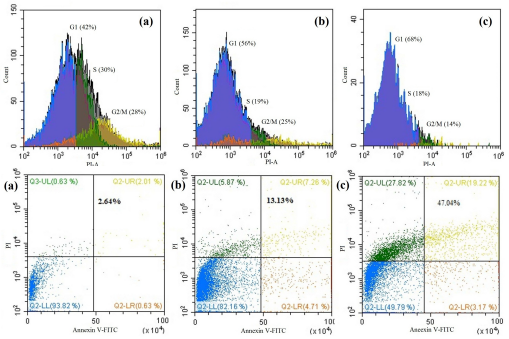
Lc-ms chromatogram of dunaliella bardawil biomass.jpg



Average particle size of nsc-nps.jpg



Hrtem image of nsc-nps.jpg



Flowcytometric analysis of apoptotic cell cycle.jpg

Apoferritin-affibody conjugates for cancer therapy

Tuesday, 26th September - 17:38 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 134

Prof. Neil Thomas [1], ***Dr. Lei Zhang*** [1], ***Dr. Tracey Bradshaw*** [1]

1. *University of Nottingham*

Introduction

Apoferritin is a ubiquitous protein nanocage, 12 nm in diameter with an 8 nm diameter hollow core that is used in nature as a store for up to 4,500 iron (III) ions forming ferritin. Removal of the Iron(III) ions by reduction leaves the protein cage which reversibly disassembles below pH 4.0. The apoferritin nanocage is very robust being stable in aqueous buffer upto 80 ^{o}C and pH 4.5-10.0. A number of groups including ours have encapsulated drugs (gefitinib, doxorubicin, cis-platin) or nanocrystals (magnetic iron, PbS quantum dots) in the apoferritin core using either a 'nanoreactor' or pH shift disassembly-reassembly route. This composites have been shown to be actively uptaken by a range of different cell types, typically via transferrin-receptor mediated endocytosis. Progression through the endosome-lysosome system with a gradual decrease in pH results in the disassembly of the apoferritin and release of its cargo. To take advantage of these 'trojan horse'-like properties we have modified the apoferritin with an affibody that targets the Her2 receptor that is overexpressed in >20% of breast cancer cell lines in order to specifically target tumorogenic cells.

Results and Discussion

By mixing together Her2-modified and unmodified apoferritins in different ratios we can significantly attenuate the biological effects these constructs have on Her2 receptor degradation and downstream signalling which then affects both apoptosis and cell proliferation *in vitro.* The results of these studies together with those in which the affibody-apoferritins are combined with a variety of cargoes will be presented.

References

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Apoferritin-encapsulated PbS quantum dots significantly inhibit growth of colorectal carcinoma cells, Bradshaw, Tracey D.; Junor, Marc; Patane, Amalia; Clarke, Phil; **Thomas, Neil R.**; Li, Mei; Mann, Stephen; Turyanska, Lyudmila, *J. Mat. Chem. B*, **2013**, 1, 6254-626

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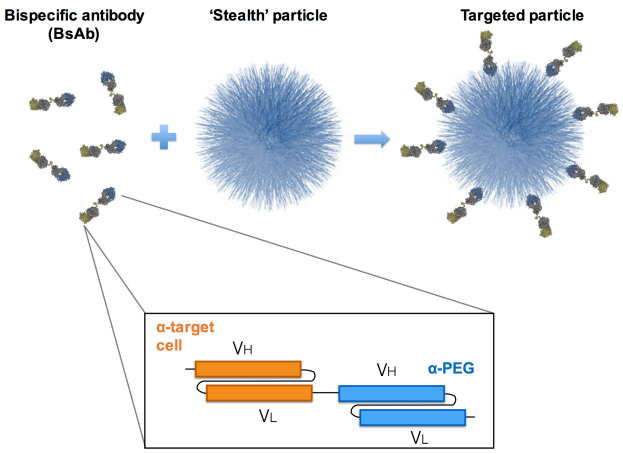
Harnessing bispecific antibodies to target immunologically stealth particles

Tuesday, 26th September - 17:55 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 252

Mr. Joshua J Glass [1], *Prof. Jiwei Cui* [2], *Dr. Christopher Howard* [3], *Mr. Yi Ju* [1], *Prof. Stephen Mahler* [3], *Prof. Frank Caruso* [1], *Prof. Kristofer Thurecht* [3], *Dr. Robert De Rose* [1], *Prof. Stephen Kent* [1]

1. *University of Melbourne*, **2.** *Shandong University*, **3.** *University of Queensland*

Although essential for human health, the innate immune system represents a major biological barrier to the medical applications of nano-engineered particles. Opsonisation and phagocytosis sequester particles from the circulation to the detriment of therapeutic delivery. We have previously developed long-circulating polyethylene glycol (PEG) particles with a unique ability to evade phagocytic cell uptake. However, these 'stealth' particles lack cell targeting abilities. Now, in an effort to engineer cell targeting into stealth materials, we developed bispecific antibodies (BsAb) that display dual specificity; one arm binds PEG while the other targets a cell antigen, such as the cancer marker epidermal growth factor receptor (EGFR) or the T cell marker CD3. We used flow cytometry and confocal microscopy to investigate how the addition of BsAb targeting moieties (1) modulates cell targeting, (2) alters particle stealth properties in human blood, and (3) is influenced by the protein corona formed in autologous human plasma. Cancer cell line targeting by EGFR-targeted PEG particles increased as a factor of BsAb density. In the more complex environment of whole human blood, the highest BsAb density examined resulted in a modest 1.48- to 1.52-fold mean increase in association with primary monocytes and granulocytes, respectively. Association with blood phagocytes occurred primarily in the presence of a plasma protein corona, whether PEG particles were targeted or not. Importantly, in fresh human blood, anti-CD3 targeting substantially increased particle delivery to T cells (23.8- and 18.2-fold after 1 and 5 h, respectively), while non-specific uptake by monocytes and granulocytes increased just 1.5-fold at the highest BsAb density examined. *In vivo* studies will ultimately determine whether the benefits of targeted delivery outweigh the disruption to stealth properties. BsAb-targeted stealth particles have the potential to deliver therapeutics with minimal off-target effects and/or image cells with high specificity.



Bsab-targeted stealth particle.jpg

Investigating the Internalization of the Polyelectrolytes Microcapsules Using Scanning Ion Conductance Microscopy

Tuesday, 26th September - 18:12 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 451

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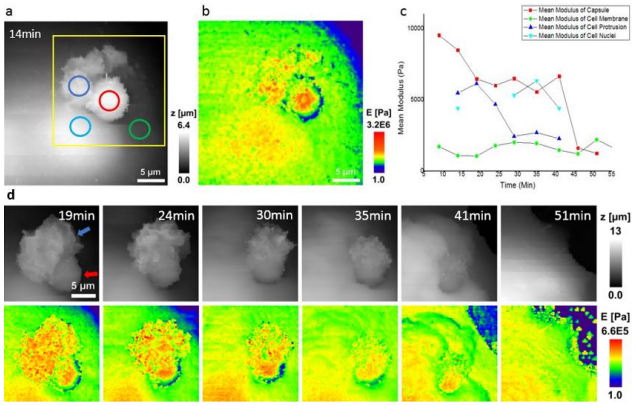
Introduction:
Polyelectrolyte multilayer microcapsules represent one of the promising strategies for intracellular drug delivery. However, the underlying mechanism of cellular internalization remains poorly understood due to limited availability of specific fluorescent markers and blockers of the processes involved in the internalization. Here we employ an emerging scanning probe microscopy technique called Scanning Ion Conductance Microscopy (SICM) to study these complex processes at nanoscale resolution.

Methods:
In SICM, an electrolyte-filled glass nanopipette, vertically positioned above a sample, serves as the scanning probe using a small reduction (≈0.3 %) in the ionic current flowing out of the nanopipette to detect proximity of the sample surface. To record topography of the sample surface, the nanopipette periodically approaches the surface at selected x-y coordinates and records the relative height of a sample. To estimate elasticity of the sample simultaneously with topography, the nanopipette is allowed to continue closer to the sample surface at each imaged point until current drops by 1 - 3%. Changes in the sample height in response to stress exerted by the nanopipette tip at these higher current drops is used to estimate elasticity.
Results:
We were able to obtain high resolution topographical recordings of 12 cases of single capsule internalization events with mean duration of internalization of 46 minutes (ranging from 17 to 71 minutes). The recordings provide a first direct proof that capsules landing on the cell membrane trigger formation of membrane protrusions in its immediate vicinity. The protrusions gradually formed a complex and rather irregular mesh enveloping the capsule and finally pulling the capsule into the intracellular space (Fig. 1a). Simultaneous measurement of elastic modulus revealed quick build-up of membrane engulfing the capsule, followed by relatively long plateau, and surprisingly abrupt sinking of the capsule deep into the cell (Fig. 1b-c).
Discussion:
According to our knowledge these are the first live recordings of complex topographical changes during the internalization process of microcapsules demonstrating the response triggered by capsules and the importance of membrane protrusions. Furthermore, our data show that mapping of elastic modulus has a potential to provide new insights into the mechanobiology of cellular uptake of microcapsules.



Time lapse images with stiffness data.jpg

Halloysite – quantum dots nanocomposites for the intracellular labeling

Tuesday, 26th September - 18:29 - Nano-Imaging for diagnosis, therapy and delivery - Room 207 - Oral - Abstract ID: 446

Dr. Anna Stavitskaya [1], Dr. Andrei Novikov [1], Dr. Elvira Rozhina [2], Prof. Rawil Fakhrullin [2], Dr. Evgenii Ivanov [1], Prof. Yuri Lvov [3], Prof. Vladimir Vinokurov [1]

1. *Gubkin University,* ***2.*** *Kazan Federal University,* ***3.*** *Louisiana Tech University*

Introduction

Quantum dots (QD) are widely used for cellular labeling due to enhanced brightness, resistance to photobleaching and multicolor light emissions, thus being superior to traditional organic fluorescent dyes. The inherent problem of quantum dots applications is the toxicity of the ligands used in the synthesis or of the quantum dots themselves.

Methods

We have synthesized CdS, $Cd_xZn_{1-x}S$, CdSe, and FeSe quantum dots in the presence of halloysite – tubular clay serving as the stabilizing agent instead of the potentially toxic ligands. The obtained nanotubule-QD composites were characterized by transmission electron microscopy, and by reflectance and fluorescence spectroscopy. The halloysite–QD composites were tested for labeling of human skin fibroblasts and prostate cancer cells.

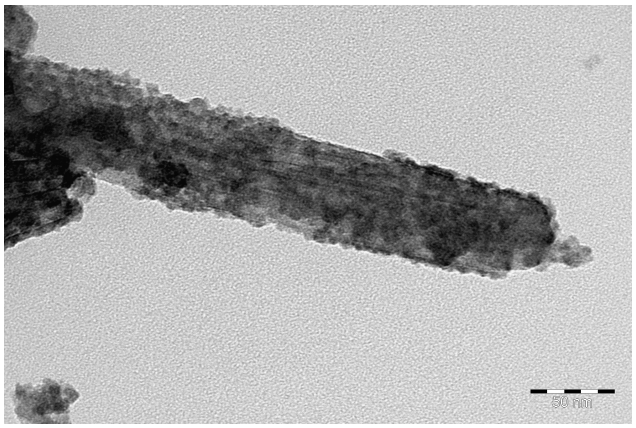
Results

We synthesized halloysite nanotubes with 2-3 nm diameter nanoparticles adsorbed into the lumen or onto the outer surface of halloysite (see Figure 1). In the absence of halloysite, analogous synthetic procedures led to the agglomeration of particles into a bulk precipitate. The obtained composites exhibited spectral properties typical for the quantum dots. The uptake of the halloysite–quantum dots composites by human cells was confirmed by dark-field and fluorescence microscopy. The human cells both QD-halloysite treated and untreated completely covered the substrates grown in Petri-dish within 4–5 days. Cell morphology and cellular proliferation were not affected by the QD-halloysite treatment, contrary to the poisonous effect of pure uncoated QDs.

Discussion

Adsorption of quantum dots on halloysite nanotubes prevents them from aggregation. The pronounced scattering and fluorescence demonstrated by halloysite–quantum dots composites allow using them as intracellular markers. Depending on the chemical composition, halloysite-based QD-markers are either diffusely distributed within the cytoplasm or predominantly agglomerated in perinuclear regions. Thus, the obtained tubular clay encapsulations of quantum dots are the perspective markers for the applications in biomedical studies.

This work is funded by the Ministry of Education and Science of the Russian Federation (grant 14.Z50.31.0035).



Cdxzn1-xs-halloysite tem 150kx.jpg

Quatsomes for the topical delivery of the recombinant human epidermal growth factor (rh-EGF) to treat complex wounds

Tuesday, 26th September - 16:30 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 413

Dr. Elisabet González-Mira [1], ***Dr. Lidia Ferrer-tasies*** [2], ***Dr. Santiago Sala*** [3], ***Prof. Jaume Veciana*** [1], ***Dr. Nora Ventosa*** [1], ***Dr. Héctor Santana*** [4], ***Dr. Haydee Gerónimo*** [4], ***Dr. Jenay Díaz*** [4], ***Dr. Milagros Font*** [4], ***Dr. Fidel Raul Castro*** [4], ***Dr. Dinorah Torres*** [4], ***Dr. Eduardo Martínez*** [4]

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Introduction

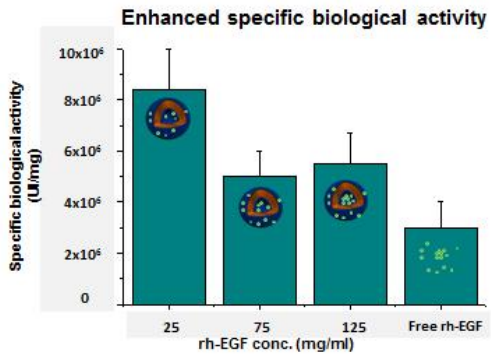
Complex wounds, such as diabetic foot ulcers and chronic venous ulcers, defy cure using conventional and simple “dressings” therapy and, in most cases, surgical treatments are unavoidable. Therefore, new technologies that improve the conventional treatments should be introduced. EGF is a small polypeptide which exerts a potent mitogenic activity through binding to a specific cell membrane receptor promoting the epidermal regeneration and the corneal epithelialization. Some institutions of the Havana Western Scientific Pole in Cuba have been developing and marketing novel therapeutic products using the rhEGF, known as Heberprot-P® and Cimavax EGF®. Nevertheless, the proteolytic environment present in the complex wounds affects the bioavailability and effectiveness of this rhEGF-based treatment. Consequently, the encapsulation of this drug within nanocarriers, such as vesicles, can provide protection together with an effective delivery of the active molecules in the site of action. Here, we present a new highly stable nanovesicular system named Quatsomes, which confers several advantages over conventional liposomes, for the topical delivery of EGF.

Methods

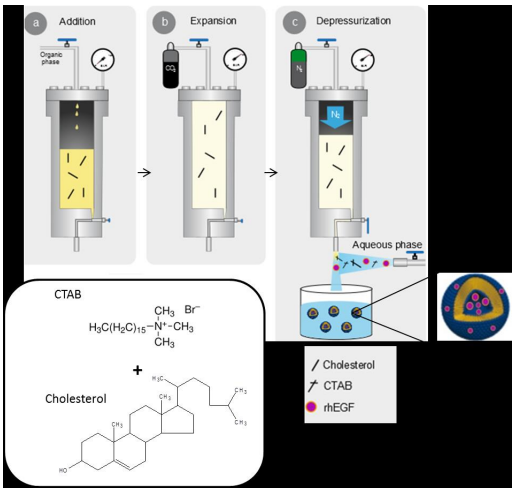
rhEGF-quatsomes, composed of cholesterol and CTAB, were prepared by the DELOS-SUSP methodology (Figure 1) and deeply characterized to determine their physico-clemical properties (size and zeta potential by dynamic light scattering, morphology by cryo-TEM, entrapment efficiency and protein loadings by an enzyme-linked immunosorbent assay) and their biological properties (bioactivity, resistance to proteases, and *in vivo*efficacy).

Results and Discussion

rhEGF-quatsomes with nanometric sizes (100-150 nm), with a great degree of unilamellarity, and with high entrapment efficiencies (~99 %), were successfully prepared. These conjugates resulted very stable along time, keeping the same protein/lipid loadings for more than one year. The specific enzymatic activity of the rhEGF entrapped in the quatsomes was at least two times higher than that for the free protein (Figure 2). rhEGF-quatsomes also showed antimicrobial activity and 8 times more resistance to degradation by proteases compared to free rhEGF. The pharmacological efficacy of the developed rhEGF-quatsomes was successfully demonstrated in a rat model of chronic ulcer as well as when used for the compassionate treatment of 12 patients, showing outstanding results in the cicatrization of the treated complex wounds.



Rhegf-quatsomes induce higher 3t3 a31 mouse cells proliferation.jpg



Schematic representation of delos-susp method for the rh-egf-quatsomes preparation.jpg

Drug-loaded micelles in bio-relevant dissolution media: Impact of surfactant-bile interactions on drug solubility

Tuesday, 26th September - 16:47 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 360

Dr. Zahari Vinarov [1], ***Mr. Vladimir Katev*** [1], ***Prof. Slavka Tcholakova*** [1]

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Introduction

More than 50 % of new drug molecules have poor water solubility which limits their clinical application. Oral delivery presents an additional challenge due to the endogenous surface-active substances in the gut that can interact strongly with the surfactants used to increase drug solubility, which results in variable and difficult to predict oral bioavailability. We aim to reveal the impact of bile salt-surfactant interactions on drug solubility by studying the influence of surfactant molecular structure on drug solubilization in biorelevant media.

METHODS

We studied the solubilization of Fenofibrate (Log P = 5.3) by 13 surfactants with different chain length (C_{12}-C_{18}) and head group (cationic, anionic, nonionic and zwitterionic). Porcine bile extract containing bile salts and phospholipids was used for biorelevant media preparation and sodium taurodeoxycholate (97 %) was used as pure bile salt for model experiments. Drug solubility was determined by HPLC. Bile salt-surfactant interactions were studied by determining the critical micellar concentration (CMC) of the single and mixed systems by surface tension measurments.

RESULTS AND DISCUSSION

Drastic decrease of Fenofibrate solubility in presence of bile extract was observed for anionic and cationic surfactants, whereas drug solubilization in nonionic surfactants was not significantly affected. Experiments at different surfactant-to-bile extract ratios showed linear decrease in Fenofibrate solubility with increasing bile extract fraction for Tween 20. However, increasing bile extract fraction from 0 to 20 % resulted in pronounced drop in Fenofibrate solubility for both anionic and cationic surfactants, see Figure 1. Identical results were obtained when bile extract was replaced with a pure bile salt (sodium taurodeoxycholate), which confirmed that surfactant-bile salt interactions are key. Measurements of CMC showed that nonionic surfactants do not form mixed micelles with the bile salts, thus retaining their solubilization capacity, whereas charged surfactants form mixed micelles with bile, which have low drug solubilization capacity. These findings advance the understanding of drug solubility in complex, biorelevant media and could be used to improve the *in-silico* models for prediction of oral bioavailability that currently neglect such type of effects.

Acknowledgements

The financial support of Project BG05M2OP001-2.009-0028 of Sofia University is gratefully acknowledged

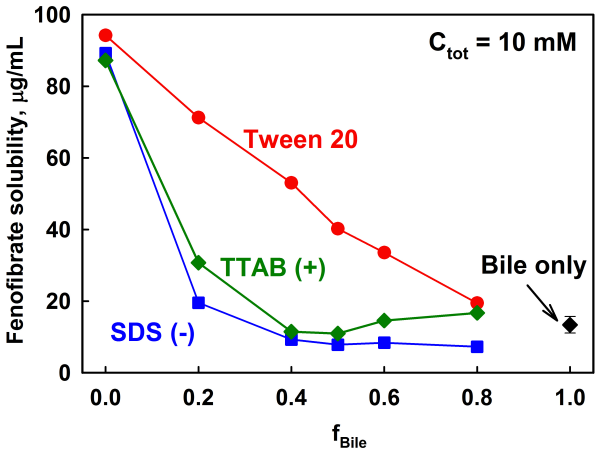

Figure 1. Fenofibrate solubility as a function of the fraction of bile in bile + surfactant mixtures for Tween 20 (red circles), tetradecyl-trimethylammonium bromide (green diamonds) and sodium dodecyl sulfate (blue squares). The total concentraiton of surfactant + bile is constant at 10 mM.

Figure 1.jpg

Inhalation of peptide-loaded nanoparticles improves heart failure

Tuesday, 26th September - 17:04 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 254

Dr. Michele Miragoli [1], Mrs. Paola Ceriotti [2], Dr. Michele Iafisco [3], Mr. Marco Vacchiano [2], Dr. Nicolò Salvarani [3], Dr. Alessio Alogna [4], Mr. Pierluigi Carullo [3], Dr. Stefano Rossi [1], Dr. Gloria Rodriguez [3], Dr. Tatiana Patricio [3], Dr. Silvana Pinelli [1], Dr. Rossella Allinovi [1], Dr. Marco Erreni [2], Prof. Gianluigi Condorelli [2], Prof. Heiner Post [5], Dr. Anna Tampieri [3], Dr. Daniele Catalucci [3]

1. *University of Parma,* ***2.*** *Istituto Clinico Humanitas,* ***3.*** *Consiglio Nazionale delle Ricerche,* ***4.*** *Berlin Institute of Health,* ***5.*** *University Medicine Berlin*

Peptides are recognized for being highly selective and efficacious for the treatment of cardiovascular as well as other diseases but their administration via a non invasive procedure is currently not possible, thus representing scientific and technological challenges. Here we demonstrate that inhalation of small sized (<50nm) biocompatible and biodegradable calcium phosphate nanoparticles (CaPs) allows for rapid translocation of CaPs from the pulmonary tree to the blood stream and thus to the myocardium where their cargo is quickly released. In particular, treatment of a rodent model of diabetic cardiomyopathy through inhalation of CaPs loaded with a therapeutic mimetic peptide that we previously demonstrated to improve myocardial contraction resulted in restoration of cardiac function. When translated to large animals, tangible evidence that inhalation of a peptide-loaded CaP-formulation is a safe and valid method of cardio-targeted administration was provided. Altogether, these results demonstrate that inhalation of biocompatible tailored peptide nanocarriers represents a pioneering approach for the pharmacological treatment of heart failure.

Hydroxypropyl-beta-cyclodextrin as cryoprotectant in nanoparticles prepared by nano-spray drying technique

Tuesday, 26th September - 17:21 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 411

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1. *Department of Pharmaceutical Technology, Collage of Pharmacy, Future University,* ***2.*** *Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt,* ***3.*** *Department of Pharmaceutics, Faculty of Pharmacy, Cairo University*

Nano-spray dryer is advanced instrument to produce a stable and spherical nanoparticles with high yield. In this study, econazole nitrate nanoparticles were formulated by nano-spray dryer using 1:1, 1:2 and 1:3 weight ratios of drug to hydroxypropyl-beta-cyclodextrin and stabilizer. The prepared samples were sprayed through nozzle size of 7.0 µm using 95 ºC and 45 ºC as inlet temperature and outlet temperatures, respectively. The prepared nanoparticles were evaluated for process yield and percent drug loading. Furthermore, the drug nanoparticles were dispersed in isotonic buffer solution and examined for drug release and their stability at room temperature. The spray dried particles were in the nano-range (148 to 294 nm) and their yield values ranged between 79.1 and 84.9 %. Increasing weight ratio of drug to hydroxypropyl-beta-cyclodextrin to 1:2 and 1:3 showed increase in percent drug release compared to formulation containing 1:1 weight ratio of drug to hydroxypropyl-beta-cyclodextrin but the prepared econazole nitrate nanosuspension containing 1:1 weight ratio of drug to hydroxypropyl-beta-cyclodextrin revealed best stability study during storage period at room temperature compared to other formulations. As a result of *in-vitro* drug release and stability studies, the optimum weight ratio of 1:1, drug to hydroxylpropyl-beta-cyclodextrin, was chosen as a best weight ratio duo to its good balance between drug release and stability of drug nanoparticles.

Preparation of nanoparticle-protein complexes for therapeutic application of nanoparticles in ocular diseases

Tuesday, 26th September - 17:38 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 250

Dr. Dong Hyun Jo [1], ***Dr. Jin Hyoung Kim*** [1], ***Dr. Jin Gyeong Son*** [2], ***Mr. Ki Soon Dan*** [1], ***Dr. Sang Hoon Song*** [3], ***Dr. Tae Geol Lee*** [2], ***Dr. Jeong Hun Kim*** [1]

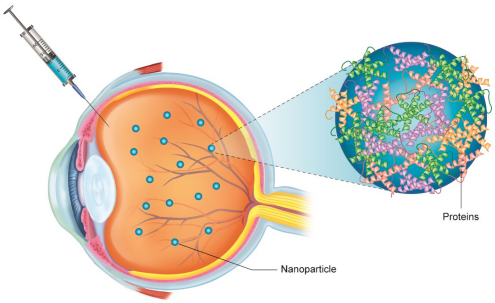
1. *Seoul National University Hospital,* ***2.*** *Korea Research Institute of Standards and Science,* ***3.*** *Seoul national university*

Introduction: Nanoparticles are thought to be novel platforms for therapeutic agents. However, clinical application of nanoparticles lags behind rapid progress in nanotechnology. One of the problems is uncontrolled protein adsorption around nanoparticles in the biological fluids, which prohibits intended biological activity. In this study, we investigated the therapeutic potential of nanoparticle-protein complexes which were formed before intraocular injection.

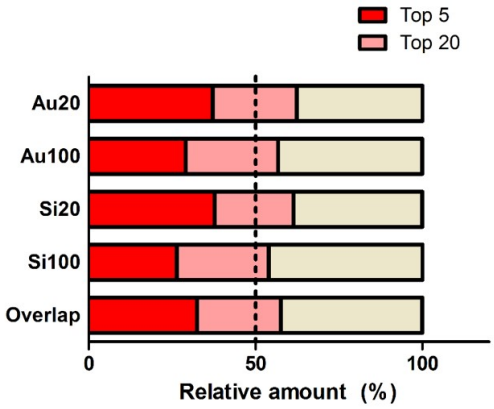
Methods: To investigate corona formation in the ocular environment, we incubated 20- and 100-nm gold and silica nanoparticles in the vitreous fluid of the eye for 24 hours. Then, the corona proteins were identified using proteomic analyses. Biological activities of nanoparticle-protein complexes which were formed with self-assembly of 20-nm gold nanoparticles and the top 5 corona proteins were analyzed with *in vitro*angiogenesis assays and an *in vivo*choroidal neovascularization model in mice.

Results: Interestingly, although the compositions of the corona proteins were different from those of the vitreous samples, they each displayed similar patterns independent of the core material or size of nanoparticles. In the vitreous, where protein concentration is less than that of serum or plasma, corona formation is different in its composition patterns. These results highlight the tissue-specific nature of corona formation in the ocular environment. As a novel bio-inspired approach to overcome the loss of intended activity of the nanoparticles due to uncontrolled corona formation, we suggested priming the nanoparticles with tissue-specific corona proteins. Interestingly, the nanoparticle-protein complexes exhibited therapeutic effects by binding with vascular endothelial growth factor that promotes pathological angiogenesis, even *in vivo*by intraocular injection,better than naïve nanoparticles, which were inevitably exposed to nonspecific protein adsorption.

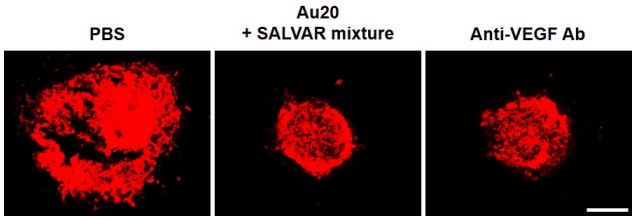
Discussion: In this study, we investigate tissue-specific formation of corona around locally administered nanomaterials and biological impacts of nanoparticle-protein complexes which were formed before intraocular injection. This strategy, using tissue-specific corona for protection of nanoparticles from non-specific protein adhesion, might extend the applicability of nanoparticle-based therapeutic approaches against various human diseases, especially in local administration.



Corona formation around nanoparticles after intraocular injection.jpg



Composition of corona proteins around 20- and 100-nm nanoparticles.jpg



Therapeutic effects of nanoparticle-protein complexes on choroidal neovascularization in vivo.jpg

Improvement of the Enzymatic Activity of α-Galactosidase Using Nanovesicles with application to Fabry Disease treatment

Tuesday, 26th September - 17:55 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 347

Dr. Solène Passemard [1], ***Dr. Elisabet González-Mira*** [1], ***Ms. Anna Lechado*** [2], ***Ms. Natalia Garcia Aranda*** [2], ***Dr. Ibane Abasolo*** [2], ***Dr. José Luis Corchero*** [3], ***Dr. Santiago Sala*** [4], ***Dr. Daniel Pulido*** [5], ***Dr. Edgar Cristobal*** [5], ***Dr. Míriam Royo*** [5], ***Prof. Antonio Villaverde*** [3], ***Dr. Simó Schwartz*** [2], ***Prof. Jaume Veciana*** [1], ***Dr. Nora Ventosa*** [1]

1. *ICMAB-CSIC/CIBER-BBN,* ***2.*** *VHIR/CIBER-BBN,* ***3.*** *Institut de Biotecnologia i de Biomedicina/CIBER-BBN,* ***4.*** *ICMAB-CSIC/CIBER-BBN/NT,* ***5.*** *Combinatorial Chemistry Unit/CIBER-BBN*

Introduction:

Fabry disease is a rare inherited disease caused by loss of function of the enzyme α-Galactosidase A (GLA) [1]. Commercially available treatments are based on the intravenous administration of GLA demonstrated positive short-term effect, reducing the progression of the disease and improving the quality of life in patients. However, GLA replacement therapy exhibits drawbacks such as the degradation of the exogenously administered enzyme, its limited efficacy in patients with an advance stage of the disease and the extremely high cost of the treatment. In order to improve the delivery efficacy and the systemic circulation of the current treatment, nanoliposomes containing GLA were prepared as novel drug delivery systems (DDS).

Methods:

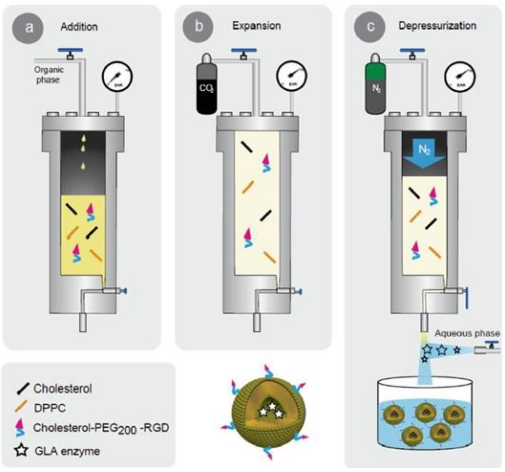
The incorporation of GLA in liposomes was obtained following the DELOS-SUSP process (**Figure 1**) which use compressed CO_2 [2] as co-solvent. Liposomes were constituted from phospholipids (DPPC) and cholesterol-based compounds. In addition, c(RGDfK) peptide ligand was incorporated in the membrane bilayer of the vesicles to enhance the targeting and the uptake efficiency of the GLA-loaded conjugates to the diseased cells. The conjugate was further characterized to obtain information on its physico-chemical characteristics and morphology entrapment efficiency and also biological efficacy and cell uptake.

Results:

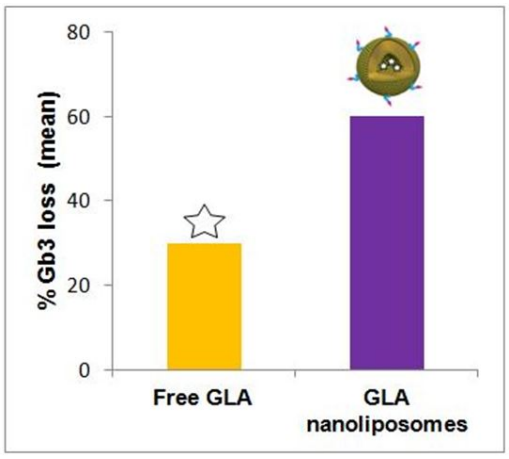
Through the DELOS-SUSP process, nanometric liposomes containing GLA were successfully prepared with an entrapment efficiency of about 40 %. *In vitro* efficacy studies in GLA deficient cells of Fabry KO mice showed that the GLA-nanoformulations were able to reduce lysosomal Gb3 deposits more efficiently than the free enzyme in agreement with a greater specific activity also encountered (**Figure 2**).

Discussion:

This finding indicates that (i) such multifunctional nanovesicles are uptake by GLA deficient cells, (ii) the GLA-nanovesicles reach the lysosomal compartment, and (iii) the cargo (GLA) is efficiently released so that the GLA activity in the cells is restored. The results obtained prove the great potential of DELOS-SUSP method for the production of new nanomedicine candidates based on enzyme-nanovesicle conjugates. The development of these new GLA-nanoconjugates up to the end of the regulatory preclinical phase will be carried out under the frame of the European Smart-4-Fabry project (H2020-NMBP-2016-2017 GA 720942).



Nano-gla multifunctional nanoformulation manufactured by the delos-susp platform .jpg



Effect of free gla and gla-nanoliposomes in the reduction of gb3 deposits in aortic endothelial cells of fabry ko mice.jpg

Glycan-functionalized liposomes as a cell-selective drug delivery approach for the treatment of Tuberculosis

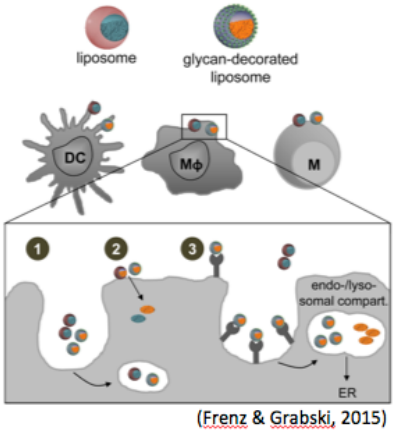
Tuesday, 26th September - 18:12 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 386

Mr. Verónica Durán [1], ***Dr. Elena Grabski*** [1], ***Dr. Theresa Frenz*** [2], ***Mr. Constantin Hozsa*** [3], ***Dr. Robert Gieseler*** [3], ***Prof. Ulrich Kalinke*** [1]

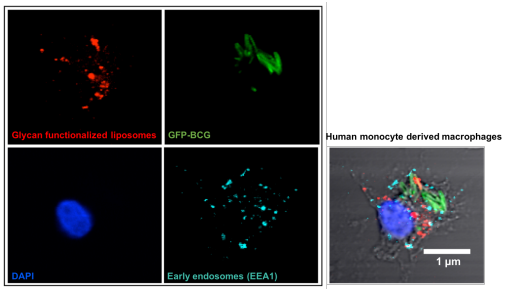
1. *TWINCORE, Centre for Experimental and Clinical Infection Research,* ***2.*** *TWINCORE, Centre for Experimental Infection Research,* ***3.*** *RODOS BioTarget*

Tuberculosis (TB) is one of the top 10 causes of death worldwide. The current treatment standard regimen requires a 6 to 9 months course combination therapy of antimicrobial drugs that are provided daily, which leads to low compliance and failure in the treatment resulting in emergence of multi-drug-resistant (MDR) and extensively-drug-resistant (XDR) cases. For this reason, improved treatment of TB is a major medical need. Nanocarriers such as liposomes offer a promising approach for drug delivery due to their size and hydrophobic/hydrophilic character which makes them highly biocompatible and because their surfaces can be modified in order to functionalize them for cell-selective drug delivery. In the present study we use a glycan-decorated liposomal formulation to target antigen-presenting cells (APC) by binding of C-type lectin receptors (CLR) exposed on the surface of these cells. CLR play a crucial role in pathogen uptake by sensing carbohydrate structures that are prominent constituents of various pathogens such as HIV-1, dengue virus, *C. albicans* and *M. tuberculosis* and therefore can be exploited by various glycan-functionalized particles.

The CLR expression profile as well as the uptake of glycan-decorated liposomes was studied in human immune cell subsets derived from blood, secondary lymphoid organs such as tonsils and lymph nodes, as well as in cells derived from human lung tissue. Furthermore, the potency of such liposomes to deliver antibiotics was assessed with an *in vitro*BCG-infection model in human monocyte derived macrophages. Our results demonstrate that dendritic cells and macrophages have a superior uptake of liposomes in comparison to other non-APC like T and B cells. Glycan-decorated liposomes showed a preferential subcellular localization in endosomal compartments, presumably due to receptor-mediated endocytosis through CLR, whereas non-decorated liposomes appear to be ingested by receptor-independent mechanisms and are mainly trafficked into the cytoplasm. Most importantly, liposomes that are both glycan-decorated and loaded with antibiotics effectively inhibited bacterial growth in BCG-infected macrophages. Thus, functionalized liposomal formulations might be suitable to treat tuberculosis, while minimizing adverse effects even upon effective dosage escalation by directing active compounds directly into infected target cells.



Endo lysosomal localization of glycan decorated liposomes.png



Glycan decorated liposomes for tuberculosis treatment.png

Design of magnetic/mesoporous silica nanocomposites as a delivery platform of antineoplastic drugs

Tuesday, 26th September - 18:29 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 88

Prof. Margarita Popova [1], ***Dr. Ivalina Trendafilova*** [1], ***Prof. Ágnes Szegedi*** [2], ***Dr. Judith Mihály*** [2], ***Prof. Denitsa Momekova*** [3], ***Prof. Spiro Konstantinov*** [3], ***Prof. Neli Koseva*** [4]

1. *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria,* ***2.*** *Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, Hungarian Academy of Sciences, 1525 Budapest, Hungary,* ***3.*** *Faculty of Pharmacy, Medical University of Sofia, 1000 Sofia, Bulgaria,* ***4.*** *Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

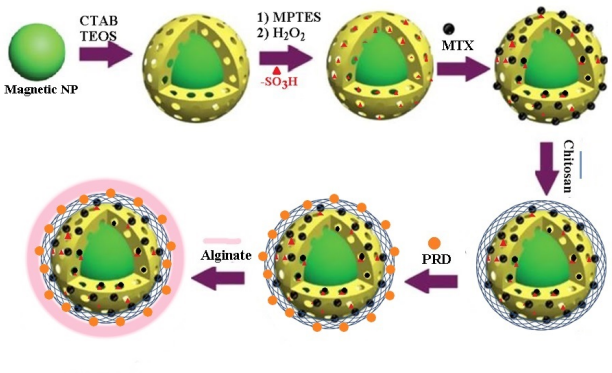
The application of mesoporous silica materials as carriers in the drug delivery systems is a promising and new approach in nanomedicine. Mesoporous silicates are characterized by narrow distribution of pores with a controlled size, large pore volume, high surface area, dual-functional surface (external and internal) and good chemical and thermal stability. New perspective in targeted delivery of antineoplastic drugs is given by mesoporous silicas integrated with magnetic nanoparticles. These composites allow selective supply of the drug to the targeted organ of the body when an external magnetic field is applied. The preparation of appropriate delivery systems based on these materials can solve some problems such as low concentration in the region of interest and toxic side effects of antineoplastic drugs.

Mitoxantrone is a synthetic antineoplastic drug, widely used as a chemotherapeutic agent in the treatment of various types of cancer. Glucocorticoids, i.e. prednisolone, have been used in clinical oncology for more than three decades because of their anti-inflammatory action that additionally influences the oncological therapy.

In the present study we developed dual delivery system of mitoxantron/prednisolone on the basis of magnetic/mesoporous silica nanocomposite carriers.

Magnetic/mesoporous silica nanocomposites were synthesized and modified by post-synthesis method with –SO_3H groups (Scheme 1). Mitoxantrone was loaded by incipient wetness impregnation on the mesoporous support (Scheme 1). The functionalized and drug loaded formulations were characterized by powder XRD, N_2 physisorption, TEM, TG analysis, ATR-FT-IR and Mössbauer spectroscopy. The results from TG and N_2 physisorption measurments show that mitoxantrone was successfully loaded in the pores of the carrier. Additionally, coating by polyelectrolyte complex containing two layers, chitosan:prednisolone and sodium alginate, resulted in controlled release of loaded drug molecules (Scheme 1). The cytotoxicity of free and formulated mitoxantrone and prednisolone was evaluated using MTT-dye reduction assay in a panel of human tumor cell lines (HL-60/Dox (acute promyelocyte leukemia), K-562 (chronic myeloid leukemia), HD-MY-Z (Hodgkin lymphoma) and EJ (bladder carcinoma). The results obtained showed that encapsulated drugs evoked prominent concentration-dependent cytotoxic effects even at low tested concentrations.

Acknowledgements: Financial support from the Bulgarian-Hungarian Inter-Academic Exchange Agreement and the National Science Fund of Bulgaria (Grant DH 09/18) is acknowledged.


CTAB
TEOS
Magnetic NP
1) MPTES
2) H_2O_2
$-SO_3H$
MTX
Chitosan
PRD
Alginate

Scheme 1.jpg

Bio-inspired, multifunctional Block Copolymers to absorb Cholesterol and Calcium – Towards dissolution of Atherosclerotic Plaques

Tuesday, 26th September - 18:46 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 157

Mr. Philipp Keckeis [1], ***Prof. Helmut Cölfen*** [1]

1. *University of Konstanz*

Coronary atherosclerosis and the related cardiovascular diseases constitute one of the most serious health problems in the Western Hemisphere, which causes around 40 % of all deaths.[1] Based on the high lethal number of coronary diseases, we introduce a novel strategy (Figure 1). Plaque-sensitive macro-surfactants were synthesized to target arterial plaques, extract partially hydrophobic and ionic compounds, encapsulate them within stable aggregates and finally, excrete over the renal pathway. The plaque is very complexly composedof solid blood components, hydrophobic components like cholesterol, but also of inorganic deposits of calcium minerals.[2],[3] The class of synthesized multifunctional triblock copolymers with covalently attached cholesteryl and anionic moieties is assumed to achieve interactions with hydrophobic and mineral parts of the arterial deposit. Moreover pendant polyethylene glycol increases the water solubility. Complementary analytical techniques (DLS, AUC, cryo-TEM, CMC fluorescence measurements and zeta-potential measurements) suggest the self-oriented arrangement of the polymers into distinct shaped, spherical particles in the lower nanometer range with a negatively charged surface. The polymeric dispersions (Figure 2) were able to dissolve and absorb solid cholesterol (up to 5.6 wt%) and calcium ions bound in mineral structures and entrap them within micellar nanoassemblies. Furthermore, ex-vivo alamar blue assays against human kidney epithelial cells negated toxic impacts of the polymers.

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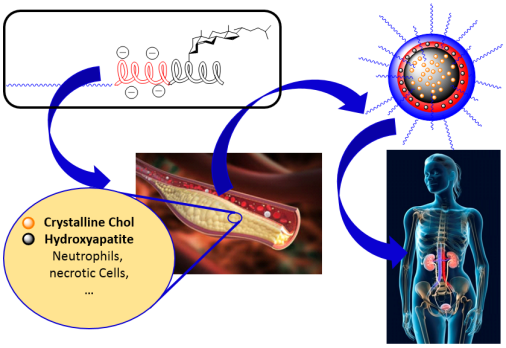


Figure 1.png

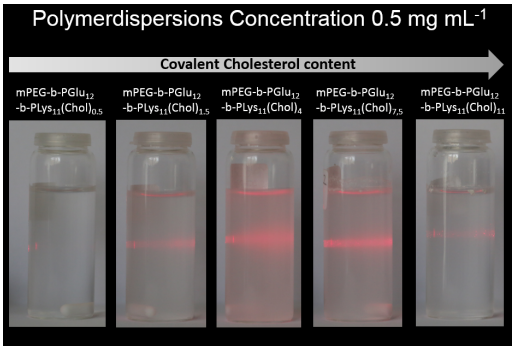


Figure 2.png

Stimuli-responsive polysaccharide nanogels for the targeted treatment of cancer

Wednesday, 27th September - 09:00 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 454

Prof. Rachel AUZELY [1]

1. *Grenoble Alpes University and CERMAV-CNRS*

Self-assembled nanogels, nanometer-sized hydrogels obtained by physical self-assembly of interactive hydrophilic polymers, have attracted growing interest for drug delivery as these systems combine the advantages of hydrogels with nanoscale formulations.[1-3] Such systems can be designed to facilitate the encapsulation of diverse classes of bioactive compounds as well as to release them in response to stimuli. Moreover, their hydrophilic shell can be exploited to control their biological fate and targeting ability. In this regard, self-assembled nanogels made of polysaccharides hold promise as a versatile nanocarrier, due to the presence of various functional groups on shell-forming polysaccharides in addition to their unique physicochemical properties, including biocompatibility and biodegradability. Among them, hyaluronic acid (HA) has been widely explored to fabricate nanogels as anti-cancer drug carriers due to the interesting biological properties of this natural polysaccharide.[4,5] Indeed, HA is a glycosaminoglycan specifically recognized by the CD44 receptor that is overexpressed by several cancer cells. In this respect, we recently focused our efforts on engineering HA-copolymer conjugates to produce nanogels exhibiting a favorable balance between long circulation and moderate stability to release their payload at the targeted location (i.e. tumor site). We will show how precise control over functionalization of HA, as well as other glycosaminoglycans, and copolymer architecture can induce original physico-chemical and biological properties of polysaccharide-based nanogels. We will also present a new family of self-assembled nanogels obtained by boronate ester bond formation between biocompatible polysaccharides. The original feature of these gels nanoparticles, in particular their sensitivity to acidic pH, can be exploited for intracellular triggered disassembly and drug release in cancer cells.

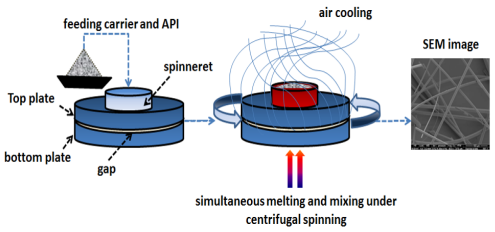
Nano-engineered drug delivery systems; from oral administration to compartmentalised particles

Wednesday, 27th September - 09:40 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 412

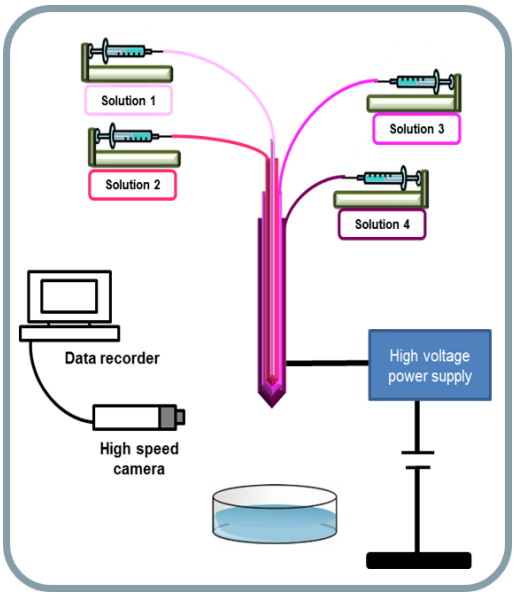
Prof. Duncan Craig [1]

1. *UCL School of Pharmacy*

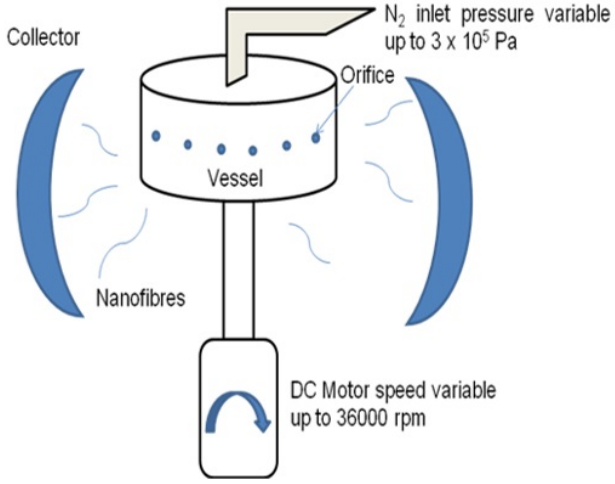
Drug delivery requires increasingly sophisticated approaches to engineer systems with release and distribution properties appropriate to the disease state in question, often at medium to large scale in order to be commercially feasible. Nano-engineered systems, including fibres and particles, offer both opportunities and challenges and this presentation will address some of these with particular emphasis on developing systems with potential for specific patient benefit. The use of nanofibres for oral delivery will be outlined, including consideration of large scale production, development of supersaturated systems on release in the gastrointestinal tract and stability characterisation. Similarly, fibres for vaginal delivery and application to wounds will be outlined. Nanoparticles produced via electrohydrodynamic approaches will be described including the development of compartmentalised systems which may incorporate both low molecular weight and biological therapeutic agents at a nanoscale. The images associated with the abstract outline three approaches to medium to large scale drug-loaded nanosystem production, namely pressurized gyration, centrifugal spinning and electrohydrodynamic spraying.



Picture5.png



Picture2.png



Picture6.jpg

Multivalent Nanosystems to Target Inflammation

Wednesday, 27th September - 10:45 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 472

Dr. Kai Licha [1]

1. *Institut für Chemie und Biochemie, Freie Universität Berlin, Takustr. 3, 14195 Berlin*

Polymer therapeutics in medicine are increasingly gaining acceptance and recognition as an independent area of scientific endeavor and pharmaceutical development. The combination of a high density of endgroups and a compact well defined molecule structure makes particularly dendritic architectures attractive for biomedical applications.[1] Due to their low degree of molecular weight dispersity and flexible design, dendritic polyglycerols (PGs) have a broad range of potential applications in medicine.[2] Dendritic polyglycerol architectures have already been demonstrated to be useful in therapeutic approaches related to multivalency because of the synergy between the nano-sized dimensions combined with the high density of functional groups.[3,4] Based on polyglycerols, several attempts have been made to mimic specific glycoarchitectures, (i) with neutral hydroxyl end groups representing analogues of polysaccharides, and (ii) polyanionic derivates similar to negatively charged polysaccharides, such as heparin.

Most recently, our group demonstrated that polyanionic, dendritic polyglycerol sulfates (dPGS) exert strong binding affinity to cellular targets involved in the inflammatory process by inhibiting leucocyte infiltration.[4] Translation into the diagnostic application was accomplished by *in vivo*fluorescence imaging in a rat rheumatoid arthritis (RA) model, demonstrating fast and highly selective targeting of tissue inflammation.[5] We also demonstrated that dPGS acts favorably in RA and osteoarthritis models, leading to chondroprotective properties[6]. Furthermore, we demonstrated chemical versatility by synthesizing shell-cleavable dPGS and mixed polyanions to modify pharmacokinetics and selectivity in bone targeting[7]and include dPGS into micelles or nanogels for targeting and drug transport[8]

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New Polymers and Strategies for Drug Delivery Applications

Wednesday, 27th September - 11:25 - Plenary Speeches - Auditorium - Plenary Speech - Abstract ID: 481

Dr. Julien Nicolas [1]

1. *Institut Galien Paris-Sud, CNRS, Univ Paris-Sud*

This lecture will present our recent achievements in the field of macromolecular engineering for biomedical applications, and especially the use of controlled radical polymerization as a tool for the design of polymer prodrug nanoparticles.[1] We developed of new class of polymer prodrug nanoparticles by using the “drug-initiated” method (see Figure),[2] which consisted in the controlled growth of hydrophobic polymers from anticancer drug-bearing alkoxyamines or chain transfer agents to prepare, by NMP or RAFT, well-defined and high drug content polymer prodrug nanoparticles with in vitro and in vivo anticancer activity.[3,4] Degradability will also be conferred to those materials by using radical ring-opening polymerization (rROP) from cyclic ketene acetal (CKA) monomers.[5,6,7]

References

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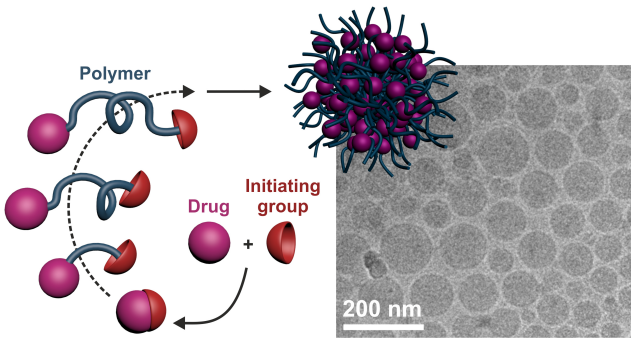

Fig.jpg

A novel six-armed PEG-b-PCL copolymer based on G1 PETIM dendrimer for nano delivery of vancomycin

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 103

Mr. Calvin Omolo [1], ***Dr. Rahul Kalhapure*** [1], ***Dr. Mahantesh Jadhav*** [1], ***Dr. Sanjeev Rambharose*** [1], ***Prof. Thirumala Govender*** [1]

1. *University of KwaZulu-Natal*

Background

Bacterial infections and antibiotic resistance are becoming major health concerns globally. Nanosized drug carrier systems are innovative strategies to overcome the limitations with conventional dosage forms for improved delivery and efficacy of antibiotics and also to overcome resistance. The synthesis of novel materials for the design of nanoantibiotics with enhanced performance is therefore essential. Therefore, the aim of this study was to synthesize a novel multiarm polymer (G-1-PETIM-PEG-*b*-PCL) using G1-poly(propyl ether imine) dendrimer (G1-PETIM) and a copolymer of methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone) (PEG-*b*-PCL) to offer high loading capacity and sustained delivery of vancomycin against susceptible and resistant *Staphylococcus aureus*.

Methods

The hybrid G1-PETIM was synthesized following a literature procedure and coupled to PEG-*b*-PCL to obtain G-1-PETIM-PEG-*b*-PCL. (Fig. 1). The synthesized G-1-PETIM-PEG-*b*-PCL was characterized for its structure by FTIR, ^{1}H and ^{13}C NMR, and MTT assay for biosafety. Vancomycin loaded micelles were prepared from G-1-PETIM-PEG-*b*-PCL, and characterized for size, surface charge, morphology, aggregation behavior, drug loading, drug release, stability and in vitro efficacy against sensitive and resistant *S. aureus.*

Results

The novel polymer G-1-PETIM-PEG-*b*-PCLwas successfully synthesized and characterized with FT-IR, ^{1}H and ^{13}C NMR. MTT assays showed a high percentage of cell viability ranging from 77% to 85% at all concentrations against all cell lines MCF 7, A549 and Hep G2 cells (Fig. 2), which confirmed the nontoxic nature of the polymer. Ultra-small unimolecular spherical micelles with average size, surface charge and polydispersity index of 52.48±2.6 nm (Fig. 3), -7.3±1.3 mV, 0.103 ±0.047 respectively and drug entrapment efficiency of >60% were successfully prepared. Vancomycin was released in a sustained manner and showed 91% release of the drug after 72 hours. The long circulation of the particles was further confirmed by the bovine serum albumin test. The formulation was found to be stable for more than 3 months. *In vitro* antibacterial tests revealed that at the end of 24 h period vancomycin-loaded micelles had 8- and 16-fold greater activity against *S. aureus* and MRSA respectively compared to bare vancomycin with prolonged activity for 5 days (Table 1). These findings confirmed the potential of G-1-PETIM-PEG-*b*-PCL as a promising nanocarrier for efficient antibiotic delivery.

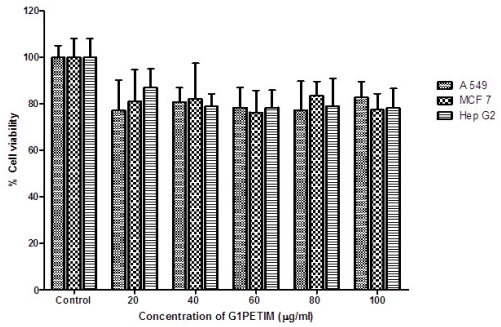

Figure 2.jpeg

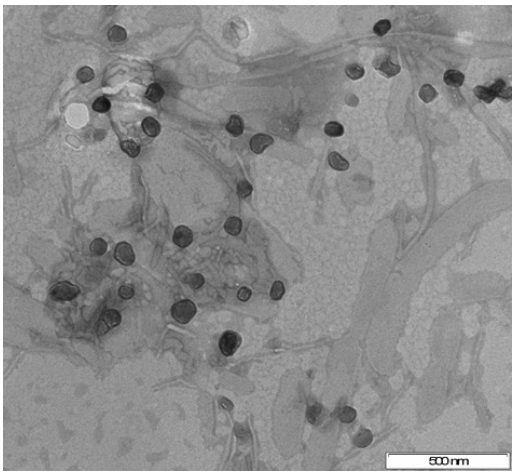

Figure 3.jpeg

Time (hours)	24	48	72	96	120	24	48	72	96	120
	S. aureus (MIC µg/mL)					MRSA (MIC µg/mL)				
Bare VCM FB	3.9	NA	NA	NA	NA	15.65	NA	NA	NA	NA
G-1-PETIM-PEG-b-PCL	0.488	0.488	0.488	0.488	0.488	0.98	0.98	0.98	0.98	0.98
Blank	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA = No activity. The values are expressed as mean ±SD, n=3.

Table 1.jpeg

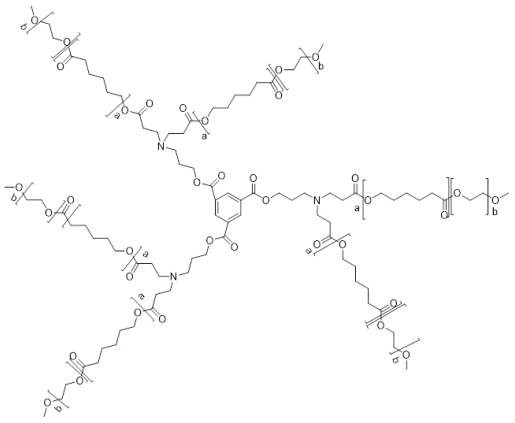

Figure1.jpeg

Changing enzyme kinetic parameters of chymotrypsin and NAD-dependent formate dehydrogenase under low-frequency magnetic field

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 359

Mr. Maxim Veselov [1], ***Prof. Natalia Klyachko*** [1], ***Ms. Mariia Efremova*** [2], ***Prof. Alexander Majouga*** [1], ***Dr. Igor Uporov*** [1], ***Dr. Yuriy Golovin*** [3], ***Mr. Ivan Kargov*** [1], ***Dr. Vladimir Tishkov*** [1]

1. *Lomonosov Moscow State University,* ***2.*** *National University of Science and Technology MISiS,* ***3.*** *Nanocenter, G. R. Derzhavin Tambov State University, Tambov, Russia*

Nanomechanical approach for biochemical reaction management means that, rotation of magnetic nanoparticles (MNPs) with immobilized on them enzyme under expose of alternative magnetic field is applied for changing an enzyme structure-activity [1, 2] (fig. 1). When enzyme immobilized between two MNPs, rotation of MNP under low-frequency magnetic field (LFMF) can significantly change the enzyme structure leading to the decrease of the enzyme activity. In case of enzyme molecules immobilized on single MNP, the hydrodynamic forces changing the enzyme structure more mild. In this work we demonstratethe effect of LFMF on activity of different enzymes, immobilized on single-domain magnetite@gold nanoparticles.

Previously we have shown [1] that enzymatic activity of chymotrypsin, immobilized on the aggregates of magnetite nanoparticles, decrease under impact of LFMF. To determine the mechanism of such inactivation we carried out a molecular modeling experiment where we applied stretch forces to chymotrypsin molecule. As a result of modeling we have found that enzyme catalytic center had no difference from the initial structure while the binding site has becomein more closed state. As a result of LFMF expose to immobilized on dimeric magnetite@gold nanoparticles chymotrypsin, we observed an increase in K_m value while V_m remained unchanged (fig 2). Thus, experimental data of kinetic parameters measurements confirmed our data on molecular modeling.

Another example of nanomechanical "devices" that could change structure-activity properties of enzyme is a single magnetic nanoparticle with enzyme, immobilized on its surface. Here, we demonstrate changing enzymatic parameters of immobilized on a single magnetite@gold nanoparticle NAD-dependent formatedehydrogenase after expose to LFMF (Table 1).

Figure 1.Different forces and deformations that appearin enzyme macromolecule, immobilized on single-domain MNP, as a result of Brownian relaxation under expose of LFMF.

Figure 2.Impact of low-frequency magnetic field(50 Hz, 0.14 T) on enzymatic rate of chymotrypsin immobilized on aggregates of magnetite@gold nanoparticles

Table 1. Changing enzymatic parameters of NAD-dependentformate dehydrogenase immobilized on magnetite@gold nanoparticles after expose to 77 Hz and 0.1 T magnetic field for 5 min

This workwas supported by RSF-14-13-00731P grant.

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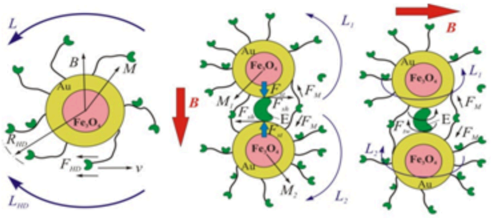


Fig 1.png

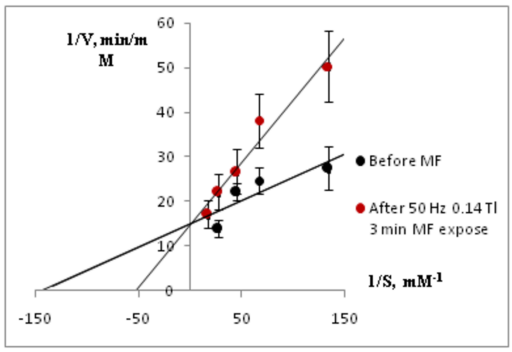


Fig 2.png

	K_M		V_M	
	Before MF	After MF	Before MF	After MF
NAD	(0.12±0.02)μM	(0.15±0.02)μM	(100±2) μM/min	(121±4) μM/min
NADP	(0.28±0.01)mM	(0.35±0.03)mM	(92±3) μM/min	(124±2) μM/min

Table 1.png

Cytotoxicity evaluation of functionalized nanocarriers on HepaRG cells

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 117

Dr. Odette Prat [1], ***Mr. Cédric Pisani*** [1], ***Dr. Estelle Rascol*** [2], ***Mr. Christophe Dorandeu*** [2], ***Dr. Clarence Charnay*** [2], ***Dr. Yannick Guari*** [2], ***Dr. Joel Chopineau*** [3], ***Prof. Jean-marie Devoisselle*** [2]

1. *CEA/ DRF/ BIAM,* ***2.*** *université de Montpellier,* ***3.*** *universite de Nîmes*

Magnetic mesoporous silica nanoparticles (M-MSN) represent possible tools for cancer diagnostic and therapy. Nevertheless, once functionalized, their biocompatibility must be assessed before to be used as nanocarriers. We investigated the cellular impact of pristine, PEGylated M-MSN, and lipid bilayer (DMPC) covered M-MSN on HepaRG cells. Based on MTT assay and real-time cell impedance, none of these NPs presented an extensive toxicity on hepatic cells. However, we observed by transmission electron microscopy that the pristine and DMPC M-MSNs were internalized. In comparison, PEG M-MSNs showed a slower cellular uptake. We carried out whole gene expression profiles in a time-and dose-dependent manner and biological data mining with a pathway-driven analysis using Ingenuity Pathways Analysis. The lowest dose tested (1.6 µg/cm²) induced no molecular effect and was defined as 'No Observed Transcriptional Effect level'. The dose 16 µg/cm² revealed nascent but transient effects. At the highest dose (80 µg/cm²), adverse effects have clearly arisen and increased over time. The limit of biocompatibility for HepaRG cells could be set at 16 µg/cm² for these NPs. At the highest dose, we highlighted the sequence of events that leads to the disruption of hepatobiliary system, elicited by the three types of M-MSNs. The Adverse Outcome Pathway of hepatic cholestasis was implicated. Toxicogenomics applied to cell cultures is an effective tool to characterize and compare the modes of action of many substances. This strategy might be an asset for upstream selection of the safest nanocarriers in the frame of drug safety regulation.

Delivery of Paclitaxel and Everolimus in HER2-Targeted Polymeric Nanoparticles to HER2 Positive Metastatic Breast Cancer

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 395

Mrs. Loujin Houdaihed [1], ***Dr. James Evans*** [1], ***Prof. Christine Allen*** [1]

1. *University of Toronto*

Introduction: The combination of paclitaxel (PTX) and trastuzumab (TmAb) has been the first line therapy for metastatic HER2+ breast cancer (BC) for nearly two decades. However, the majority of patients develop TmAb resistance and the conventional PTX currently used (Taxol®) has been associated with dose-limiting toxicities. mTOR inhibitors, such as everolimus (EVER), were found to overcome TmAb resistance and increase sensitivity to PTX. Importantly, administering PTX and EVER at a synergistic ratio could allow for reducing the dose and toxicities of PTX. This research aims to develop a HER2-targeted polymeric nanoparticle (NP) formulation encapsulating PTX and EVER to improve tumor growth inhibition and reduce PTX-induced toxicities in TmAb sensitive/resistant HER2+ metastatic breast cancer in vivo.

Methods: The combination of PTX and EVER was evaluated in BC cell lines. PTX+EVER-NPs were prepared and characterized for physicochemical properties. HER2-targeted Fab fragments were obtained by digestion of TmAb and conjugated to NPs by Amine Coupling. Internalization of FITC-labeled TmAb(Fab)-NPs vs untargeted-NPs were studied in the SKBR3 BC cell line (HER2+) using flow cytometry after 24 h exposure.

Results: The synergistic ratio of PTX: EVER combination was defined at 1:0.5 in all BC cell lines tested. PTX+EVER-NPs were spherical, ~55nm in diameter, had a total drug loading of 9% (wt%), and exhibited sustained drug release *in vitro* under physiological conditions for 168 h. A significant shift in FITC fluorescence intensity for TmAb(Fab)-NPs was observed in comparison to untargeted-NPs, indicating a significant increase in cellular uptake (3.3 fold) for TmAb(Fab)-NPs relative to untargeted-NPs in SKBR3 cell line (HER2+).

Discussion: The combination of PTX and EVER increased the growth inhibition of BC *in vitro*, including a TmAb resistant cell line, compared with PTX alone, and was encapsulated in polymeric NPs at the synergistic ratio. TmAb(Fab) fragments were prepared, characterized and conjugated to the NPs. From flow cytometry data, the amount of TmAb(Fab)-NPs internalized in SKBR3 BC cells (HER2+) was significantly increased relative to that of untargeted NPs. These data show high potential for a HER2-targeted formulation of PTX and EVER combination to improve tumor growth inhibition and reduce PTX toxicities in TmAb sensitive/resistant BC *in vivo*.

Combination Index (CI) – Paclitaxel:Everolimus (72 h)									
	1:0.05	1:0.1	1:0.2	1:0.5	1:1	1:2	1:5	1:10	1:20
TrR1	0.431	0.545	0.442	0.447	0.536	0.339	0.254	0.198	0.215
MDA-MB-231-H2N	0.830	0.735	0.847	0.701	0.681	0.366	0.204	0.239	0.189
SKBR3	0.736	0.524	0.163	0.219	0.306	0.233	0.125	0.19	0.292
MDA-MB-468	1.272	1.196	0.745	0.867	0.779	0.59	0.06	0.421	0.099
MDA-MB-231	1.147	0.759	0.837	0.429	0.167	0.423	0.148	0.158	0.152
MCF-7	0.884	1.009	0.726	0.673	1.097	1.136	0.143	0.339	0.289

Combination Index (CI)	>1.3	1.1-1.3	0.9-1.1	0.8-0.9	0.6-0.8	0.4-0.6	0.2-0.4
Description of combination effect	Antagonism	Moderate antagonism	Additive	Slight synergism	Moderate synergism	Synergism	Strong synergism

Table 1. Summary of combination index (CI) values for PTX-EVER combination at fraction of affected cells of 0.75 (Fa 75) (i.e. at 75% cell death). The combination of PTX and EVER exhibited synergistic effects in BC cell lines, including a TR HER2+ BC cell line.

Ci heat map.png

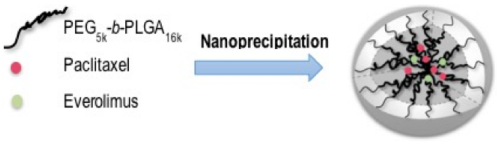


Figure 2. Schematic design of PTX+EVER-loaded polymeric NPs.

Schematic design of nps.png

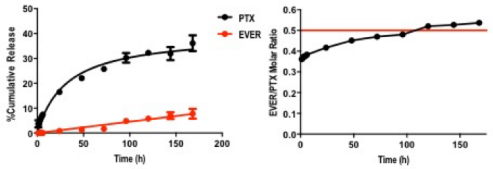


Figure 3. In vitro drug release of PTX and EVER from NPs in PBS containing BSA (50 mg/ml) at 37°C. Each data point represents the mean ± SD of 3 different experiments. *In vitro* drug release studies revealed sustained release for both drugs under physiological conditions for 168 h. The synergistic molar ratio of PTX to EVER (1:0.5) has been maintained within the NPs for over 168 h.

In vitro drug release.png

Table 1. Summary of characteristics of PTX+EVER-loaded polymeric NPs.

PTX:EVER Loading Efficiency (L.E.%)	PTX:EVER Loading Content (L.C.%)	PTX (mg/ml)	EVER (mg/ml)	PTX:EVER Molar Ratio	Size (nm)	Zeta Potential (mV)
40.1 ± 3.2	9.1 ± 0.7	1.4 ± 0.0	0.62 ± 0.1	1:0.39	55.2 ± 5.4	-17.2 ± 2.6

Characteristics of nps.png

Evaluation of adenoviral dodecahedron in vivo tropism.

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 339

Mrs. Marta Jedynak [1], ***Dr. Inga Szurgot*** [1], ***Dr. Monika Zochowska*** [1], ***Mrs. Malgorzata Podsiadla-Bialoskorska*** [1], ***Dr. Jadwiga Chroboczek*** [2], ***Dr. Ewa Szolajska*** [1]

1. *Institute of Biochemistry and Biophysics Polish Academy of Sciences,* ***2.*** *Université Grenoble Alpes, CNRS, Grenoble INP, TIMC-IMAG*

Introduction. Development of efficient non-toxic delivery systems remains a big challenge for targeted cancer therapy. Adenoviral dodecahedron (Dd), a virus-like particle with outstanding cell penetration capabilities, is able to deliver many copies of bioactive molecules into one cell. Cellular and organ tropism evaluation is essential for selecting therapeutic targets for carrier applications. Here we present vector distribution upon intravenous and intratumoral injection in human melanoma xenografts in mice model. Moreover, we demonstrate *in vitro* analysis of passage accross blood brain barrier (BBB) and Dd translocation between cancer cells.
Methods. To follow Dd distribution in melanoma xenografts 2D fluorescence imaging and real time imaging were used. Dd transport via BBB in MDCKII-MDR1 model was analyzed by Western Blot. To gather the data about cell-to-cell translocation we used fluorescence microscopy.
Results. Upon intratumoural injection, Dd displays over 24-hours persistence in tumours, while nearly no signal is observed in the isolated organs. Intravenous injection results in a spread distribution and the *ex vivo* imaging of organs and tumours isolated 5 hours after injection shows the highest level of Dd in the liver and the skin. Moreover, *ex vivo* imaging of isolated brains as well as *in vitro* analysis in MDCKII-MDR1 model show that Dd is unable to pass through BBB. Results of *in vitro* studies show that Dd is able to translocate between adjacent cancer cells.
Discussion. Dd persistence in tumour upon intratumoral injection, without spreading on healthy organs suggests that it may be considered as the optimal route of administration in animal studies. Moreover, Dd translocation between neighboring cells, may explain our previous observation, concerning delivery of Dd-drug conjugate to solid tumours in rat model, which resulted in tumour growth inhibition. Dd inability to pass through the BBB on the one hand excludes delivery of factors acting on the central nervous system (CNS), however on the other hand enables therapeutic applications of the carrier, without causing side effects on CNS.
Acknowledgements. This work was financially supported by the National Science Centre (Poland) (DEC-2013/09/B/NZ3/02327)

Exploiting the Protein Corona around Gold Nanorods for Laser-Triggered Tri-modal Cancer Therapy

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 80

Ms. Eugenia Li Ling Yeo [1], Mr. Joshua Cheah [2], Mr. Bing Yi Lim [1], Dr. Patricia Soo Ping Thong [3], Dr. Khee Chee Soo [3], Dr. Kah Chen Yong James [1]

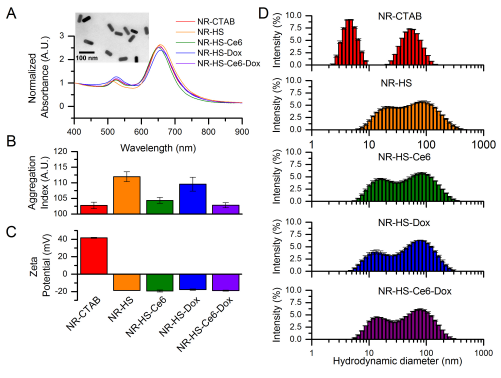
1. *National University of Singapore,* ***2.*** *NUS Graduate School for Integrative Sciences and Engineering,* ***3.*** *National Cancer Centre Singapore*

A novel nanoparticle-based multi-modal phototherapy consisting of gold nanorods (AuNRs) co-loaded with the photosensitizer Chlorin e6 (Ce6), and chemotherapeutic agent Doxorubicin (Dox) within a pre-formed human serum (HS) protein corona, i.e. AuNR-HS-Ce6-Dox, was synthesized in order to perform simultaneous photothermal (PTT), photodynamic (PDT) and chemotherapy (CTX) under single laser irradiation at a wavelength of 665nm.

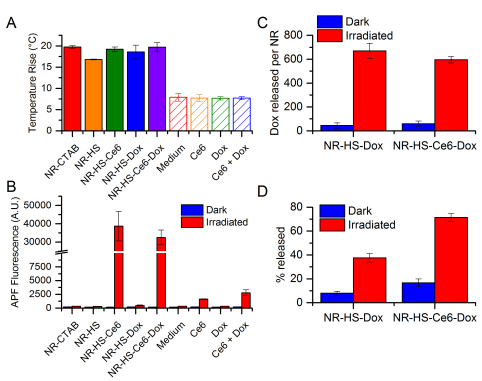
The excitation of AuNRs and Ce6 resulted in photothermal ablation (PTT) and production of reactive oxygen species (ROS) to elicit oxidative stress (PDT) in Cal 27 oral squamous cell carcinoma (OSCC) cells, while laser-triggered Dox release and subsequent DNA intercalation resulted in additional DNA damage (CTX) – eventually resulting in cell death.

High laser-triggered Dox release efficiency of 71.5 % as well as strong AuNR plasmonic enhancement of ROS production – as verified with 3'-(p-aminophenyl) fluorescein (APF) – by loaded Ce6 (11.7-fold increase as compared to free Ce6) was observed. Uptake of both Ce6 and Dox co-loaded on AuNRs by Cal 27 cells was also greatly enhanced, as demonstrated using flow cytometry and confocal laser scanning microscopy (CLSM), with 3.3 and 52 times higher intracellular Dox and Ce6 uptake fluorescence observed respectively after a 6 h dose of AuNR-HS-Ce6-Dox as compared to the use of free Ce6/Dox.

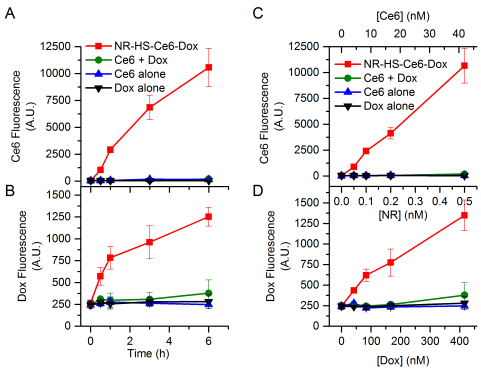
The simultaneous laser-triggered tri-modal therapy also achieved a near complete eradication of cancer cells (98.7% cell death) with an extremely low dose of AuNR-HS-Ce6-Dox (15pM AuNR loaded with 1.26 nM Ce6 and 12.5 nM Dox), and low dark toxicity was observed. Additionally, the loaded drug concentrations were far lower than any previously reported *in vitro*, thereby greatly minimizing potential systemic toxicity of these agents. Finally, enhanced treatment efficacy was attributed to strong synergistic enhancement between therapies, as compared to individual therapies performed separately.



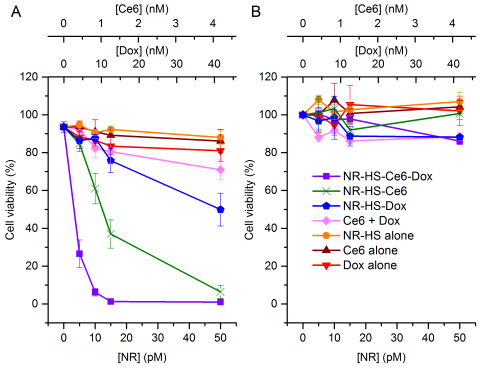
1. physical characterization of aunr-hs-ce6-dox with controls.jpg



2. effect of 665nm laser irradiation of aunr-hs-ce6-dox with controls.jpg



3. uptake of ce6 and dox by cal 27 oscc cells as measured by fluorescence under flow cytometry.jpg



4. cell viability of cal 27 oscc cells when dosed with increasing concentrations of aunr-hs-ce6-dox.jpg

Fabrication and functionalization of biocompatible Fe3O4@Graphene Oxide hybrid for biomedical applications

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 452

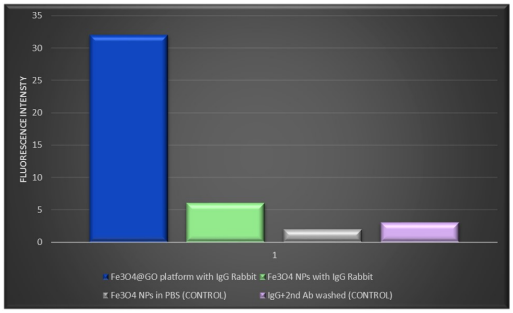
Ms. Katarzyna Karpinska [1]

1. *The University of Manchester*

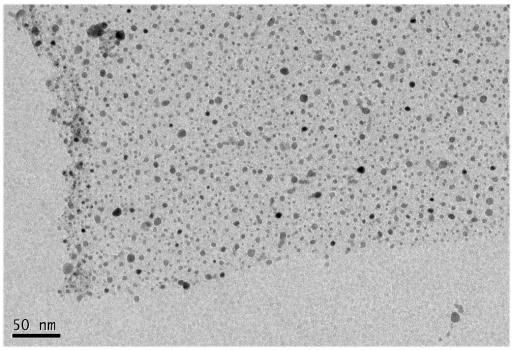
Functionalized nano platforms have enormous potential in targeted therapies and controlled delivery of drugs. Although different production techniques have been developed in recent years, methods used to generate uniform in size, stable in a solution and pure NPs are still waiting to be optimised. Functionalization of the NPs and further conjugation with different biomolecules remain a great challenge.

Using chemical and laser methods we have generated different biocompatible and biodegradable nanoparticles e.g. Chitosan NPs, Polylactic acid NPs, Iron Oxide NPs and NPs modified with Poly-L-lysine. Among these, Iron Oxide NPs with additional magnetic properties, were further functionalized by simultaneous *in situ* attachment to Graphene Oxide (GO) using one–step chemical method. Fe_3O_4@GO hybrid have uniform ~10nm magnetic NPs decorated on top of the GO nano/micro flakes and can be easily separated by magnetic field. Using carbodiimide crosslinker chemistry we have successfully conjugated IgG molecules as well as antimicrobial peptide on to the Fe_3O_4@GO nano hybrids, respectively.

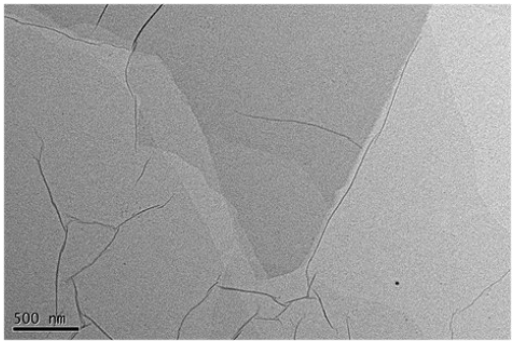
Magnetic properties of functionalized Iron Oxide@GO hybrid could be very useful for different biosensors and directing the nano-hybrids to target tissues by external magnetic field. Future work will aim to link cell-type specific antibodies and nucleic acids such as siRNAs to achieve precisely targeted therapy.



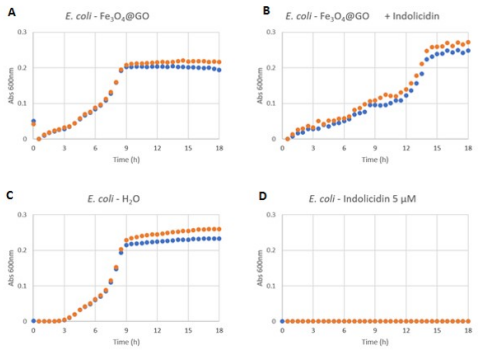
Fluorescent detection of igg in conjugates with fe3o4 go composites.jpg



Tem pictures of fe3o4 go hybrid.jpg



Tem pictures of unmodified go.jpg



Test of the susceptibility of e. coli to fe3o4 go with indolicidin.jpg

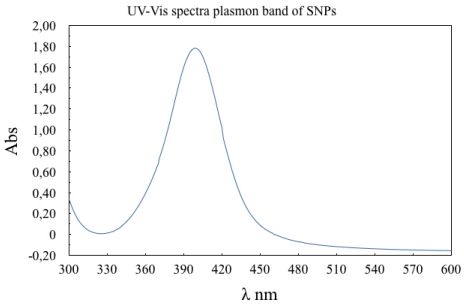
Fast and reproducible electrochemical synthesis of negatively charged Silver Nanoparticles (nSNPs) for microbiological application.

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 337

Dr. Luca Scotti [1], ***Dr. Angelini Guido*** [1], ***Dr. Carla Gasbarri*** [1], ***Prof. Tonino Bucciarelli*** [1]

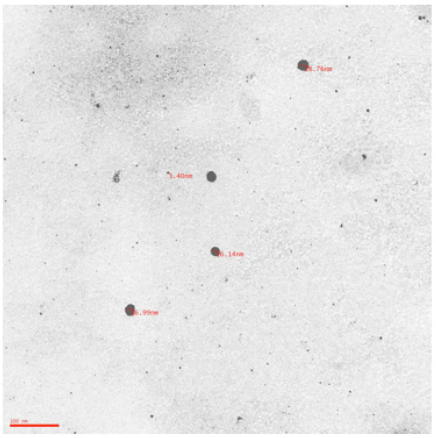
1. *University of Chieti-Pescara*

Introduction:
Metal nanoparticles are an effective antibacterial solution as an efficient and safe solution for the standard additive (such as the aromatic compound) in food, pharmaceuticals, cosmetics, …
Nanoparticles have a single chemical-physical property for microbiological application (such as the antimicrobial agent for infectious diseases).
In this preliminary study fifteen different species of procariota microorganism are used for tested the efficacy of silver NPs. Eucariota microorganism selected rapresent a major severe compliance for one specific pathology (cistic fibrosis).
The synthesis process is total controlled by electrical equipment assembled and designed in our laboratory.
Methods:
Electrochemical synthesis was used for produced an silver nanoparticle solution with pH < 8 and was loaded with analytical techniques (TEM, SEM-XRD, UV-Vis, Light Dispersion) and tested with microbiological microorganisms (> 10 different species: pseudomonas, coli, streptococcus …).
Microbiological data are important evidence for application to pathological diseases.
All synthesis proces are controlled by supervisor on electronic device assembled and designed in our laboratory; this controller is necessary for reproducible method.
Parameters contolled: temperature, reaction time, time stirrer and electrochemical parameter (such as Intensity-mA/cm, ddp, wave form).
Results and Discussion:
We present a fast, reproducible and inexpensive electrochemical method for synthesizing a smoler silver nanoparticle (8.34 ± 4.09 DS particles) with negative charge (-50 ± 5 mV) without chemical stabilization (without citrate, ascorbate …) in ultra pure aqueous solution .
Low reaction time (< 10 minute) and silver concentration is 30-40 ppm depending of running time.
The solution is stable for 3 months if is capped under nitrogen and stored at 0-5 °C in a dark box .
Tha stability are confirmed by UV-Vis,Light scattering,TEM,SEM analitical technichs.
The SNPs synhtesised are tested and we have evidence of bacteriostatic and bactericide effect (MIC,MBC,Time killing).
Low MIC and MIB concentration (1:30;1/16) are found for any microorganism tested.
Experiment evidence an probaly mechanism of cellular damage (ultrastructure TEM analysis) are present.

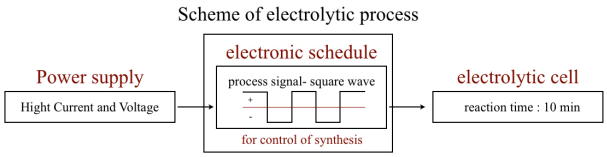


Uv-vis plasmon snps.jpg

TEM image of SNPs suspended in water



Tem image of snps.jpg



Scheme of electrolytic process.jpg

Favouring T cell migration using nanoparticle mediated hyperthermia to modulate the tumor extracellular matrix.

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 439

Ms. Alba Nicolas-Boluda [1], ***Ms. Sarah Barrin*** [2], ***Dr. Chahrazade Kantari-Mimoun*** [2], ***Dr. Javier Vaquero*** [3], ***Dr. Gilles Renault*** [2], ***Dr. Stéphane Roux*** [4], ***Dr. Laura Fouassier*** [3], ***Dr. Amanda A K Silva*** [1], ***Dr. Emmanuel Donnadieu*** [2], ***Prof. Florence Gazeau*** [1]

1. *Laboratoire Matière et Systèmes Complexes (MSC), CNRS-UMR 7057, Université Paris Diderot,* ***2.*** *INSERM U1016, Institut Cochin , Paris , France ; CNRS, UMR8104 , Paris , France ; Université Paris Descartes, Sorbonne Paris Cité , Paris , France,* ***3.*** *Sorbonne Universités, UPMC Université Paris 6, INSERM, Saint-Antoine Research Center, F-75012, Paris, France,* ***4.*** *Université Bourgogne Franche-Comté, Institut UTINAM - UMR CNRS 6213*

Solid tumors are characterised by an aberrant organisation of the extracellular matrix (ECM) in the form of highly reticulated and long linear collagen fibres. The excessive and aberrant deposition of collagen contributes to tumor stiffness, generally correlated to a negative prognosis in many human carcinomas. Also, the aberrant organisation of the ECM directly affects the migration of T lymphocytes, preventing their penetration into the tumor islets and precluding them from exerting their cytotoxic action. There are currently many strategies to modify tumor ECM based on ECM-targeting chemical agents, however these have showed deleterious side-effects. The use of excitable nanoparticles, allowing localised and remote heating in a spatio-termporal controlled manner, has shown being able to perturb tumor ECM. Here we characterise the effect of tumor-localised hyperthermia therapy mediated by gold-decorated iron oxide nanoflowers in the evolution of tumor stiffness and hence, extracellular matrix integrity in a xenograft model of choloangiocarcinoma (biliary duct tumor). Shear wave elastography (SWE) was used to map tumor stiffness. In addition, the effect of the modification of the tumor ECM on the distribution and migration of T cells was characterised using confocal microscopy.

Functionalization of harmonic nanoparticles for targeted tumor imaging and multimodal cancer diagnosis

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 86

Mr. Raphaël de Matos [1], ***Mr. Jérémy Vuilleumier*** [1], ***Dr. Solène Passemard*** [1], ***Dr. Davide Staedler*** [2], ***Dr. Samuel Constant*** [3], ***Dr. Luigi Bonacina*** [4], ***Prof. Sandrine Gerber-Lemaire*** [1]

1. *EPFL Lausanne,* ***2.*** *Tibio Sagl, CH-1131 Tolochenaz,* ***3.*** *Epithelix, CH-1228 Plan les Ouates,* ***4.*** *Université de Genève*

The recent and rapid progress in nanotechnologies has paved the way for the investigation of nanomaterials in clinical settings for early detection, diagnosis and targeted treatment of cancer, which represents a major health burden in developed countries.[1] The ability to produce inorganic nanoparticles of tunable size and composition, combined with their surface properties suitable for chemical functionalization have generated intense efforts to develop novel theranostic tools based on multifunctional nanomaterials.[2]

In this work, we present the synthesis of an Erlotinib analogue as targeting ligand for epithelial growth factor receptor (EGFR) which is an important prognosis biomarker for breast cancer. This compound was evaluated for its selective association to cancer cells and was further conjugated to poly(ethylene glycol) coated bismuth ferrite (BiFeO3, BFO) nanoparticles (NPs) through click reaction.[3] Development of a synthetic pathway for fibroblast activation protein α inhibitors suitable for post-conjugation to coated imaging harmonic NPs is currently investigated for targeting the tumor microenvironment.

Taking advantage of the second harmonic generation properties of the BFO NPs, the resulting nanomaterials were evaluated for their ability for cancer cells and tissue imaging by multiphoton microscopy.

References

[1] Tassa, C.; Shaw, S. Y.; Weissleder, R. *Acc. Chem. Res.* **2011**, *44*, 842-852; Yigit, M. V.; Moore, A.; Medarova, Z. *Pharm. Res.* **2012**, *29*, 1180-1188.

[2] Smith, L.; Kuncic, Z.; Ostrikov, K. K.; Kumar, S. *J. Nanomater.* **2012**, Article ID 891318, 7 pages.

[3] Passemard, S.; Staedler, D.; Sonego, G.; Magouroux, T.; Schneiter, G. S.; Juillerat-Jeanneret, L.; Bonacina, L.; Gerber-Lemaire, S.

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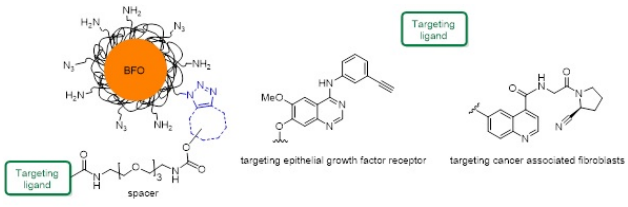


Functionalized bfo nps and ligands.jpg

Graphene plates and their derivates in glioma therapy.

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 492

Dr. Marta Grodzik [1], ***Mr. Jarosław Szczepaniak*** [1], ***Dr. Sławomir Jaworski*** [1], ***Ms. Barbara Strojny*** [1], ***Ms. Joanna Jagiełło*** [2], ***Ms. Emilia Sołtan*** [3]

1. *Warsaw University of Life Sciences, Faculty of Animal Sciences, Department of Animal Nutrition and Biotechnology,* ***2.*** *Institute of Electronic Materials Technology,* ***3.*** *Center of Oncology - Maria Sklodowska-Curie Memorial Institute*

Glioblastoma multiforme (GBM) is a malignant primary tumor. The most important feature of this type of cancer is the presence of necrotic areas and atypical vascularization as well as the presence atypical cells with nuclear pleomorphism and high proliferative ratio. Despite many years of research glioma is still the most deadly form of human cancer. It is therefore necessary to seek innovative experimental therapies to treat this type of tumor. Graphene and their derivates are a 2D carbon sheet with a honeycomb structure that exhibits extraordinary physical and electrical properties. One outstanding electrical feature of graphene is that its electron mobility derives from the presence of pi (π) electrons located above and below the graphene sheet. Graphene has many biological activity, including anticancer properties. In this work 3 forms of graphene oxide plate (GO), 2 forms of graphene plates (GR), and 4 forms of reduced graphene oxide plate (rGO) were synthesis, described and its influence on glioma IV cells was investigated, using U87, T98G and A172 cell lines as a model. There were tested five concentrations of each plates: 5, 10, 25, 50, 100 μg/ml. The morphology of cancer cells treated with GO, GR and rGO was assessed using the light microscopy. To determine the cytotoxic effect of plates on U87, T98G and A172 cell line, were used Cell Proliferation Assay (MTT), that allow to asses metabolic activity of cells. The viability of the cell lines was evaluated using PrestoBlue® viability test. Each of plates type had a differential effect on U87, T98G and A172 cell line. Six types of platelets reduced cell viability and proliferation with increasing doses, but three of them increased the number of glioma cell and the mitochondrial metabolism. There were any interaction between type of plates and cell effects. Graphene and their derivates have potential in glioma therapy but only after individual adjustment.

This work is supported by the National Centre for Research and Development (NCBiR) under Grant LIDER/144/L6/14/NCBR/2015

High-throughput method screening of small bispecific antibody for cancer therapy

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 200

Mr. Aruto Sugiyama [1], ***Prof. Mitsuo Umetsu*** [1], ***Dr. Hikaru Nakazawa*** [1], ***Dr. Ryutaro Asano*** [1], ***Dr. Teppei Niide*** [1], ***Prof. Izumi Kumagai*** [1]

1. *Tohoku University*

Various types of the recombinant antibody format have been designed from the domains. One of them, Bispecific diabody is designed only from two kinds of the fragments of variable region (Fvs). The diabody composed of the Fvs from anti-cancer and anti-lymphocyte antibodies can recruit cancer cells and T-lymphocyte, and induce T-cell–mediated cytotoxicity (Fig. 1). However, their cytotoxicity is critically dependent on their structural and functional properties. Here, we constructed an optimized procedure for identifying highly cytotoxic antibodies from a variety of the T-cell–recruiting antibodies engineered from a series of antibodies against the epidermal growth factor receptor family for cancer and antibodies against CD3 and CD28 for T-cell retargeting. By developing and applying a set of rapid operations for expression vector construction and protein preparation (scheme. 1), we screened the cytotoxicity of 104 small antibodies with diabody format and identified some with 10^3-times higher cytotoxicity than that of previously reported active diabody.

The results demonstrate that cytotoxicity is enhanced by synergistic effects between the target, epitope, binding affinity, and the order of heavy-chain and light-chain variable domains. We demonstrate the importance of screening to determine the critical rules for highly cytotoxic antibodies.

In conclusion, a domain library approach generated various bispecific diabodies with a wide-range of cytotoxicity. The results of this screening process demonstrate that cytotoxicity changes drastically according to the Fv used and the domain order, and provide critical rules for the designing diabody with high cytotoxicity, effective Target on the T-lymphocyte is CD3, the domain order is LH-type than HL-type, effective target on the TFK-1 cancer cells is EGFR, using anti-EGFR antibody recognizing EGF-bound area on folded EGFR with high affinity. In general, parental antibodies binding the desired target are selected from large-scale libraries, but combinatorial optimization of the choice of Fv fragments and domain order to construct highly cytotoxic bispecific antibodies has not been attempted previously. Our results reveal that the construction of a diabody library from parental antibodies, used in combination with our novel screening method enables the rapid selection of Fvs suitable for constructing highly cytotoxic bispecific antibodies.

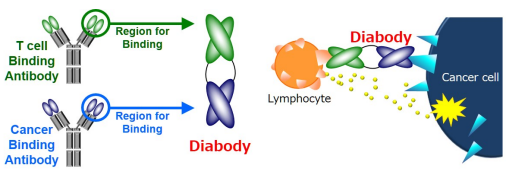
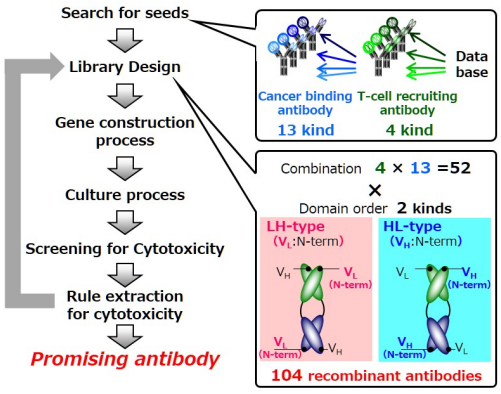


Figure 1 structure of the diabody and principle of the cancer therapy.jpg



Scheme 1 scheme for screening promising antibodies.jpg

Hybrid metal-polymer nanoparticles as promising radiosensitizers for cancer treatment

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 133

Ms. Marine Le Goas [1], ***Dr. Aurélie Paquirissamy*** [1], ***Dr. Béatrice Cambien*** [2], ***Dr. Thierry Pourcher*** [2], ***Dr. Geraldine Carrot*** [1], ***Dr. Jean-Philippe Renault*** [1], ***Dr. Serge Palacin*** [1]

1. *NIMBE UMR 3685, CEA, CNRS, Université Paris-Saclay, CEA Saclay 91191 Gif sur Yvette Cedex,* ***2.*** *Laboratoire TIRO, UMRE 4320, iBEB, DSV, CEA, Nice / Université de Nice-Sophia Antipolis, Nice*

Nanotechnologies are being widely studied for medical applications, both diagnosis and treatment. They have already shown great promise, especially to treat cancer through various strategies such as chemotherapy, photothermal therapy or radiation therapies. High-Z elements nanoparticles are of particular interest for the latter, considering their ability to amplify the damaging effects of both photon and ion radiations: gold, platinum and gadolinium are amongst the most investigated elements. [1][2]

A well-controlled synthesis is key to obtain stable and scalable nano-objects. Here, various polymers were grafted onto metallic nanoparticles to improve stability and biocompatibility and to facilitate subsequent functionalization. Advanced methods of characterization attested to the robustness and reproducibility of the synthesis procedure. Moreover, promising results were obtained regarding the radioenhancing properties of these hybrid nanocompounds.

Polymers mainly synthesized *via* controlled radical polymerization were grafted onto gold and platinum nanoparticles by a “grafting to” or “grafting from” method. Subsequent grafting of a chemotherapy drug onto the polymer corona was also successfully carried out.

The resulting nano-objects were fully characterized by thermogravimetric analysis, transmission electronic microscopy and small-angle x-ray scattering. Small-angle neutron scattering was also performed, taking advantage of possible contrast matching. The impact of various radiation doses on the nanoparticles structure was studied. Finally, radiosensitizing effects were investigated through *in vitro* tests.

Under irradiation, uncoupling and cleavage of polymer chains were demonstrated, leading to an overall size reduction of the hybrid nano-objects. The location of target sites during irradiation was determined (see attached figure) and helped to better understand the underlying mechanism of the radiosensitization assessed by the *in vitro* results.

The synthesized nano-objects have therefore shown great potential to enhance radiation cancer treatment. Their stability and controlled surface chemistry will allow to develop multiple strategies to further improve their radiosensitizing effect and *in vitro* behavior. *In vivo* tests are currently under study, as well as experiments regarding radioenhancement for proton therapy.

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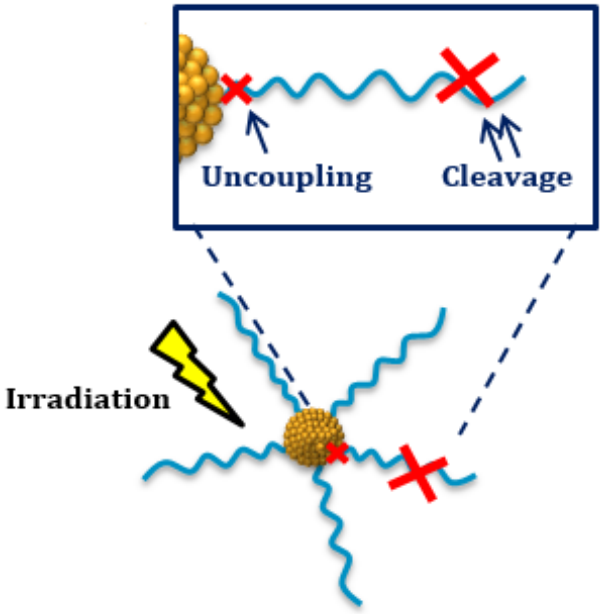


Location of radiation effects on the nano-objects.png

In vitro montmorillonite clay screening for advanced hybrid material development as chemotherapeutic drug carrier.

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 105

Mrs. Madalina Icriverzi [1], ***Dr. Paula Ecaterina Florian*** [1], ***Dr. Mihaela Trif*** [1], ***Mrs. Ioana Catalina Gifu*** [2], ***Dr. Raluca Ianchis*** [2], ***Dr. Anca Roseanu*** [1]

1. *Institute of Biochemistry of the Romanian Academy, Bucharest, Romania,* ***2.*** *National R-D Institute for Chemistry and Petrochemistry ICECHIM*

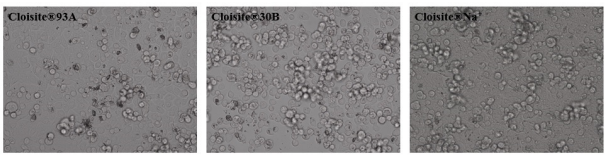
Introduction: Different anti-cancer carrier and delivery systems were developed over time. Recently montmorillonite clay particles are combined with polymers in order to create an advanced network for a better modulation of drug release. In this study the cytotoxicity effect induced by natural montmorillonite, Cloisite®Na^+ and the organically modified ones, Cloisite®30B and Cloisite®93A over time on two cell lines, MDBK and HT29 was assessed.

Methods: All mentioned clays (Southern Clay Products Inc.) were characterized by FTIR and TGA analysis. The diameter size and the polydispersity index (PDI) were determined by DLS using a ZetaPlus instrument. Cellular viability and proliferation of normal MDBK and colon adenocarcinoma HT29 cells untreated or treated with different concentrations of clays (31.25µg/ml- 500µg/ml) was evaluated using a colorimetric non-radioactive assay (MTS). Morphology of cells exposed to clays was monitored in time using bright field microscopy technique, using TissueFAXSiPlus imaging system.

Results: FTIR spectra revealed specific peaks for montmorillonite clays. TGA curves of commercial clay indicate three steps of weight loss. The z-average mean of all types of cloisite was between 2000-3000 nm. PDI, reflecting the particle size distribution in each suspension variant indicated values between 0.226 and 0.292. The shape of DLS graphs also confirmed that all variants of clays were polydisperse and had a tendency to form aggregates. The MTS assay revealed that after 24 and 48 hours of exposure, irrespective of cloisite type, high concentrations of clay particle present significant cytotoxic properties probably due to the aggregate formation which cover cell monolayer and inhibit cell proliferation. Treatment with 31.25µg/ml of Cloisite®93A for up to two days showed the highest viability on both cell lines compared with control (untreated cell), results confirmed by cell morphology analysis under the light microscope.

Conclusions: The present findings show that Cloisite®93A might be a good candidate for incorporation in a complex biopolymer semi-interpenetrated networks for the improvement of chemotherapeutic drug delivery into gastrointestinal tract.

Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2016-1896 and Romanian Academy Project 3 of the Institute of Biochemistry/2017.



Bright-field images of human coloncarcinoma HT-29 cells after exposure for 24h with 31.25µg/ml of different cloisites (magnification 20x).

Ht-29 cells-cloisite.jpg

In vivo imaging and local persistance of polymeric micro- and nanomaterials labelled with the near-infrared dye IR820

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 170

Ms. Isabel Ortiz de Solorzano [1], ***Dr. Gracia Mendoza*** [1], ***Dr. Inmaculada Pintre*** [1], ***Ms. Sara Garcia-salinas*** [1], ***Dr. Victor Sebastian*** [1], ***Dr. Vanesa Andreu*** [1], ***Dr. Marina Gimeno*** [1], ***Dr. Manuel Arruebo*** [1]

1. *Universidad de Zaragoza*

INTRODUCTION

In biomedical applications, the use of micro- and nanomaterials as drug delivery carriers can enhance the efficiency of treatments by avoiding side effects[1].The binding of a dye to a drug or to a drug-carrier has opened a wide range of possibilities for an efficient drug biodistribution tracking[2]. Optical imaging techniques are effective approaches to demonstrate drug biodistribution *in vivo* using non-invasive real-time methodologies.[3,4] The aim of this work is the development of novel polymeric micro- and nanomaterials of different sizes fluorescently labelled with the NIR dye IR820 to track their *in vivo* biodistribution after intramuscular and subcutaneous administration.

EXPERIMENTAL METHODS

Materials synthesis

Different micro and nanoparticles based on PLGA (poly(lactic-co-glycolic acid))and on PNIPAm (Poly(N-isopropylacrylamide)) have been chemically modified to include the NIR dye IR820 by using carbodiimide coupling. PNIPAM-IR820 microgels were obtained by conventional batch synthesis and PNIPAM-IR820 microparticles (MPs) were prepared by microfluidic LED polymerization.

In vitro assays

The cytotoxicity of the labelled materials was studied at three essential levels: cell metabolism, cell cycle, both on five different cell types; and endotoxin content.

In vivo studies

In vivo imaging studies were performed with five-to-eight-week-old female BALB/c nu/nu mice with the IVIS® Lumina Xenogen equipment. A complete histopathologic study was carried out.

RESULTS AND DISCUSSION

We will describe the synthesis and characterization of the resulting fluorescently labelled micro- and nanoparticles. The resulting materials showed sizes ranging from 120 nm to 410 μm. The in vitro biological studies revealed a high biocompatibility of the developed materials at doses up to 1 mg/mL on five different cell lines as well as the absence of potentially harmful bacterial contamination. The *in vivo* imaging system (IVIS) analysis performed in nude mice allowed the long-term tracing of materials biodistribution after intramuscular and subcutaneous administration showing their local persistence and the biocompatibility after histopathological studies.

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Insight into the interactions between silver nanoparticles and tumor necrosis factor signaling

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 114

Dr. Kamil Brzóska [1], ***Ms. Katarzyna Sikorska*** [1], ***Dr. Iwona Grądzka*** [1]

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Introduction

The ever-increasing development of nanoparticles with various physicochemical properties for different industrial applications has greatly enhanced human exposure to nanomaterials. This exposure can be deliberate, such as in applications where nanoparticles are used as imaging agents or drug carriers, and unintentional, e.g., through nanoparticles pollution of the environment by industrial production. The aim of the presented study was to assess the possible interference of silver nanoparticles (AgNPs, 20 nm, BSA coated) with the cellular signaling activated by the tumor necrosis factor (TNF, formerly known as TNFα) in HepG2 liver hepatocellular carcinoma cell line.

Methods

HepG2 cells were incubated with TNF or/and AgNPs for various time periods. Absorption of AgNPs was confirmed cytometrically using side-scattered light. Viability was assessed after 24 hours by neutral red uptake assay. Cell reproductive death was determined by clonogenicity test after 7-12 days. Expression of the genes associated with apoptosis and NF-kappaB signaling was analyzed by real-time PCR.

Results

During 24-hour incubation, the effect of TNF and AgNPs on viability of cells was additive. Over a longer incubation period (7-12 days), in the clonogenicity test, the effect of TNF and AgNPs on the cell survival was synergistic. Analysis of transcriptional response to TNF in the presence or absence of AgNPs revealed that the expression of *TNFSF9* was enhanced in TNF and AgNPs treated cells compared to that observed after TNF alone. This effect was observed after treatment for both 6 and 24 h. AgNPs augmented also the expression of cytokines *IL10*, *TNFSF15* and *TNFSF8* but only after longer treatment (24h). On the contrary, the expression of *BAG3* was augmented only after short treatment (6h).

Discussion

The presented results suggest that AgNPs may interfere with the cellular response to TNF and disrupt the cellular homeostasis contributing to the development of malignancies, such as cancer or autoimmune diseases at the level of the organism. Therefore, an extended study is needed to provide more information about the nature and specificity of the functional interactions between TNF and AgNPs in cells.

This work was supported by the grant 2014/13/D/NZ7/00286 from National Science Centre, Poland.

Magnetite nanoclusters as promising material for biomedical applications

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 124

Mr. Aleksey Nikitin [1], ***Ms. Mariia Fedorova*** [2], ***Ms. Natalia Fedorova*** [2], ***Mr. Igor Shchetinin*** [3], ***Dr. Victor Naumenko*** [4], ***Mr. Maxim Abakumov*** [5], ***Prof. Alexander Savchenko*** [6], ***Prof. Alexander Majouga*** [4]

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The development of nanoparticle-based systems for diagnostic and therapeutic purposes is one of the perspective areas in modern biomedicine. From the number of different nanomaterials, magnetic nanoparticles in particular magnetite nanoparticles (MNPs) are certainly the most promising material for biomedical applications, including magnetic-resonance imaging (MRI), hyperthermia, cell-labeling and others [1]. Shape- and size-controlled synthesis of nanoparticles has become a recent focus, because different shapes of particles can introduce novel magnetic and electric properties, which affect the parameters required for biomedical applications, such as MRI and hyperthermia. MNPs with strongly marked anisotropy represent a special interest for biomedical applications. In particular colloidal clusters with controlled size and shape have been an area of great interest for researchers coming from a wide range of disciplines [2]. The controlled assembly of initial small magnetic nanoparticles into cluster structures with defined shape and size opens horizons for materials which combines properties of individual nanocrystals as well as collective properties due to interactions between the single units.

In this work one-pot method for producing magnetic nanocrystal clusters was used. All samples were obtained by thermal decomposition of iron precursor in high-boiling organic solvents in the presence of different organic acids. The results show that the organic acids can directly affect the final shape and size of nanoclusters through specific absorbance onto surface of magnetite nanocrystals. Thus, nanoclusters with spherical, cubic and flower-like shape were obtained. To confirm structure of obtained nanoclusters physicochemical investigations such as transmission electron microscopy, X-ray diffraction analysis, Mössbauer spectroscopy, magnetic measurements, thermogravimetric analysis, magnetic resonance imaging and others were performed. For determination of T_2-relaxivity values as well as *in vitro* and *in vivo* testing nanoclusters were modified by polyethylene glycol derivative. All obtained magnetite nanoclusters have very high magnetic saturation and T_2-relaxivity values. Moreover they can be promising nanomaterials for MRI and hyperthermia.

This work has been financially supported by Ministry of Education and Science of the Russian Federation (14.607.21.0132, RFMEFI60715X0132).

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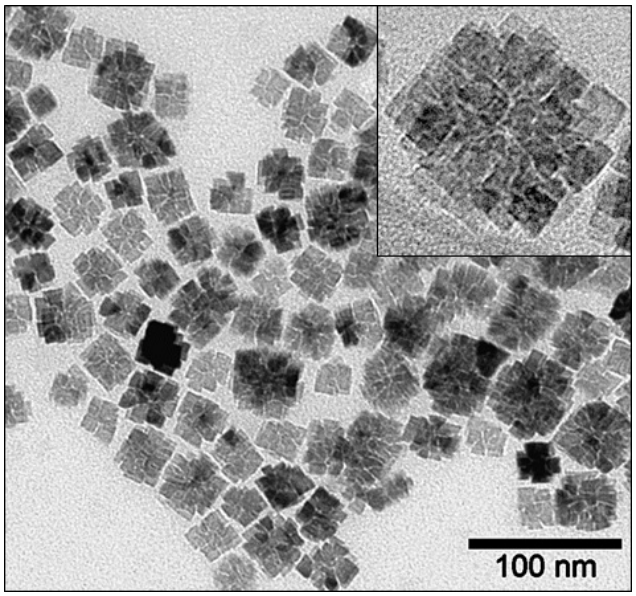


Clusters.jpg

Optimization of methotrexate ultra-permeable niosomes applying Box Behnken design for improved topical delivery: Fabrication and in vitro characterization

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 221

Dr. Wessam Hamdy [1], ***Dr. Abdulaziz Mohsen*** [1], ***Dr. Ahmed Roshdy*** [1]

1. *Faculty of pharmacy, Cairo university*

Introduction: Psoriasis is a chronic autoimmune disease predominantly appeared on the skin and joint. It occurs when immune system mistakes the skin cells such as a pathogen, and speed up the growth and division of skin cells, resulting in a well-defined erythematous together with red and white hues of scaly patches appearing on the top layer of the epidermis.

Methotrexate (MTX) is one of the drug choices for the treatment of psoriasis. It can be administered through oral and/or parenteral routes. However, various side effects can occur when administered over a long period. To reduce the adverse side effects, the topical application of MTX is preferred.

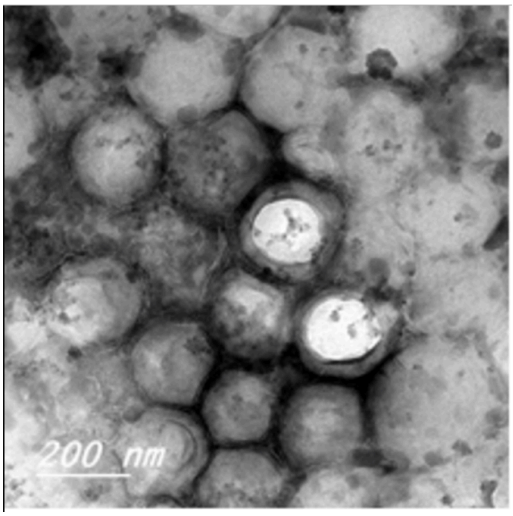
The objective of this study was to develop novel MTX ultra-permeable niosomes; containing edge activator; Cremophor RH 40 and a stabilizer; *Poly vinyl alcohol (PVA*) in order to achieve optimum concentrations in the skin tissues for extended periods of time.

Methods:MTX ultra-permeable niosomes were prepared using ethanol injection method. Box behnken design was used for planning and analysis of the experimental trials to select the optimized formulation using Design-Expert® software. MTX ultra-permeable niosomes were formulated and the influence of three variables, namely; % of Cremophor RH 40, % of stabilizer (PVA) and sonication time, was studied.

Results and discussion:MTX ultra-permeable niosomes possessed small particle size and high entrapment efficiency in comparison with conventional niosomes. In addition, the systems showed higher stability for a longer duration of time confirmed by homogenous physical appearance and high zeta potential values. The best selected formula contained Cremophor RH 40 (14.95%), PVA (4.29%) and was not subjected to sonication procedure (zero sonication time).

Factors	Levels			
% Cremophor RH 40	5	10	15	(%)
% Stabilizer (PVA)	1	3	5	(%)
Sonication time	0	1	2	min

Formulation parameters.png



Tem.png

MoS2 and graphene oxide nanoplatforms for detection of cancer

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 335

Dr. Matej Micusik [1], ***Mrs. Nikola Bugarova*** [1], ***Mr. Michal Bodik*** [2], ***Dr. Peter Siffalovic*** [2], ***Dr. Jozef Kollar*** [1], ***Dr. Zdenko Spitalsky*** [1], ***Dr. Maria Omastova*** [1]

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A systemic toxicity to the patient organism is an important issue of the conventional chemotherapy treatment of cancer. The possible route to overcome the overall poisoning of organism is targeted delivery and controlled release of cytotoxins to the cancer cells. In recent years, we witness accelerated research in the field of antibody-drug conjugates. Here the polymer backbone serves as a universally accepting base for bonding of targeting ligands and drugs. With the invent of graphene and the other 2D materials their capabilities were extensively scrutinized for biomedical applications. The field effect transistors (FETs) based on single layer of functionalized MoS_2 layer exhibit extraordinary high sensitivity for detection (down to 10 fg/ml) of cancer related antigens. The rapid progress in covalent and non-covalent functionalization of exfoliated MoS_2 layers opens new opportunities for binding of biocompatible molecules and proteins.

In this work we prepared exfoliated MoS_2 nanoplatelets and modified with suitable photo-activated or biodegradable linker molecules to which the cytotoxins in form of small molecules or proteins will be attached. The linker molecules allow controlled release of cytotoxin in cancer cells. The added value of MoS_2 is the strong Raman signal and photoluminiscence in red part (above 600 nm) of visible spectrum.

The alternative way would be to use graphene based nanoplatform. In particular, the hydrophilic character of graphene oxide (GO) permits the manufacture of reliable, highly sensitive and ultrafast biosensing nanoplatforms.

Basic characterization of MoS_2 modified with polyethylene glycol or zwitterions (sulfobetain, carboxybetain) and modified GO and rGO, in terms of the degree of oxidation, exfoliation and nanoparticle size, are performed by the small-angle X-ray scattering (SAXS) and by XPS. The monoclonal antibodies (MAb) specifically obtained from the medium of hybridoma cells are proteins and therefore have free NH_2 and COOH groups. The COOH group was used for binding to nanoplatform with the amino groups on their surfaces. In the next step binding, internalization and effects of the functionalized GO-nanoplatform on the living cells using biological approaches will be studied.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0641, and APVV-14-0120.

Osteogenic differentiation of bone marrow – derived mesenchymal stem cells (BMMSCs) on nanotextured Ti6Al4V sterilized by air plasma

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 215

Dr. Leonardo Marasca Antonini [1], ***Dr. Melissa Camassola*** [2], ***Dr. Antonio Shigueaki Takimi*** [1], ***Dr. Célia de Fraga Malfatti*** [1]

1. *Universidade Federal do Rio Grande do Sul,* ***2.*** *Universidade Luterana do Brasil*

The Ti6Al4V surface treatment by electropolishing is able to induce a bioactive behavior using nanotexturing processes preserving the mechanical properties of Ti6Al4V. In this work, three treatments were studied to modify the Ti6Al4V surface aiming to promote the osteogenic differentiation of bone marrow – derived mesenchymal stem cells (BMMSCs) on nanotextured Ti6Al4V. Mechanical polishing and electropolishing for 4 and 12 minutes were used as surface treatments. The samples were characterized by atomic force microscopy (AFM) (Fig. 1), optical interferometry and wettability. After the air plasma sterilization process, the samples were seeded with bone marrow – derived mesenchymal stem cells (BMMSCs). The cell growth, osteogenic differentiation and mineralization (Fig. 2) were evaluated. The electropolishing surface treatments modified the nanometric morphology and wettability. The nanostructured Ti6Al4V surface, electrochemically treated for 4 minutes, presented a greater cell growth, and it also increased the osteogenesis rate and the mineralization of the BMMSCs.

Phosphonium carbosilane dendrimers are efficient non-viral vectors for siRNA cell delivery on model cell lines in vitro.

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 50

Mrs. Regina Herma [1], ***Dr. Jan Maly*** [1], ***Dr. Dominika Wrobel*** [2], ***Dr. Marek Maly*** [3], ***Dr. Tomas Strasak*** [4]

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Gene therapy is a rapidly growing field of biomedical research which brought a new hope in the fight against genetic-based diseases. The success depends on the development of suitable vectors for the nucleic acid cell targeting. Dendrimers have number of favourable properties, such as good transfection efficiency and relatively low toxicity. This work introduces results from the comparative study of two types of cationic carbosilane dendrimers terminated with ammonium or phosphonium groups for their use as non-viral vectors for transfection of fibroblasts (cell line B14) with siRNA. We present a part of work devoted to characterization of dendriplexes formed from generation 1-3 (G1-3) of carbosilane dendrimers and model siRNA. Dendriplexes were characterized by Gel retardation electrophoresis, DLS, ξ-Potential, Fluorescence Anisotropy, Nuclease Protection Assay and AFM. Transfection efficiency was evaluated by Fluorescence Microscopy. We found that both types of G2-G3 dendrimers form stable complexes with siRNA due to positive charge of surface groups of dendrimers and negative charge of siRNA backbone. Formation of dendriplexes was investigated at different charge ratio (1/5 – 10/1 (+/-)) to find an optimal properties of complexes for cell transfection. All types of dendriplexes show quite similar characteristics (stability, surface charge, dimensions etc.), based on the charge ratio and generation of dendrimer used. *In vitro* transfection experiments proved an ability of both G3 dendriplex structures to enter the cells, with maximal achieved transfection efficiency at 7/1 (+/-) charge ratio. Ammonium dendrimers achieved max. 30% of transfected cells, contrary more than 70% were transfected at the same conditions with phosphonium terminated dendrimers.

With the aim to further optimize the properties of phosphonium dendriplexes we incorporated new periphery substituents ($P(Et_2)_2(CH_2)_3OH$, $P(C_6H_4\text{-}OMe)_3$, P(Ph)3, PBu_3) into the dendrimer structure. Similar cytotoxicity and transfection efficiencies were obtained, with the exception of $P(C_6H_4\text{-}OMe)_3$ and $P(Ph)_3$peripheral substituent. These dendrimers exhibits more than 80% transfection efficiency and dendrimer $P(C_6H_4\text{-}OMe)_3$ also very low toxicity at comparable experimental conditions. Therefore, these dendrimers types seems to be “hot” candidates for further improvements of gene delivery by phosphonium carbosilane dendrimer vectors.
Research was supported by project 15-05903S of Czech Science Foundation and partially by Internal Grant Agency UJEP.

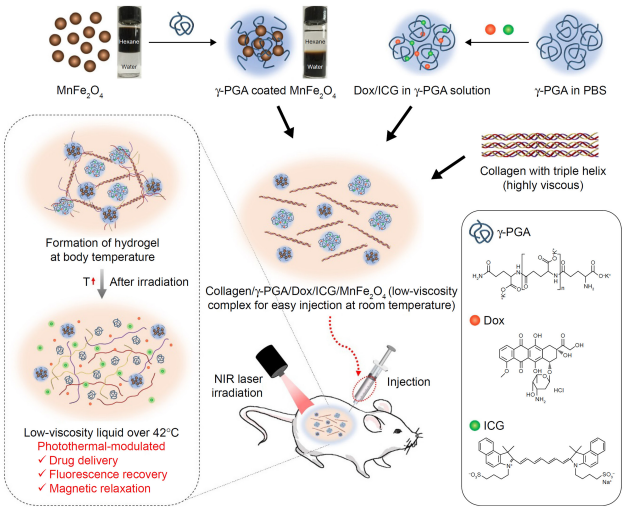
Photothermal-modulated drug delivery and magnetic relaxation based on collagen/poly(γ-glutamic acid) hydrogel

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 81

Dr. Jee-Hyun Cho [1], ***Ms. Sun-hee Cho*** [2], ***Dr. Kwan Soo Hong*** [1], ***Dr. Yong Taik Lim*** [2]

1. *Korea Basic Science Institute, Korea Research Institute of Bioscience & Biotechnology,* ***2.*** *Sungkyunkwan University*

The design and fabrication of multifunctional hydrogels are important issues in both the pharmaceutical and medical research fields because of their high potential for use in molecular imaging, drug delivery, and tissue engineering. In this study, we developed a novel injectable and photoresponsive composite hydrogel composed of anticancer drugs, imaging contrast agents, bio-derived collagen, and multifaceted anionic polypeptide, poly(γ-glutamic acid) (γ-PGA). By the introduction of γ-PGA, the intrinsic temperature-dependent phase transition behavior of collagen was modified to a low viscous sol state at room temperature and nonflowing gel state around body temperature (ie, injectable hydrogel);. The modified temperature-dependent phase transition behavior of collagen/γ-PGA hydrogels was also evaluated after loading of near-infrared (NIR) fluorophore, indocyanine green (ICG), which could transform absorbed NIR photonic energy into thermal energy. By taking advantage of the abundant carboxylate groups in γ-PGA, cationic-charged doxorubicin (Dox) and hydrophobic $MnFe_2O_4$ magnetic nanoparticles were also incorporated successfully into the collagen/γ-PGA hydrogels. By illumination of NIR light on the collagen/γ-PGA/Dox/ICG/$MnFe_2O_4$ hydrogels, the release kinetics of Dox and magnetic relaxation of $MnFe_2O_4$ nanoparticles could be modulated. The experimental results suggest that the novel injectable and NIR-responsive collagen/γ-PGA hydrogels developed in this study can be used as a theranostic platform after loading of various molecular imaging probes and therapeutic components.



Schematic illustrations.jpg

Paclitaxel-Loaded Selenium Nanoparticles Induce Apoptosis in HeLa and MCF-7 cells Through Mitochondrial Damage and Reactive Oxygen Species Generation

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 216

Mr. Anil Bidkar [1], ***Dr. Pallab Sanpui*** [2], ***Prof. Siddhartha Sankar Ghosh*** [1]

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Increasing research on selenium nanoparticles (SeNPs) makes it a potential nanocarrier to deliver anticancer drugs. Herein, we reported synthesis, characterization of F-127 stabilized paclitaxel loaded SeNPs (PTX-SeNPs) for evaluating chemotherapeutic effects on human cervical cancer (HeLa) and human breast cancer (MCF-7) cells. Characterizations of the synthesized SeNPs and drug loaded SeNPs were performed using Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FE-SEM) and Atomic Force Microscopy (AFM). Paclitaxel loading on SeNPs and its release were monitored using UV visible spectroscopy. Cell viability assays indicated dose dependant growth inhibition of HeLa and MCF-7 cell lines by PTX-SeNPs. Experimental results indicate that SeNPs treatment could cause cell cycle arrest, reactive oxygen species generation and mitochondria damage, which subsequently leads to apoptosis.

Self-assembled lipid nanostructures containing oleic acid for skin purposes

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 354

Mr. Kirian Talló [1], ***Ms. Verónica Moner*** [1], ***Mr. Martí De Cabo*** [2], ***Dr. Manel Bosch*** [3], ***Dr. Alfonso de la Maza*** [1], ***Dr. Olga López*** [1]

1. *Institute of Advanced Chemistry of Catalonia (IQAC-CSIC),* ***2.*** *Autonomous University of Barcelona (UAB),* ***3.*** *Scientific and Technological Centers of the University of Barcelona (CCiTUB)*

The treatment of the skin is challenging due to the strong barrier function of the outermost skin layer, the stratum corneum (SC). This layer is formed by dead flat cells embedded into a lipid matrix forming a lamellar structure which exhibits spacing between 6-10 nm. Drug delivery across the skin requires the modification of the tissue permeability, without promoting non-desirable effects.

The use of nanostructured lipid systems, with size small enough for passing through the narrow intercellular spaces of the SC has demonstrated to be a good alternative for skin treatment[1]. In the present work, oleic acid (OA), a skin penetration enhancer with recognized skin benefits [2] was combined with different phosphatidylcholines to form new nanostructures for topical application.

Size and polydispersity index of the nanostructures were characterized by dynamic light scattering (DLS), while shape and morphology were evaluated using cryogenic transmission electron microscopy (Cryo-TEM). Additionally, the phase behavior of the systems was determined by differential scanning calorimetry (DSC). The interaction of these systems with the skin was assessed in vitro by treatment of pig skin with Rhodamine B labeled systems and subsequent visualization of skin sections using fluorescence microscopy.

Images acquired by Cryo-TEM showed discoidal shaped structures or polyhedral vesicles depending on the constitutive molecules used (Figure 1). Average size of these systems determined by DLS was found to be around 20 and 100 nm respectively. According to DSC experiments, alkaline pH and increased amounts of OA produced a decrease in the main transition temperature of the membranes.

Fluorescence microscopy images showed a different permeation behavior of the nanostructures as a function of their composition. Overall, the main part of the molecules was retained inside the SC and follicles, while a smaller fraction was able to reach deeper layers of the skin (Figure 2).

In conclusion, the versatility of these systems and the enhancing effect of OA regarding skin permeation make evident the potential use of these nanostructures to act as delivery systems for skin and follicular applications.

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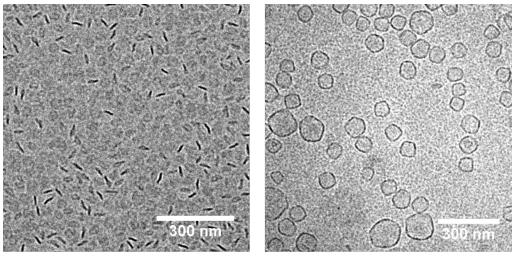


Fig.1. Cryo-TEM micrographs of discoidal (left) and vesicualr (right) structures

Structure of lipid systems.png

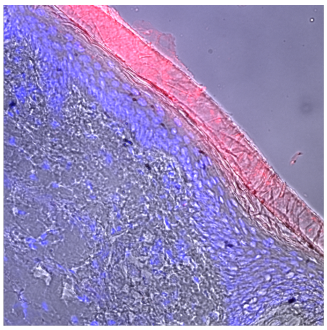


Fig. 2. Fluorescence microscopy image showing the permeated lipid system (red) across porcine skin. Cell nuclei were stained in blue to enhance contrast between skin layers.

Percutaneous penetration.png

Stability and drug release of anti-cardiac hypertrophic peptide loaded in new nanohybrids based on gold nanoparticles embedded into a polymeric matrix

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 399

Ms. Sabrina Sepulveda Rivas [1], ***Dr. Javier Morales*** [1], ***Dr. Maria Paz Ocaranza*** [2]

1. *Universidad de Chile,* ***2.*** *Pontificia Universidad Católica de Chile*

Introduction

Angiotensin-(1-9) has been reported as a peptide of renin-angiotensin system (RAS) and a novel anti-cardiac hypertrophy agent (1). The clinical application of Angiotensin-(1-9) is restricted due to its pharmacokinetic properties including a short half-life *in vivo* attributed to enzymatic metabolism. Nanoparticles are promising vehicles that can provide protection, controlled drug release, improving bioavailability, and transport of numerous bioactive molecules (2). The aim of this study was to prepare nanohybrids as a carrier system for angiotensin-(1-9), and then to investigate the colloidal stability and the release profiles.

Methods

Nanohybrids which consists in the mixture of Eudragit E (EE), Alginate (Alg), gold nanoparticles (AuNPs) and Angiotensin-(1-9) (EE/Alg/AuNPs/Ang-(1-9)) were obtained by coacervation of aqueous EE (polycation) and Alg (polyanion) solutions and then loaded with gold nanoparticles and angiotensin-(1-9). Controlling the amounts of charges in play during the coacervation process, we evaluated negatively charged systems. Size, polydispersity index and zeta potential of nanohybrids were characterized by dynamic light scattering and laser Doppler electrophoresis in a Zetasizer Nano ZS. Nanoparticle tracking analysis (NTA) were performed using a NanoSight NS300 instrument. The stability parameters were studied at different temperatures (room temperature or 37°C), media (phosphate buffer pH 7.4, DMEM/M199 and plasma) and time. For determining encapsulation efficiency and *in vitro* drug release, angiotensin-(1-9) was quantified by HPLC Flexar (Perkin Elmer) detecting at a 220 nm wavelength.

Results

All studied conditions allowed the formation of nanohybrids by coacervation of [EE/Alg/AuNPs/Ang-(1-9)]. Hydrodynamic diameters and zeta potential of nanohybrids were media, temperature and time. Nanohybrids showed high encapsulation efficiencies (>68%), providing further evidence of the high affinity of the nanocarriers for the encapsulated peptide. Release studies showed a rapid release of the peptide during the first minutes, 75% after 15 minutes and the release was 100% at 105 minutes. These results provide evidence of the capacity of these nanohybrids, which could act as a protective vehicle for this peptide.

Discussion

Overall, these data suggest that the nanohybrids developed are promising nanocarriers for the delivery of angiotensin-(1-9), providing the insights of the application of nanohybrids as delivery systems of angiotensin-(1-9) for cardiovascular therapy.

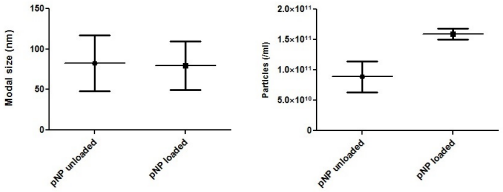


Figure 1. Particle size (a) and particles concentration (particles/ml) (b) in pNP (EE/Alg/AuNPs) unloaded and EE/Alg/AuNPs loaded angiotensin-(1-9).

Modalandconcent.jpg

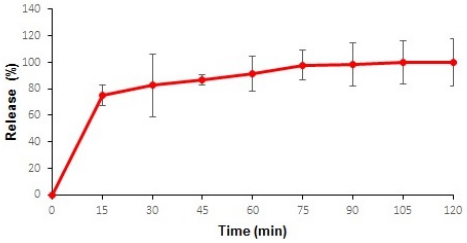


Figure 2. In vitro release profile of angiotensin-(1-9)- loaded EE/Alg/AuNPs. Values are expressed as mean ± standard deviation (n=3).

Releaseabsct.jpg

Sterilization processes effects on the properties of a hybrid coating applied on Ti6Al4V alloy

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 162

Mr. Estela Baldin [1], ***Dr. Rosmary Brandalise*** [2], ***Dr. Célia de Fraga Malfatti*** [1]

1. *Universidade Federal do Rio Grande do Sul,* ***2.*** *Universidade de Caxias do Sul*

The sterilization method, which is the last stage before the implantation of biomaterial, can influence on the material properties implanted. There are several sterilization methods being used, each one with their advantages and disadvantages. These methods can even modify the materials surface, as well as, cause chemical, physical, morphological and biological changes. In the case of metallic implants, these mentioned properties affect directly the interactions with the host tissue and its biocompatibility. Hybrid coatings based on silicon precursors have been studied for application in the biomedical area aiming to improve the corrosion performance of metallic prostheses and also as an alternative method to improve the osteointegration process. In this context, three different types of sterilization were tested: steam autoclave, hydrogen peroxide and ethylene oxide, in order to evaluate their action on the surface properties of the hybrid coating. The hybrid coatings were obtained on Ti6Al4V substrate by dip-coating process from a sol constituted by TEOS and MTES. Their morphological changes and electrochemical behavior were evaluated after sterilization. Besides, the cytocompatibility study from viability, adhesion and morphology of MG-63 cells were carried out. The results showed that the steam autoclave sterilization method promoted greater changes in the morphological properties of the hybrid coating. In addition, the sterilization processes did not affect the cytotoxicity. However, the cell viability and morphology were affected by surface modifications due to the different sterilization methods tested.

Surface-modified PLGA nanoparticles for ocular use: in vitro and in vivo evaluations

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 493

Dr. Alaa H. Salama [1], ***Dr. Azza A. Mahmoud*** [1], ***Dr. Rabab Kamel*** [1]

1. *Department of Pharmaceutical Technology, National Research Center, Cairo, Egypt*

This study is concerned with preparing nanoparticulate system as an ophthalmic delivery system for fluocinolone acetonide to improve its ocular bioavailability using a simple yet attractive method. Thin film hydration method was used to prepare poly(lactic-co-glycolic acid) (PLGA) nanoparticles. Two different types of PLGA was used (75/25 and 50/50 copolymer molar ratio of DL-lactide/glycolide). Results demonstrated that using PLGA with lower glycolic acid monomer ratio exhibited high values for particle size (PS), zeta potential (ZP) and drug encapsulation efficiency (EE) along with slow drug release pattern. Moreover, increasing the drug concentration during nanoparticles preparation enhanced its EE to reach almost 100%. Surface modification of optimized formulation was done using different amount of stearylamine and chitosan HCl aiming at increasing nanoparticles mucoadhesion ability. PLGA nanoparticles coated with 0.1% w/v chitosan HCl attained most suitable results of PS, ZP and EE values as well as high drug release properties. Transmission electron microphotographs illustrated the deposition of chitosan molecules on the nanoparticles surfaces. *In-vivo* study was performed on Albino rabbit's eyes and pharmacokinetic studies revealed that the prepared novel chitosan-coated PLGA nanoparticles showed rapid and extended drug delivery to the eye.

Synthesis and toxicity of peptide coated polyethylene glycol gold nanoparticle for targeting and uptake in colon tumours

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 355

Ms. Lynn Cairncross [1], ***Mr. Olusola Adewale*** [1], ***Prof. Saartjie Roux*** [1], ***Prof. Mervin Meyer*** [2]

1. *Nelson Mandela University,* ***2.*** *Universisty of Western Cape*

Introduction: Gold nanoparticles (AuNPs) possess several features that make them well suited for applications in medicine. AuNPs functionalized with cancer targeting peptides can be applied in the diagnosis and/or treatment of cancer. We investigated the application of two cancer targeting peptides, namely p.L and p.14 to produce AuNPs that can specifically bind to colon carcinoma cells.

Methods: Citrate capped AuNPs (cAuNPs) of 14 nm in size were synthesized and coated with PEG-OH and PEG-biotin to produce PEG-AuNPs. Streptavidin tagged p.L and p.14 peptides were conjugated to PEG-AuNP using the biotin moiety on PEG-biotin to facilitate the conjugation. UV-Vis spectroscopy, TEM, FTIR spectroscopy and the zetasizer measurements were used to characterize the physico-chemical properties of the AuNPs. The cytotoxicity of the AuNPs was evaluated on human colon carcinoma (Caco-2, HT-29), human breast carcinoma (MCF-7) and non-cancerous human fibroblast cells (KMST-6). The cells were treated with increasing concentrations (1 – 4nM) of the cAuNP, PEG-AuNP, and peptide conjugated PEG-AuNP (p.L-AuNPs and p.14-AuNPs) after which cell viability was assessed using the MTT assay. The cellular uptake of AuNPs was confirmed using ICP-MS.

Results: The AuNPs displayed a typical surface plasmon resonance (SPR) band of 520 nm. TEM micrographs demonstrated relatively spherically shaped and reasonably monodispersed AuNPs. PEG-AuNPs and peptide-AuNPs showed a red shift in SPR band, size and charge. cAuNPs were highly toxic even at the lowest concentration (1nM) tested. PEGylated AuNPs were less toxic with more than 80% of the cells still viable. The cytotoxicity assay also revealed reduced Caco-2 viability at 4nM for p.L-AuNPs. Similarly, p.14-AuNPs showed reduced HT-29 and Caco-2 viability (below 80%) at 3 and 4nM. No cytotoxicity was observed for MCF-7 and KMST-6 cell lines.

Conclusion: This study provides more information on the successful conjugation of peptides to AuNPs using streptavidin-biotin chemistry, and also gives preliminary information on the toxicity of the synthesized-AuNPs *in vitro*. This study warrants further investigation into the cellular uptake and localization of p.L-AuNP and p.14-AuNP in the colon carcinoma cell lines.

Keywords: Gold nanoparticles; Colon cancer; Toxicity;Polyethylene glycol;Peptides

Synthesis of gold nanoparticles conjugated lectin and using it for enhancement the antibacterial activity of lectin purified from Acinetobacter baumannii

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 294

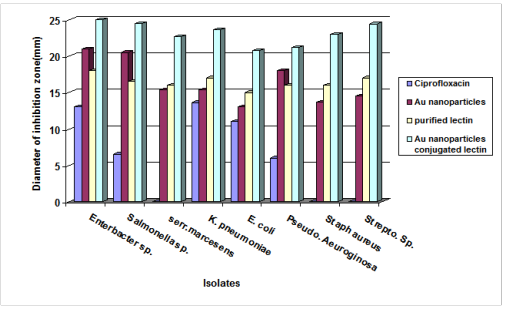
Ms. Israa Al-Kadmy [1], ***Dr. Sahira Nsayef Muslim*** [1], ***Dr. Sraa Nsayef Muslim*** [2], ***Dr. Nadheema Hammood Hussein*** [3], ***Mrs. Alaa Naseer Mohammed Ali*** [1], ***Mrs. Saba Saadoon Khazaal*** [1], ***Ms. Buthainah Mohammed Taha*** [3], ***Ms. Istabreq Muhammed Ali Salman*** [1], ***Ms. Sarah Naji Aziz*** [1]

1. *1Branch of Biotechnology, Department of Biology, College of Science, AL-Mustansiryia University,* ***2.*** *2Department of Geophysics, College of Remote sensing and geophysics, AL-Karkh University,* ***3.*** *3Branch of microbiology, Department of Biology, College of Science, AL-Mustansiryia University*

Introduction: Lectin was originally called hemagglutinin or agglutinin due to its ability to agglutinate of human and animal erythrocytes. Lectins are heterogeneous group of proteins or glycoproteins of non-immune origin. These proteins are ubiquitous in nature, and occur in animals, plants, bacteria, viruses, and fungi. Lectin has a wide range of applications in many fields as anti-tumor, anti-insect, antiviral and antifungal drug.

Methods: Here we reported lectin production from *Acinetobacterbaumannii* isolated from sewage water by microscopic glass slide and microtiter plate methods and detection the best producer. Lectin was extracted with glass beads method and purified with three steps including ammonium sulfate precipitation, ion exchange chromatography by using DEAE -cellulose column and gel filtration chromatography by sephadex G-150 column. the purified lectin was pulsed with Au nanoparticles by using laser ablation technique to produce Au nanoparticles conjugated lectin. Different UTI causing bacteria were collected UTI cases from many hospitals in Baghdad city and separately treated with ciprofloxacin as antibiotic disc, purified lectin, Au nanoparticles and Au nanoparticles conjugated lectin for determining their effects as antibacterial agents.

Results and discussion: In the present study a novel strain, *Acinetobacter baumannii*W_{13} isolated from water sewage samples gave the highest production level of lectin bymicroscopic glass slide and microtiter plate methods. Lectin was purified to homogeneity by using ammonium sulfate at 35% saturation followed by DEAE -cellulose ion exchange chromatography and sephadex G-150 gel filtration chromatography with 45.3 fold of purification and a yield of 57.1%. Au nanoparticles was prepared by laser ablation and conjugated to lectin by pulses method. The Au nanoparticles conjugated lectin led to enhancement of lectin activity against all tested UTI causing in comparison with ciprofloxacin antibiotic that used for treatment of UTI infections, where used it showed high effectiveness against *Enterobacter*sp. followed by *Salmonella* sp. and *Streptococcus*sp. in comparison with the other tested bacteria. So that Au nanoparticles conjugated lectin may be used as a useful antibacterial agent for the treatment of increasing urinary tract infections.



3.png

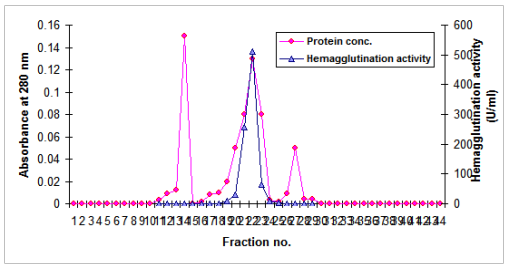


Figure -1.png

Synthesis, surface modification, structural and magnetic study of dual contrast agents based on Gd:Fe3O4

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 121

Ms. Iana Tcareva [1], ***Mr. Constantine Fedotov*** [1], ***Mr. Mark Zheleznyi*** [1], ***Mr. Igor Shchetinin*** [1], ***Mr. Maxim Abakumov*** [2], ***Prof. Alexander Savchenko*** [3], ***Prof. Alexander Majouga*** [4]

1. *National University of Science and Technology, MISiS,* ***2.*** *Pirogov Russian National Research Medical University,* ***3.*** *National University of Science and Technology MISiS,* ***4.*** *Lomonosov Moscow State University, National University of Science and Technology, MISiS*

Magnetic Resonance Tomography, MRI, is one of the most used instruments for non-invasive clinical diagnostics. In contrast to radiological investigations, MRI has no danger of radiation exposure to produce images using radio frequency electromagnetic radiation with very low energy. The most researches in this area are focused on the development of contrast agents, that can provide a clearer distinction between healthy and diseased tissue. The majority of contrast agents are magnetic nanoparticles (MNP) [1]. MNPs made from iron oxide are used for the diagnostics of many diseases such as cardiovascular, neurological and cancer. Liver and prostate cancer are the most prevalent of malignant tumors. Hybrid contrast agents are among most popular trends for MRI providing comprehensive data on disease progression. Gadolinium chelates and magnetite are the most appropriate T1 and T2 contrast agents, respectively, but these compounds can provide toxic effect on healthy cells. One of the ways to prevent toxicity is the creation of hybrid contrast agents based on gadolinium doped magnetite [2], [3]. In this work gadolinium doped magnetite nanoparticles were prepared by thermal decomposition of iron-gadolinium complex in dibenzyl ether and Gd(III) acetate and $Fe(acac)_3$in dibenzyl ether in addition oleic acid, oleylamine and 1,2-hexadecandiol. These nanoparticles are designed to be used as a hybrid contrast agents for hepatocellular and prostate carcinomas visualization. Obtained nanoparticles were investigated by methods of transmission electron microscopy, X-ray diffraction, Mössbauer spectroscopy, dynamic light scattering, zeta potential and thermogravimetric analysis. Also the toxicity of nanoparticles and their T1 and T2-relaxation time were measured in vitro.

The authors knowledge financial support from Ministry of Education and Science of the Russian Federation (№ K2-2016-069).

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[2] C. R. De Silva, J. AM. CHEM. SOC. 131, 6336 (2009)

[3] F.J. Douglas, RSC Adv. 6, 74500 (2016)

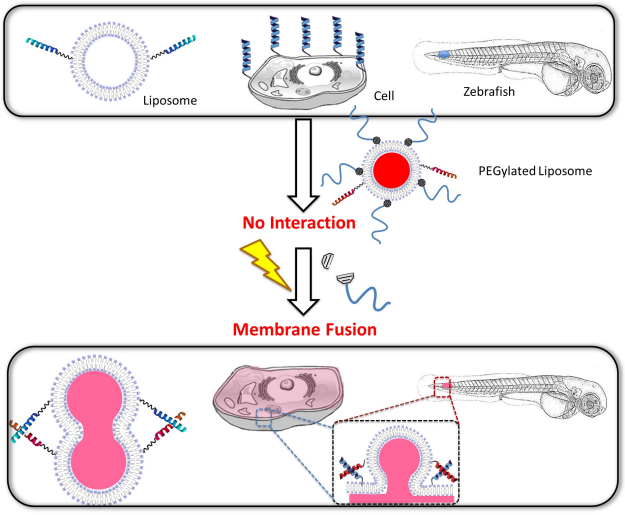
Temporal Controlled Membrane Fusion as a Drug Delivery Tool in Vitro and in Vivo

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 429

Ms. Li Kong [1], ***Mr. Quanchi Chen*** [1], ***Dr. Frederick Campbell*** [1], ***Dr. Jian Yang*** [1], ***Prof. Ewa Snaar-jagalska*** [1], ***Prof. Alexander Kros*** [1]

1. *Leiden University*

Membrane fusion results in the transport and mixing of (bio)molecules across otherwise impermeable barriers. In our group, we have reported a new method of delivering drug directly into the cytosol of live cells utilizing targeted membrane fusion between liposomes and live cells. A pair of complementary coiled-coil lipopeptides (CP_4E_4&CP_4K_4) was embedded in the lipid bilayer of liposome and counter cell membrane respectively resulting in targeted membrane fusion with concomitant release of liposome encapsulated cargo. To further regulate the fusion process, we applied steric PEG shielding and rapid, photo-induced de-shielding method to temporal control the whole process. With PEGylation, E_4 peptide on liposome was shielded and the fusion ability with K_4-liposome or K_4-cell was inhibited. However, as soon as light was applied to remove the PEG shielding, the interaction between E_4&K_4 was regained, accompanying fast membrane fusion. With this strategy, the fusion in vivo was also successfully controlled. The original freely circulated PEGylated E_4-liposomes could selectively accumulate at tumor sites modified with K_4 after light-activated removal of the PEG corona. Furthermore, the growth of tumor was successfully suppressed by ingesting the doxorubicin encapsulated in liposomes. In conclusion, by this photo-activated membrane fusion, we could deliver drug in a controlled manner with high efficiency.



Light controlled membrane fusion in vitro and in vivo.jpg

Drug-loaded magnetoliposomes with controlled release by low-frequency alternating magnetic field

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 127

Ms. Kseniia Vlasova [1], ***Mrs. Tatiana Abakumova*** [2], ***Mr. Pavel Melnikov*** [2], ***Mrs. Irina Le-Deygen*** [1], ***Prof. Vladimir Chekhonin*** [2], ***Dr. Yuriy Golovin*** [3], ***Prof. Alexander Kabanov*** [4], ***Prof. Natalia Klyachko*** [1]

1. *Lomonosov Moscow State University,* ***2.*** *V. Serbsky Federal Medical Research Centre of Psychiatry and Narcology of the Ministry of Health of the Russian Federation,* ***3.*** *Nanocenter, G. R. Derzhavin Tambov State University, Tambov, Russia,* ***4.*** *University of North Carolina at Chapell Hill*

Controlled release for magnetoliposomes (MLs) can be achieved by using magnetic hyperthermia. However, such approach is not appropriate in the case of the brain tissues and highly perfused organs (kidney, liver, lung). Moreover, application of high-frequency magnetic field with high intensity during a long time makes the method inconvenient. Our research deals with the non-heating low-frequency alternating magnetic field (LF-AMF). MNPs incorporated into liposomes can respond to an external magnetic field by mechanical rotation (Brown relaxation) and thus destabilize liposomes and induce the release of encapsulated drugs or biomolecules. **The aim of this study** was to formulate doxorubicin loaded magnetoliposomes (MLs-DOX) for theranostic application and to show the efficiency of LF-AMF application in controlled drug release.

Liposomes were prepared as follows: the film was produced from MNPs, egg lecithine, DSPE-PEG(2000) and cholesterol and then dispersed in phosphate buffe with Dox. Finally, the mixture was sonicated and free MNPs were separated by passing the emulsion through extruder with pore size filter of 400 and 200 nm. Drug excess was removed by centrifugation through NAP-25 desalting column.Animal stage IV human breast cancer 4t1 cells were used for confocal microscopy.

Results and discussion. Under LF-AMF exposure of 15 min the MNPs in MLs firstly aggregated into clusters, then these clusters rotated and destroyed the MLs membranes (data from TEM). As was shown by IR-spectroscopy, the MF application caused “melting” of the MLs membrane, while the “melting” range depended on MF exposure time and MF intensity. *In vitro*DOX release from MLs under AMF exposures was in 1.5-2 times more effective, than in control experiment (total release for 6 hr under AMF and without was 45% and 25%, respectively). Cellular internalization was enhenced with MLs-DOX under LF-AMF. It was shown, under 15 min AMF exposure free Dox was around the cell nucleus and lipids were located in cytoplasm as aggregates, in the case of non-treated MLs-DOX there was not free DOX in the cells. The effect depended on AMF parameters. Thus, we showed the possibility of new approach for remote controlled drug release from liposomes.

Grants support: RSF 14-13-00731 and RFBR 16-33-01023.

A liposome coated with serum albumin via long alkyl chain as a ligand

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 380

Ms. Hikari Sato [1], ***Mr. Yuta Nakamura*** [1], ***Prof. Akihiro Kishimura*** [1], ***Prof. Takeshi Mori*** [1], ***Prof. Yoshiki Katayama*** [1]

1. *kyushu University*

Introduction: A polyethylene glycol (PEG)-modified liposome is a representative drug carrier because of its long blood half-life. However, in the second time injection, PEG-liposome is rapidly removed from blood by produced antibody against PEG. Here, we focused on serum albumin to camouflage the surface of liposome to prolong its blood circulation time. Human serum albumin (HSA) is the most abundant protein in plasma (~40 g/L). Since serum albumin can avoid from removal via glomerular filtration and degradation in endocytosis, it has a long blood half-life (20 days). Here we propose modification of the liposome surface via serum albumin-binding ligands for reversible coating with HSA. As such a ligand, we selected alkyl ligand with various hydrophobicity; stearic acid (SA), octadecanedioic acid (OA), and decanoic acid (DA).

Method: Three types of ligand-modified phospholipids were synthesized. Then, liposomes containing these lipidswere prepared by a hydration method(SAL, OAL, and DAL) (Fig. 1). To confirm the stability of these liposomes in physiological saline, these liposomes were incubated in DPBS containing human serum albumin (HSA) at r.t. The size of liposome was measured 20 h after the incubation. The exposure of OA to out-phase was determined from the change of dispersion stability in acidic solution.

Results and Discussion: OAL and DAL aggregated in DPBS in the absence of HSA, while SAL dispersed stably (Fig. 2). This result indicated that the less hydrophobic ligands OA and DA are exposed on the liposomal surface, resulting in aggregation via interparticle hydrophobic interaction, while the highly hydrophobic SA may be buried in liposomal bilayer, leading to stable dispersion. In the presence of HSA, OAL and DAL dispersed stably, indicating that HSA interacts with the ligand of OAL and DAL to suppress the aggregation. Interestingly, the aggregation of OAL in the absence of HSA was suppressed in acidic condition, indicating that the protonated OA is buried into bilayer like SA (Fig. 3). The unique behavior of OAL indicates that negatively charged OA at neutral condition is exposed on the liposomal surface to readily bind with HSA. The present finding will provide useful information to design an albumin-coated liposome.

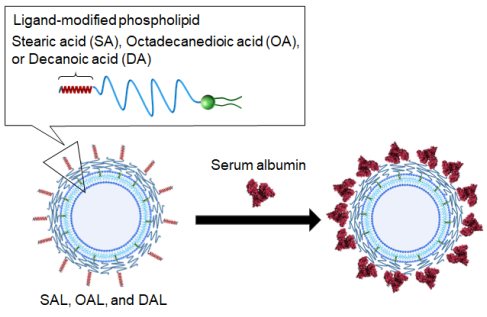


Fig. 1 Schematic illustration of HSA-coated liposome

Fig 1 schematic illustration of hsa coated liposome.png

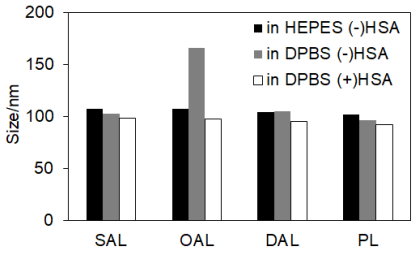


Fig. 2 Average size in HEPES and DPBS in the absence or presence of HSA
PL; PEG-modified liposome as a control

Fig 2 average size in hepes and dpbs in the absence or presence of hsa.png

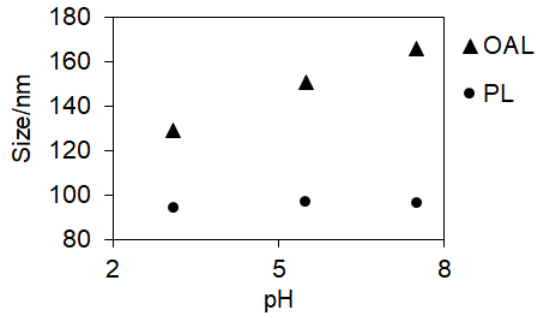


Fig. 3 Plot of average size in a different pH salt solution
PL; PEG-modified liposome as a control

Fig 3 plot of average size in a different ph salt solution.png

In vivo drug delivery system for colorectal cancer therapy based on doxorubicin-loaded oligonucleotide conjugated gold nanoparticles

Wednesday, 27th September - 13:30 - Poster Session - Gallery - Poster - Abstract ID: 509

Prof. TAE HYUN KIM [1], ***Mr. Chang-seuk Lee*** [1]

1. *Department of Chemistry, Soonchunhyang University*

In the present study, we have examined the effect of gold nanoparticle (AuNP)-based drug delivery system for doxorubicin (DOX) in colorectal cancer (CRC), which was prepared by using G-C rich oligonucleotide (ONT) coated AuNPs into which DOX was loaded by intercalation (Fig. 1). ONT-modified AuNPs present numerous binding sites for DOX, thereby facilitating the delivery of significant amount of DOX to cancer cells. The anticancer effect of DOX-loaded AuNPs coated with ONTs (Doxorubicin-Oligomer-AuNP, DOA) was analyzed in human CRC cell line SW480 by using MTT assay. *In vivo*, inhibition ratio of tumor growth in treated-to-control (T/C) tumors was also examined in tumor-bearing mouse models.

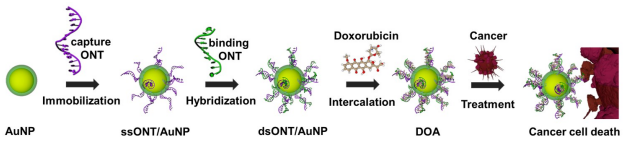


Fig1.jpg

High sensitive detection of cell membrane proteins by enzyme-catalyzed amplification of fluorescent immunolabeling

Wednesday, 27th September - 15:00 - Biological & medical nanodevices and biosensors - Room 207 - Oral - Abstract ID: 297

Mr. Takanobu Nobori [1], ***Mr. Kenta Tosaka*** [1], ***Mr. Akira Kawamura*** [1], ***Prof. Akihiro Kishimura*** [1], ***Prof. Takeshi Mori*** [1], ***Prof. Yoshiki Katayama*** [1]

1. *kyushu University*

Introduction

In the personalized medicine, patients are separated into different groups based on companion diagnostics (CoDx). One of the representative methods for CoDx is immunolabeling with fluorescence-labeled antibodies. However, this conventional cell labeling is applicable only for abundant antigen proteins (> 10^4 protein copies/cell). Therefore, we propose here a novel fluorescent immunolabeling technique, which enables amplification of dye staining via enzymatic reaction. First, antigen proteins on cell surface are marked with enzyme-modified antibodies. Then, the enzymatic reaction of dye-modified substrates on cell surface results in the staining of not only surface but also inside of cells because the hydrophilic substrates become hydrophobic, which results in the interaction and cell membrane penetration of catalyzed substrates. In this way, our staining method permits fluorescent labeling only for target cells based on the difference of hydrophilicity between before and after enzymatic reaction.

Methods

For proof of concept, we selected alkaline phosphatase (AP) as an enzyme and applied this system for detection of CD20 and EGFR on JY25 and A549 cells, respectively. Our substrate is composed of hydrophobic part, hydrophilic alkaline phosphatase (AP) cleavable part and rhodamine. Each cell was incubated with AP-modified antibodies specific for each target antigen protein at 4□. After washing of cells, the substrates were added to each cell and incubated at 37□. Then, each cell was washed, followed by flow cytometric analysis.

Results and Discussion

We found that cells stained with our substrates exhibited a fluorescence signal approximately 1,000 times higher than unstained cells. In addition, a fluorescence signal in our labeling was significantly greater than that in conventional cell labeling. These results indicate that our system will enable high-sensitive detection of low abundant antigen proteins on a single cell, which will be useful for CoDx for precision medicine.

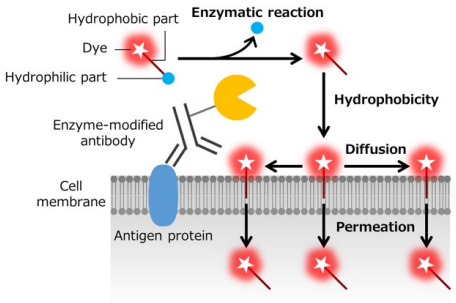


Figure 1. Schematic illustration of our staining principle

Figure 1. schematic illustration of our staining principle.jpg

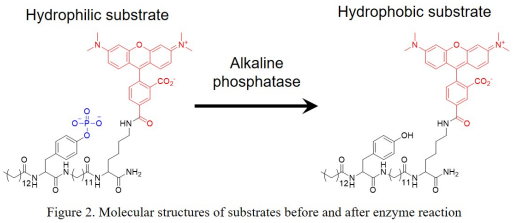


Figure 2. Molecular structures of substrates before and after enzyme reaction

Figure 2. molecular structures of substrates before and after enzyme reaction.jpg

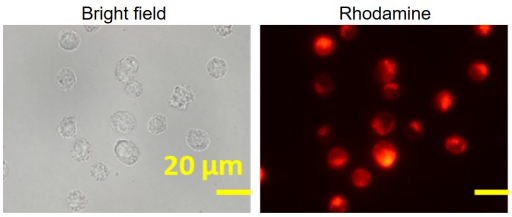


Figure 3. Fluorescence images of JY25 cells which were stained with our substrates

Figure 3. fluorescence images of jy25 cells which were stained with our substrates .jpg

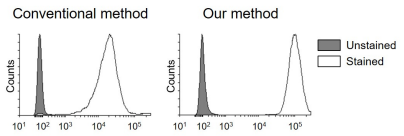


Figure 4. Flow cytometric analysis of JY25 cells stained by conventional method (left) and our method (right)

Figure 4. flow cytometric analysis of jy25 cells stained by conventional method left and our method right .jpg

Design of SERS nanotags for the multiplexed detection of Dengue and Zika in a Lateral Flow Assay

Wednesday, 27th September - 15:17 - Biological & medical nanodevices and biosensors - Room 207 - Oral - Abstract ID: 363

Dr. Maria Sanchez-Purra [1], ***Mr. Marc Carré-camps*** [2], ***Dr. Helena De Puig*** [3], ***Dr. Irene Bosch*** [3], ***Prof. Lee Gehrke*** [3], ***Prof. Kimberly Hamad-Schifferli*** [1]

1. *University of Massachusetts Boston,* ***2.*** *IQS School of Engineering,* ***3.*** *MIT*

Introduction

Zika and Dengue are mosquito-borne diseases that are currently a major global threat. They can co-circulate in endemic areas, as they are transmitted by the same vector and show similar non-specific symptoms with dramatically different outcomes. Because many outbreaks occur in areas that are resource-poor, assays that are user-friendly, inexpensive and require no power are in need for patient treatment, quarantining, and surveillance. Paper-based sandwich immunoassays, such as lateral flow assays (LFA), are attractive as point-of-care solutions as they have the potential for wider deployability than lab-based assays such as PCR. However, the low sensitivity of these assays imposes limitations on their ability to detect low biomarker levels, which is the case of Zika infections, co-infections with other viruses, and also for early diagnosis of any disease. Here, we exploit the high sensitivity of surface-enhanced Raman spectroscopy (SERS) in a multiplexed assay that can distinguish between Zika and Dengue non-structural protein (NS1) biomarkers.

Methods

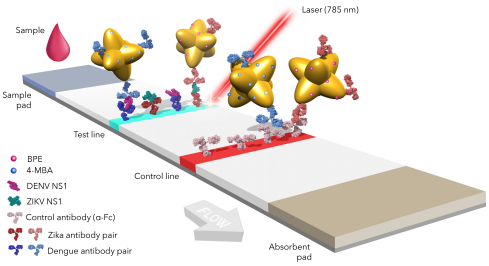
Gold nanostars absorbing in the NIR range, which can provide a high SERS effect, were synthesized. Two Raman reporter molecules, BPE and 4-MBA, were absorbed onto the nanostars that were further conjugated to specific antibodies for Zika and Dengue NS1 biomarker, respectively. LFA were run in a dipstick conformation mixing the SERS-encoded nanostars with commercial NS1. When NS1 was present in the sample, the antibody sandwich was formed rendering a colored spot in the test line, that was analyzed with a Raman microscope.

Results

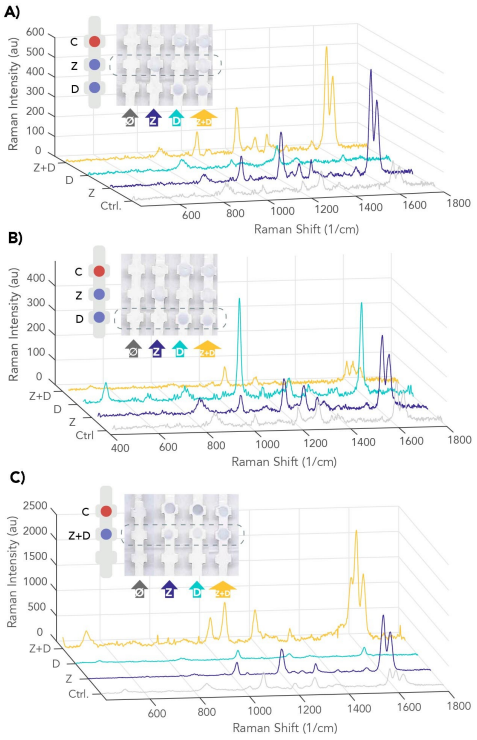
Using SERS allowed the detection of low concentrations of the viral biomarkers with a limit of detection of 15-fold and 7-fold, for ZIKV and DENV, lower than that of colorimetric LFA, being able to detect down to 0.72 ng/ml and 55.3 ng/ml of viral NS1, respectively.

Discussion

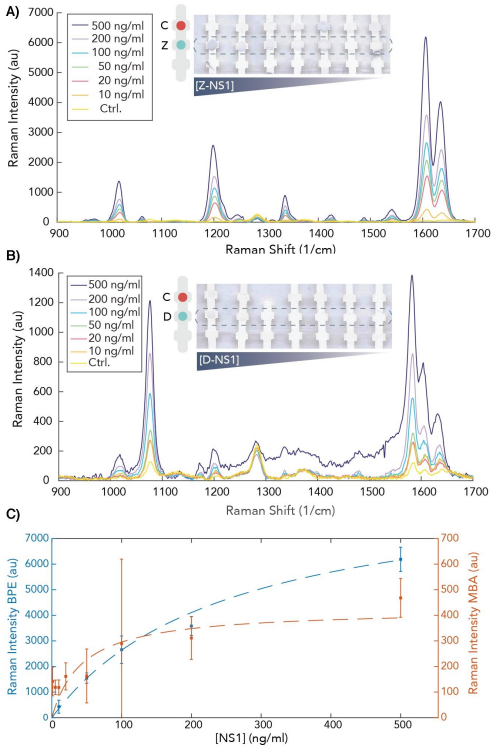
The combination of SERS with LFA can make a promising platform for a point-of-care device, as it can provide a much higher sensitivity than optical LFA devices, giving a result within minutes. In addition, it allowed the multiplexed detection of both biomarkers, which can help to decrease the amount of patient sample required for the test.



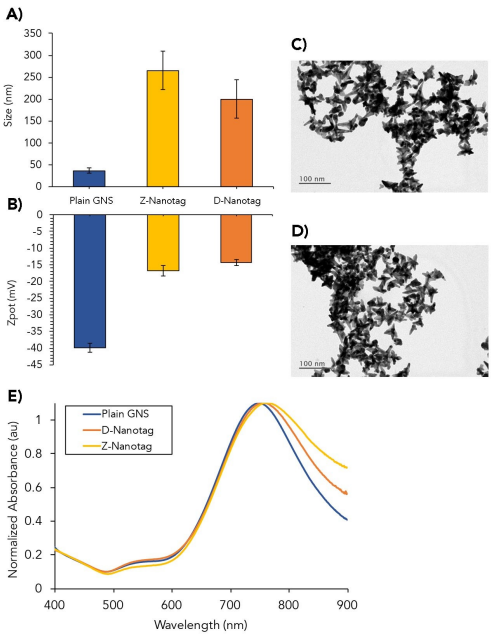
Sers-lfa approach.jpg



Multiplexed assay.jpg



Limit of detection.jpg



Ab-conjugated gold nanostars.jpg

Investigation of the Use of Nanocrater-decorated Anodic Aluminum Oxide Membranes as Substrates for Reproducibly Enhanced SERS Signals

Wednesday, 27th September - 15:34 - Biological & medical nanodevices and biosensors - Room 207 - Oral - Abstract ID: 385

Ms. Merve Celik [1], ***Ms. Sevde Altuntas*** [1], ***Prof. Fatih Buyukserin*** [1]

1. *TOBB University of Economics and Technology*

Introduction

Surface-enhanced Raman spectroscopy (SERS) is an optical phenomenon yielding enhanced Raman signals on nano-decorated conducting materials. It provides label-free analysis of molecules and has the potential to detect down to single molecule. Despite the potential sensitivity and the wide range of applications for SERS, it can not be used as a routine diagnostic tool due mainly to the poor reproducibility of the SERS signals. To obtain reproducibly strong SERS signals, both lithographic and non-lithographic approaches are investigated to produce large-area nanopatterned SERS-active substrates displaying periodical arrays of nanostructures. These methods can provide controllable periodicity of plasmonic nanostructures as well as tune hot-spot density and geometry which are known to influence the electromagnetic enhancement, the major contributor to SERS signal intensities. Also the control over the structure and the periodicity results in minimum sample-to-sample variation ensuring reproducibility.

Methods

We studied a non-lithographic method for fabricating periodically decorated nanoparticle arrays by utilizing the barrier sides of anodic aluminum oxide(AAO) membranes. These membranes are a class of special biomaterials that are produced from high purity aluminum via two step anodization. The production of the substrates are easy and highly controllable and compared to lithography it is cost-effective. The obtained barrier side of AAO membranes which are periodically nanobump-decorated(NBDS) are further treated with wet etching to create periodic arrays of nanocraters(NCDS).(Fig.1) After gold-coating on the surfaces, the intensity of SERS signals for both fabricated surfaces were compared by using two different dyes, Methylene Blue and Congo Red.

Results

The optimized thickness of gold was found to be 20 nm for both surfaces.(Fig.2) NCDS displayed intensified SERS signals compared with the NBDS counterparts.(Fig.3) This result was also confirmed with computer simulation studies and it was related to the increased surface roughness for the NCDS substrates. The fabricated Au@NCDS nanoplatforms were stable for extended periods and allowed enhanced ($2.3x10^5$ enhancement factor) and reproducible SERS signals(Fig.4) with RSD values 10% from independently prepared samples and LOD levels down to 10^{-7} M for Methylene Blue.

Discussion

Our current studies are focused on the potential use of these SERS substrates for sensing biomarker molecules including myoglobin and troponin-T.

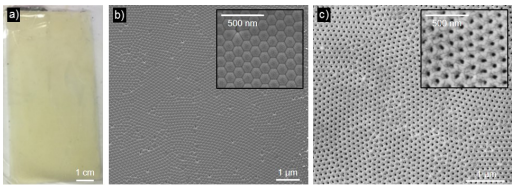


Figure1.jpg

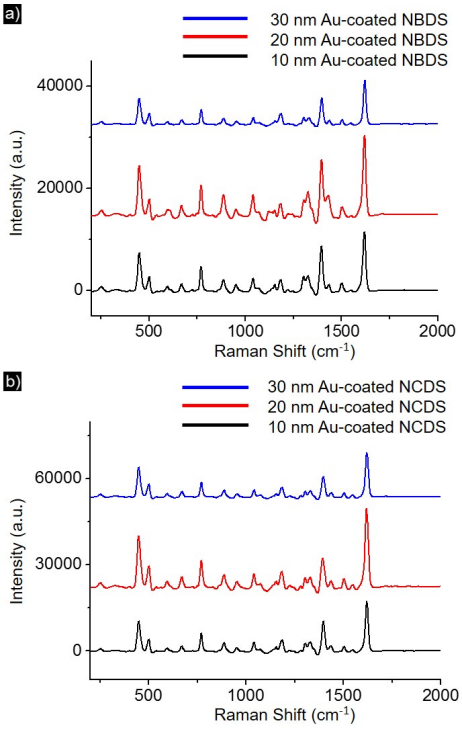


Figure2.jpg

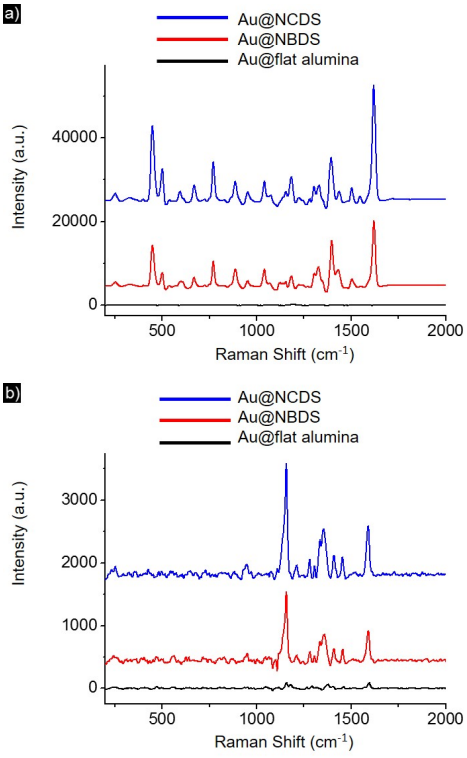


Figure3.jpg

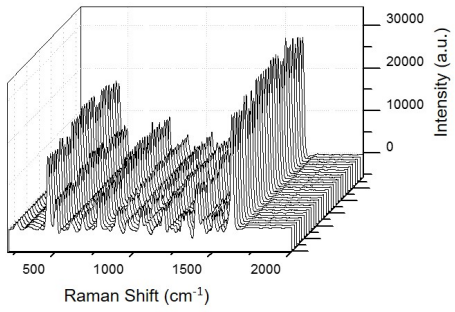


Figure4.jpg

Impact of amphiphilic peptide dendrimers on proteins self-assembly

Wednesday, 27th September - 15:51 - Biological & medical nanodevices and biosensors - Room 207 - Oral - Abstract ID: 119

Prof. Zofia Lipkowska [1], ***Dr. Maja Morawiak*** [1]

1. *Institute of Organic Chemistry, Polish Academy of Sciences*

Introduction. Improper peptide/protein folding followed by fibrillation is regarded a main reason of neurodegenerative diseases. For this reason major efforts have been achieved to find molecules that can inhibit or interfere with aggregation process.

Methods. We will present studies on interactions of low molecular weight amphiphilic peptide dendrimers with pH- and temperature-dependent secondary structures generated by model polypeptide systems: cationic polylysine (PLL) and anionic polyglutamic acid (PLGA). Interactions of dendrimers with self-assembled peptides were investigated in detail by application of circular dichroism (CD), UV and fluorescence spectroscopy. To follow conformational transitions (self-assembly – disassembly processes) three dyes: Congo Red, curcumin and Thioflavin T were applied independently. Parallel microscopic studies showed macroscopic manifestation of the formed secondary structures and mechanism of their propagation.

Results. It was found that an optimized balance between hydrophilicity and lipophilicity as well as concentration of the designed dendrimeric molecules is the critical condition for their assembling (or not) with model proteins. Moreover, careful controlling of the commercial mixtures of polypeptides used in aggregation studies seems to be another important point that will be addressed.

This work was supported by grant from the National Science Centre, UMO-2015/19/B/ST503547.

Study of the effects of xenobiotics on attached mammalian cell line by label-free biosensor

Wednesday, 27th September - 16:08 - Biological & medical nanodevices and biosensors - Room 207 - Oral - Abstract ID: 93

Ms. Enikő Farkas [1], ***Prof. András Székács*** [2], ***Mrs. Boglárka Kovács*** [1], ***Dr. Róbert Horváth*** [3], ***Dr. Inna Székács*** [3]

1. *University of Pannonia; Hungarian Academy of Sciences, Research Centre for Natural Sciences, Institute for Technical Physics and Materials Science,* **2.** *Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre,* **3.** *Hungarian Academy of Sciences, Research Centre for Natural Sciences, Institute for Technical Physics and Materials Science*

Cytotoxicity measurements predominantly apply label-based techniques (for example enzymatic and MTT assays) and animal feeding tests. In this study, we attempted to develop a new and quick cytotoxicity determination method with Epic® BenchTop label-free optical biosensor, which eliminates the drawbacks of the conventional methods (Fig. 1.).[1] Digital laser holographic transmission microscopy was applied as a label-free, non-invasive, non-destructive and non-phototoxic method for qualitative and quantitative measurement of cell morphological changes.[2]

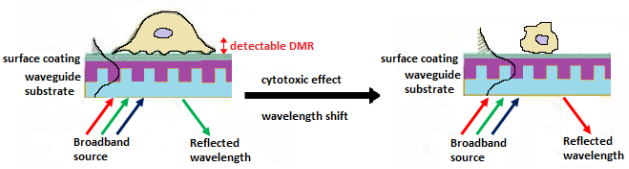
Environmental and toxicological characteristics of formulated pesticides may substantially differ from those of their active ingredients or other components alone. In the present work, Roundup herbicide, its formulant (polyethoxylated tallow amine, POEA) and its active ingredient (glyphosate) were studied on a mammalian cell lines.

Conventional method (MTT test) and holographic microscopy have proved the cytotoxicity effects of the agents on the neuroectodermal cell line.[3] Therefore, the cytotoxicity effect of this herbicide was intended to detect on the preosteoblastic cells by Epic Bt biosensor. During the Epic biosensor measurement, the reagents were added to the previously adhered on the biosensor surface cells. To characterize kinetic of cell detachment from the sensor surface a widely used for this purpose trypsin/EDTA solution was applied. Similar kinetics of biosensor signals were obtained for the treatment with xenobiotics and their magnitude and slope correlate well with the increased detachment of cells from the sensor surface. These results compare with those obtained with Holographic microscopy (Holomonitor). Holographic microscopy revealed that glyphosate influences cell division, motility, elongated morphology and causes slower cell death. Glyphosate has to be applied at higher concentration to result in cytotoxic effects than Roundup or POEA. In conclusion, the formulant POEA was determined more toxic than herbicide preparation Roundup or its active ingredient glyphosate.

[1] N. Orgovan et. al., Sci. Rep., 2014, 4: 4034.

[2] B. Peter et. al., J. Biomed. Opt., 2015, 20, 067002-067002.

[3] I. Székács et. al., Int. J. Biol. Veterin. Agric. Food Engin.,2014, 8, 212-218.



Schematic representation of epic bt setup. cytotoxic effect on the adhered cell within the close vicinity of the sensing area change the bulk index of refraction thus the wavelength value of the reflected light is altered.png

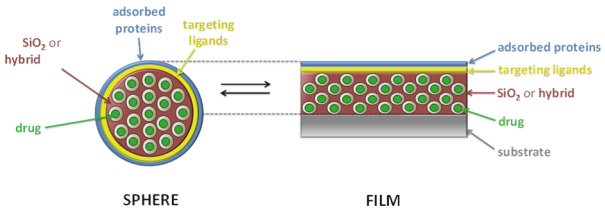
Understanding the “in vivo” interfacial dynamics of therapeutic vectors through in situ ellipsometry.

Wednesday, 27th September - 15:00 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 137

Ms. Elisa Bindini [1], ***Dr. Marco Faustini*** [1], ***Dr. Andrea Cattoni*** [2], ***Dr. Cédric Boissière*** [1]

1. *Université Pierre et Marie Curie,* ***2.*** *CNRS*

Mesoporous silica nanoparticles are now widely investigated as biocompatible vectors for drug delivery, thanks to their well-defined and tunable porosity, high loading capacity and the possibility to be functionalized with organic molecules to control cargo release, cell surface recognition, biocompatibility and stability.[1]
However, the interfacial dynamics of these systems are still unknown. In fact, investigating all the phenomena that take place *in vivo* is complicated because too many parameters are playing a role at the same time and replacing real biological environments with *in vitro* model system is often not meaningful to get useful informations. For example, in physiological conditions, the presence of proteins lead to surface adsorption of a protein layer called *corona*. The protein *corona* drives the vector’s targeting ability and the cell uptake mechanism as well as the silica degradation rate; moreover it could close the pores, affecting strongly the kinetics of the drug delivery. Furthermore, *in vivo* conditions presume a flow stream (veins, vessels) which is far from being constant all over the nanoparticle’s path and whose variations can affect greatly the vector interactions and their kinetics.
Consequently, understanding the interfacial dynamic of these therapeutic vectors in real biological environments is indispensable to formulate efficient drug delivery nanocarriers.
We reproduce the structure and composition of mesoporous silica nanoparticles, functionalized with different organic moieties, in 2D thin films and study them through *in situ* ellipsometric analysis.[2]
The ellipsometric analysis is fast and can be performed in liquid media. We can thusmonitor protein adsorption/desorption kinetics and film hydrolytic intrinsic dissolution in the chosen fluid (protein solutions, serum, blood): this information is critical for drug delivery systems since dissolution drives the drug release and defines the average stay in the body.
All the latter phenomena are studied under a flow stream, to mimic as much as possible the *in vivo* conditions, monitoring the influence of surface functionalization, pore size and geometry, drug loading and medium flow on the interfacial behavior of mesoporous silica thin films. A special ellipsometric setup has also been developed to use with opaque liquids (serum, blood) to push further the investigation.



Samples structure.png

Determination of the Adsorption Degree of Protein on Gold Nanoparticles by Gel Electrophoresis

Wednesday, 27th September - 15:17 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 47

Dr. Katarzyna Ranoszek-Soliwoda [1], ***Ms. Ewa Czechowska*** [1], ***Dr. Emilia Tomaszewska*** [1], ***Prof. Grzegorz Celichowski*** [1], ***Prof. Janusz Szemraj*** [2], ***Prof. Jaroslaw Grobelny*** [1]

1. *University of Lodz,* ***2.*** *Medical University of Lodz*

Introduction

Proteins can be immobilised on the surface of nanoparticles (NPs) by two main methods: covalent bonding and adsorption. Regardless the method, the immobilisation process allow for the design of multimodal structures that have the synergistic effect of being a protein-particle hybrid system. Although, there are several methods available to investigate NP-protein interactions, quantification of the amount of protein on the single NP surface is still challenging. In this work a simple, reproducible and highly sensitive analytical method to determine the amount of protein adsorbed on a single NP by gel electrophoresis will be presented.

Methods

Gold nanoparticles (AuNPs) synthesized via chemical reduction method were modified with proteins (catalase) by incubation. Dynamic Light Scattering was used to monitor the hydrodynamic size of AuNPs and the agglomeration state of colloids before and after the immobilisation of catalase. Morphological studies of the AuNPs before and after modification were performed by Scanning Transmission Electron Microscopy. Determination of catalase adsorption was carried out with different electrophoresis protocols to find the optimal conditions for quantification of the surface coverage of AuNPs by catalase (native-PAGE, non-reducing SDS-PAGE, b-mercaptoethanol-PAGE, and reducing SDS-PAGE).

Results

The obtained results confirmed that the modification process of AuNPs with catalase did not disturb the stability of NPs. AuNPs were colloidally stable and did not form any aggregates after modification. The hydrodynamic size of protein-modified AuNPs increased compared to non-modified which confirmed the successful modification process. The determination of the surface coverage of AuNPs with catalase was performed based on the native-PAGE for which the most sensitive conditions were found.

Discussion

The presented method allowed for the identification and quantification of the amount of CAT adsorbed on the surface of NPs in a colloidal state. The obtained results prove that the method is effective and versatile and can be successfully used for the identification and quantification of proteins adsorbed on the surface of different types of colloidal NPs.

Investigating the molecular effects of hyperbranched polycation-DNA complexes on lung cancer cells using LC-MS-based metabolite profiling.

Wednesday, 27th September - 15:34 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 331

Mr. Ali Alazzo [1], Mr. Mohammad Al-Natour [1], Dr. Keith Spriggs [1], Dr. Snow Stolnik-Trenkic [1], Dr. Dong_hyun Kim [1], Prof. Amir Ghaemmaghami [1], Prof. Cameron Alexander [1]

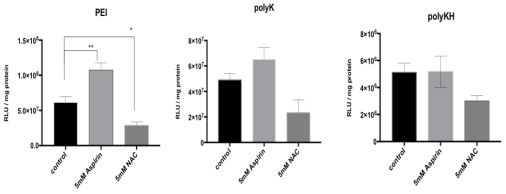
1. *University of Nottingham*

Introduction: Gene therapy has been considered a promising approach for addressing hereditary and acquired diseases since the first clinical trials in 1990. However, no gene therapy application has been approved by FDA yet due to the biological and technical barriers facing gene delivery. Hyperbranched cationic polymers have emerged as attractive gene delivery systems, they are cheap to scale up and easy to functionalise. However, the impact of their nanosized polyplexes form at the cellular and molecular level is not fully studied.

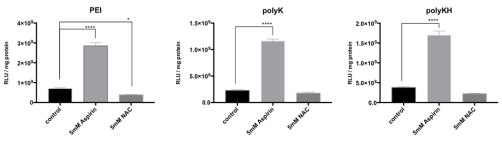
Methodology: Polylysine and polylysine-co-histidine were synthesised using conventional thermal condensation method. In addition to polyethyleneimine, the prepared polymers were used to fabricate nanosized polyplexes by mixing with noncoding plasmid DNA, cell-based global metabolic profiling was employed to investigate the impact of these polycation-DNA polyplexes (PCDP) on the metabolism of A459 and H1299 lung carcinoma cell lines.

Results: The study revealed that PCDPs have altered many metabolic pathways in both cell lines. Interestingly, PCDPs have induced oxidative stress and inflammatory response in A459 cells and only oxidative stress in H1299 cells. On the other hand, previous transfection experiments showed that the branched polymers were more efficient in H1299 than A459 which might indicate that the inflammatory response suppresses the transfection. Aspirin and N-acetyl cysteine were used to minimise the inflammatory response and the oxidative stress respectively. Aspirin has enhanced the transfection in H1299 cells more efficiently than A549 ones and has slightly decreased the transfection in both cell lines.

Discussion: Interestingly Aspirin enhanced the transfection in the cells that showed no inflammatory response which indicates that Aspirin can enhance the transfection by unknown mechanisms other than its anti-inflammatory effect. According to literature, N-acetyl cysteine is supposed to enhance the transfection by suppressing the oxidative stress, however, the results suggest that the oxidative stress aids the transfection by PCDP either by facilitating their lysosomal escape or entry to nucleus.



Effects of aspirin and n-acetyl-cysteine on transfection of pei pk and pkh polyplexes prepared in 10 m hepes buffer ph7.4 in a549 cells.png



Effects of aspirin and n-acetyl-cysteine on transfection of pei pk and pkh polyplexes prepared in 10 m hepes buffer ph7.4 in h1299 cells.png

Metabolite	Untreated Cells	PEI	PK	PKH
Formylanthranilate	0.00	54.71	33.75	1.18
L-Kynurenine	1.00	76.38	48.13	0.00
L-Formylkynurenine	1.00	8.25	7.76	0.74
Anthranilate	1.00	6.11	6.55	1.54
L-Tryptophan	1.00	0.50	0.73	0.83
Glutathione	1.00	0.65	0.79	1.04
L-Cystathionine	1.00	0.63	0.57	1.07
O-Acetyl-L-homoserine	1.00	0.41	0.79	0.90
O-Succinyl-L-homoserin	1.00	0.27	0.54	0.59
O-Acetyl-L-serine	1.00	0.47	0.84	1.16

Table 1 fold changes in inflammatory and oxidative stress biomarkers intracellular levels in a549 cells..png

Magnetoplasmonic nanoparticles for use in Nanotherapy.

Wednesday, 27th September - 15:51 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 356

Ms. Grace Brennan [1], ***Dr. Nanasaheb Thorat*** [1], ***Dr. Aladin Mani*** [2], ***Dr. Rabah Mouras*** [2], ***Dr. Joanna Bauer*** [3], ***Dr. Christophe Silien*** [1], ***Prof. Syed A. M. Tofail*** [1]

1. *University of Limerick,* ***2.*** *University of Limerick, Ireland,* ***3.*** *Wroclaw University of Technology, Wrocław*

Introduction

Photonic therapy, can treat tumors either through photothermal effect using a photothermal agent (PTA) for selective localised heating, or through photo-dynamic therapy using photosensitizers (PS) for a series of photo-chemical reactions. Many plasmonic nanoparticles can serve both as PTA and PS for stimulation in the visible and infra-red region of the optical spectra (optical stimulation). Magnetic hyperthermia (MH) based therapy [1-3] can be combined with optical photothermal stimulation. This dual therapy has been shown to be highly effective in-vitro and in-vivo in comparison to either therapy used individually[4].

Method

NANOCARGO, introduces a new paradigm that nanotherapy can be made far more effective if a multimodal action can be integrated by adding a plasmonic shell to a magnetic nanoparticle core. The development of drug resistance in tumor cells plays a major role in the failure of current cancer therapies. To overcome the drug resistance NANOCARGO with photo-magnetic properties can be developed along with simultaneous diagnosis and therapy (theranostics) approach. The plasmonic shell can be functionalized with chemotherapeutic drugs and aptamars. These nanocargos can be simultaneously simulated through magnetic hyperthermia and photonic therapy.

Results and Discussion

Anticancer therapy is thus made more effective through the multimodal stimulation of these nanocargos, minimally invasive optical stimulation and extracorporeal magnetic resonance imaging based excitation. Showing promise for a **minimally invasive procedure offering personalisation of cancer therapy**.

[1] Shete,P.B.,et al. Applied Surface Science 288(2014):149-157.

[2] Thorat,Nanasaheb.,et al. ACS applied materials & interfaces 8.23(2016):14656-14664.

[3] Thorat,Nanasaheb.,et al. Physical Chemistry Chemical Physics 18.31(2016):21331-21339.

[4] Espinosa, Ana,et al. ACS nano 10.2(2016):2436-2446.

[5] Levin,Carly,.et al. ACS nano 3.6(2009):1379-1388.

This work is supported by Science Foundation Ireland(SFI) CÚRAM funding and Government Of Ireland(GOI) Postdoctoral Fellowship by Irish Research Council(IRC).

Co-polymer layered magnetic nanoparticles for hyperthermia treatment of cancer and precise drug delivery

Wednesday, 27th September - 16:08 - Targeted drug delivery and nanocarriers - Auditorium - Oral - Abstract ID: 406

Dr. Muhammad Usman Hashmi [1]

1. *Superior University Lahore*

In this paper, we study surface-adapted multifunctional magnetic nanoparticles with bilayer oleic acid, and layered with a poly (N-isopropylacrylamide-co-acrylamide) thermo-reactive copolymer per emulsion Polymerization, for the precise management of drugs and applications of magnetic hyperthermia. Nanoparticles Anticancer drug doxorubicin was loaded onto the copolymer chains at ambient temperature. Composite (hydrated) nanoparticles with an average diameter of 50 nm were of core shell structure having a magnetic core of about 20 nm and the shell was consisted of organic compounds and water. The magnetic core was superparamagnetic devoid of coercive force and remanence because of the pseudo-single domain nanostructure. The lowest critical solution temperature (LCST) of the heat-sensitive copolymer was witnessed to be approximately 39ºC. The UV visible spectrophotometer was used to examine the loading and discharge profile of the drug at different temperatures, as well as Magnetic heating. There was almost no drug release at normal body temperature. The drug was released at temperatures higher than LCST, which is significant for controlled drug delivery. Magnetic heat generation was studied by exposing the magnetic fluid to an alternating magnetic field of 8.0 kA m^{-1} having 65 kHz frequency. Capture of magnetic nanoparticles in various fields applied to the target drug was analyzed by a simple magnetic capture system.

‘Clickable’ functionalised recombinant spider silk and its applications

Wednesday, 27th September - 16:50 - Tissue engineering and regenerative nanomedicine - Room 207 - Oral - Abstract ID: 57

Prof. Neil Thomas [1], ***Dr. David Harvey*** [1], ***Dr. Philip Bardelang*** [1], ***Dr. Sara Goodacre*** [1], ***Dr. Alan Cockayne*** [1]

1. *University of Nottingham*

Introduction

We have recently reported[1] the preparation of antibiotic and fluorophore functionalized silk fibres self-assembled from the miniaturized major spidroin protein 4RepCT. This is derived from the dragline silk of the South African nursery web spider, *Euprosthenops australis*.

Methods

The functionalized fibres were prepared by incorporating the bio-orthogonal amino acid L-azidohomoalanine (Aha) in place of the three methionines in the 4RepCT protein. This was achieved by expressing 4RepCT in an *E. coli* methionine auxotroph grown in a medium rich in Aha. Each 4RepCT protein was expressed as a fusion with the protein thioredoxin at its N-terminal in order to keep the silk protein soluble. The thioredoxin was removed by treatment with thrombin to leave $4RepCT^{3Aha}$ which then spontaneously self-assembles into fibres up to 1 m in length. The Aha residues can be selectively and efficiently modified with ligands bearing alkyne groups using a copper (I) catalysed azide alkyne cycloaddition (CuAAC) ‘click’ reaction.

Results and Discussion

We have demonstrated that we can functionalize the silk proteins with fluorophores (Figure 1) and with the broad spectrum antibiotic levofloxacin (Figure 2) using the CuAAC reaction. The antibiotic has been attached via a glycerol ester that is cleaved either through a drop in pH or by esterases released by *E. coli* as they grow. The $4RepCT_{3Aha}$ proteins can be modified either prior to or after silk fibre assembly and the tensile strength of the resulting fibres is unaffected. This has allowed fibres decorated with two or more different ligands to be prepared by mixing differently functionalized batches of fibres together prior to fibre assembly. The different ligands are found to be evenly distributed throughout the fibres as can be seen in the image of silk fibres labelled with both red and green fluorophores which produces a fibre that appears yellow when excited (Figure 2). Antibiotic functionalized silk fibres have been shown to prevent *E. coli* growth for >5 days giving potential uses in dressings for slow healing wounds such as diabetic ulcers.

1. Antibiotic Spider Silk: Site-Specific Functionalization of Recombinant Spider Silk Using “Click” Chemistry. *Adv. Mater*., 2017, **29**, 1604245 (http://dx.doi.org/10.1002/adma…)

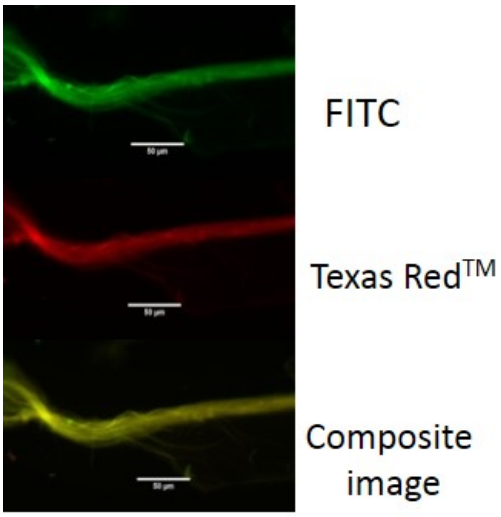


Figure 1 fluorophore functionalised spider silk.jpg

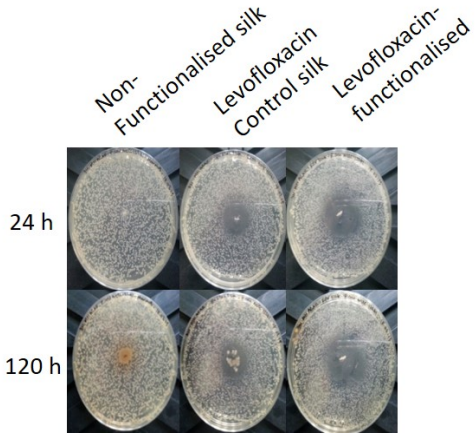


Figure 2 levofloxacin functionalized spider silk.jpg

Engineered extracellular microenvironment with in situ hydrazone-linked hydrogels for tissue engineering

Wednesday, 27th September - 17:07 - Tissue engineering and regenerative nanomedicine - Room 207 - Oral - Abstract ID: 373

Dr. Jenny Evelin Parraga Meneses [1], ***Mr. Janne Koivisto*** [2], ***Ms. Jennika Karvinen*** [1], ***Mr. Birhanu Belay*** [1], ***Prof. Jari Hyttinen*** [1], ***Prof. Katriina Aalto-setala*** [3], ***Prof. Minna Kellomaki*** [2]

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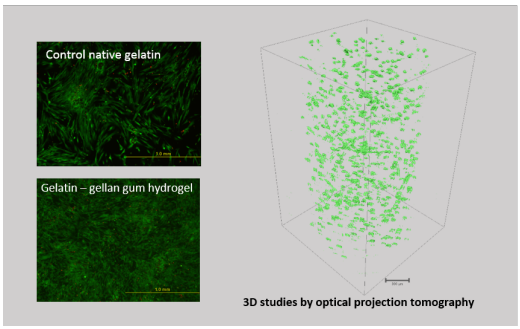
Hydrogels are exceptional biomaterials for a wide range of pharmaceutical and biomedical applications. In fact, in situ formation of hydrogels is desired for minimally invasive techniques for tissue regenerative medicine. Hydrogels have been used as scaffolds, because of their tissue like properties, biocompatibility, high water content and high permeability for nutrients and metabolites. However, engineering hydrogels with tunable mechanical properties is crucial since the extracellular matrix surrounding cells, provides mechanical signals to regulate a variety of cell behaviors.

Here, in situ crosslinkable gelatin – gellan gum hydrogels were developed via hydrazone chemistry. Hydrazide modified gelatin was synthetized in order to form hydrogels through covalent interaction with the aldehydes present in the gellan gum backbone. Aldehydes in gellan gum were generated after the cleavage of vicinal diols with sodium periodate. The hydrogels were characterized mechanically and the stability was studied in cell culture medium. To evaluate cell biocompatibility, human fibroblasts were exposed with the different gelatins and hydrogel formulations. Live/dead staining was used to visually assess cell viability and morphology of fibroblast encapsulated in hydrogels. In addition, elongation and proliferation were evaluated using confocal microscopy and optical projection tomography. Finally, the interaction of cardiomyocytes with the hydrogels was studied in 2D and 3D models.

Hydrazide modification of gelatin and oxidation of gellan gum facilitate the spontaneous covalent bonding between gelatin and gellan gum, aiding rapid gelation with and homogeneous crosslinking distribution. In addition, gelation time and mechanical properties can be controlled by varying the concentration of the components. During compression, the hydrogels showed elastic behavior, which is similar to real soft tissue. Hydrogels showed good cytocompatibility and provided a suitable microenviroment for cell culturing. In addition, human fibroblast showed elongated morphologies after 24h incubation. Preliminary study with human induced pluripotent stem cell derived cardiomyocytes showed favorable cell response to the hydrogels since they are beating spontaneously in 2D and 3D in vitro studies

Thus, we can create tunable 3D microenvironments for the cell spreading and proliferation. Our work may provide insight into the design of biomaterials for cardiac cell culture as well as tissue regeneration.



3d and 2d studies of human fibroblasts on gelatin gellan gum hydrogels.png

Fabrication and Characterization of Biomimetic Nanofibrous Scaffold as Skin Repair Graft in Treatment of Wound.

Wednesday, 27th September - 17:24 - Tissue engineering and regenerative nanomedicine - Room 207 - Oral - Abstract ID: 463

Dr. Poornima Dubey [1], ***Dr. P. Gopinath*** [2], ***Prof. Duncan Craig*** [1]

1. *UCL School of Pharmacy,* ***2.*** *Indian Institute of Technology Roorkee, Roorkee, Uttarakhand-247667, India.*

An intricate amalgamation of polysaccharides and proteins represents the key components of dermis fibrous extracellular milieu. Amongst natural polysaccharides,honey offers unique medicinal and healing properties whereas gelatin characterizes commercially available protein biopolymer which could be electrospun and that makes them an attractive choice in design of skin tissue regeneration scaffolding. In this regime, present study introduced simplest electrohydrodynamic technique based novel bio-compatible, hydrophilic scaffolding nanomaterial for skin tissue regeneration as electrospun honey/gelatin/chitosan based biopolymeric blend nanofibers with polyvinyl alcohol (PVA) as the supporting polymers. The effect of various parameters including concentration, viscosity and temperature was optimized to obtain the bead free nanofiber scaffold. Physiochemical characterization of nanofiber was done by various techniques. The FE-SEM and TEM analysis showed synthesis of bead free nanofiber for alone nanofiber and upto certain percentage of honey incorporation. Wettability and swelling studies confirms the sufficient hydrophilicity of nanofibrous scaffolds in order to permit fluid exchange and absorption of excess exudates. The scaffolding was further investigated for skin tissue engineering applications. The designed scaffolding showed enhanced adherence, growth, and proliferation of human skin fibroblast (NIH-3T3) cells seeded on the nanofibers when compared to cells seeded on TCPC and casted polymeric blend film which was further evident by FE-SEM micrograph. Additionally the cell morphological analysis showed enhanced cell proliferation with no cell death for many days. Remarkably, the honey/gelatin/chitosan blended nanofibrous scaffold offers unique biocompatible, biodegradable, biomimetic substitute as temporary biomedical grafting material for induction of‘ skin tissue regeneration.

Figure 1: FESEM micrograph showing the optimization of blended nanofibers.

Figure 2: a) The TEM micrographic and b) FESEM micrographic image depicting optimized honey incorporated nanofibers.

Figure 3: Wettability analysis of various nano-formulations (a-c).

Figure 4: FESEM micrograph showing proliferation of NIH-3T3 cells seeded nanofibrous scaffold after 3 days (a-b).

Acknowledgement:

This study was supported by DBT (no. BT/PR6804/GBD/27/486/2012), Government of India and IIC IIT Roorkee India.

References:

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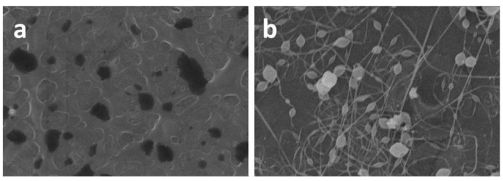

Figure.1.jpg

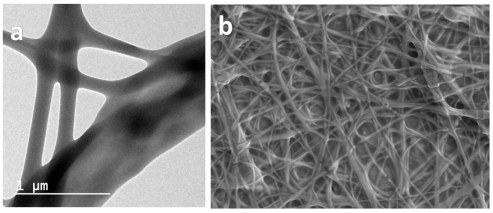

Figure.2.jpg

Figure.3.jpg

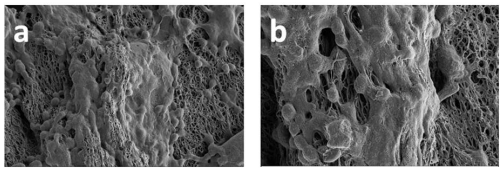

Figure.4.jpg

Fabrication and Cellular Investigation of Biomimetic Collagen-Gelatin Nanopillar Films

Wednesday, 27th September - 17:41 - Tissue engineering and regenerative nanomedicine - Room 207 - Oral - Abstract ID: 381

Ms. Pınar ALPASLAN [1], ***Ms. Sevde Altuntas*** [1], ***Prof. Fatih Buyukserin*** [1]

1. *TOBB University of Economics and Technology*

- Introduction

One of the common problems in implant dentistry is the lack of sufficient amount of bone tissue formation around the implant. Investigation of bone and teeth interaction brings out the periodontal ligament (PDL), which provides bone regeneration. Some parts of PDL include Sharpey fibers and it consists of structures linking teeth and bone. These fibers are generally positioned parallel to each other and perpendicular to the tooth surface. Besides , they have extremely rich and regular structure of collagen. Our aim in this study is to examine bone cell behaviors on arrays of collagen-gelatin mixed nanorods which are bioinspired from Sharpey fiber structures and compare them to flat collagen-gelatin films. Such nanorod arrays were fabricated using anodic aluminum oxide (AAO) molds. These nanoporous substrates are ideal molds for many applications owing to their modifiable chemistry, ultrahigh surface areas, and controllable porosity and pore dimensions that extend perpendicular to membrane surface.

- Methods

In this study, we fabricated nanoporous AAO molds by the two-step anodization of ultrapure Al foils and decreased their surface energy by using silane chemistry. Different concentrations of collagen-gelatin solution were used to obtain collagen-gelatin films that replicate the topography of the AAO molds. We have optimized the PEGDGE concentration in the collagen-gelatin solution to obtain stable nanostructures. The morphological characterization of these nanorod arrays were examined by using scanning electron microscopy (SEM) and atomic force microscopy (AFM) (Fig.1). Their stability tests in water and cell medium were conducted by utilizing SEM imaging and swelling tests. Also, cell viability and toxicity tests on SAOS-2 cells were completed.

- Results & Discussion

Improved cell adhesion and viability were observed on the nanorod collagen-gelatin films compared to flat collagen-gelatin films. Also, collagen-gelatin films were not toxic to the cells. Finally,mineralization and ALP activity of SAOS-2 cells on nanostructured surfaces will be completed and compared to flat collagen-gelatin films.

The project is partially supported by Turkish Academy of Science (TUBA).

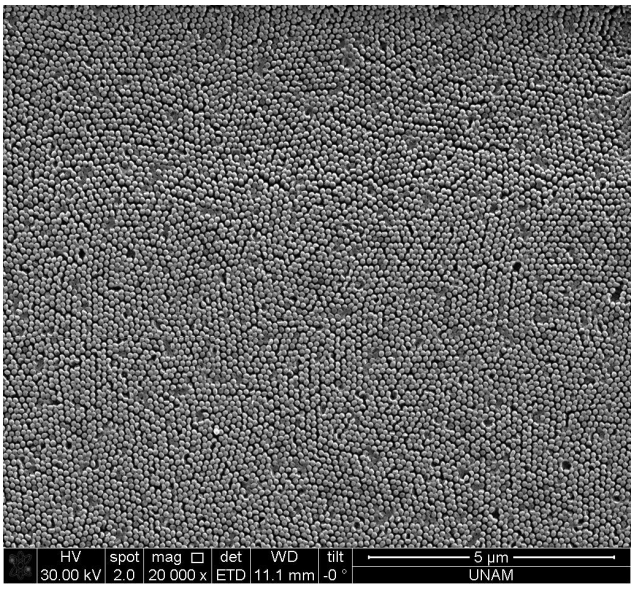

Fig.1.jpg

Improving 131I radioiodine therapy by hybrid metal-polymer nanoparticles

Wednesday, 27th September - 16:50 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 194

Ms. Béatrice Cambien [1], ***Dr. Marie Paquet*** [2], ***Ms. Marine Le Goas*** [3], ***Dr. Aurélie Paquirissamy*** [3], ***Dr. Geraldine Carrot*** [3], ***Dr. Jean-Philippe Renault*** [4], ***Mr. Thierry Pourcher*** [1]

1. *TIRO, UMRE 4320, BIAM, DRT, CEA, Université de Nice-Sophia Antipolis,* ***2.*** *TIRO, UMRE 4320, BIAM, DRT, CEA, Université de Nice-Sophia Antipolis, France,* ***3.*** *NIMBE UMR 3685, CEA, CNRS, Université Paris-Saclay, CEA Saclay 91191 Gif sur Yvette Cedex,* ***4.*** *NIMBE UMR 3685, CEA, CNRS, Université Paris-Saclay*

Introduction

^{131}I radioiodine therapy is very performant in the treatment of thyroid cancer metastases. Applicability of this approach combined with gene therapy (transfer of the Na/I symporter gene into tumors) to treat non-thyroidal neoplasms is still hampered by the reduced capacity of such tumors to accumulate ^{131}I. Various strategies have been tested so far to improve this approach, with limited efficacy. The use of metal-containing nanoparticles as radiosensitizers for cancer treatment has been reported in the context of External Beam Radiation Therapy. The present study reveals novel information on using these nano-objects to enhance the therapeutic efficacy of ^{131}I-mediated radiotherapy.

Methods

Various metal-containing nanoparticles were synthesized by reduction of corresponding metal salts. Polymethacrylate acid was grafted onto their surface (information on the poster by Le Goas et al). We performed in vitro clonogenic assays to investigate the impact of the nano-objects on the damage induced by ^{131}I (0-300 microCi) using melanoma and colorectal cancer cells expressing the Na/I symporter (NIS). Finally, the efficacy of the nanoparticles to sensitize tumors to radio-iodine treatment was explored in a preclinical model of melanoma-bearing mice receiving percutaneous intratumoral injections of Np at 10% of tumor volume.

Results

After incubation with the gold nanoparticles for 2 hours, marked radiosensitization was observed on the melanoma and colorectal cancer cells exposed to 10 microCi ^{131}I. In vivo, intratumoral injections of PMAA-GNP combined with systemic ^{131}I treatment led to a reduction in melanoma B16 tumor development compared to radio-iodine alone. Histological analysis shows increased cell death in tumors treated with combined therapies compared to radiotherapy alone.

Discussion

All together, these results open up novel perspectives for using high-Z metallic NPs in radiotherapy.

Magnetic Drug Delivery Nanoparticles for Combined Cancer Therapies

Wednesday, 27th September - 17:07 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 407

Dr. farah benyettou [1], ***Prof. Ali Trabolsi*** [1]

1. *New York University Abu Dhabi*

Introduction

A century ago, Paul Ehrlich imagined the concept of selectively target a pathogen without affecting the host using the “magic bullet”. Since twenty years, researchers in cancer therapy were particularly inspired by the idea. The engineering of tiny systems to detect, diagnose and treat disease gave rise to the most promising advances in the fight against cancer, the nanomedicine.[1]

Before becoming an extraordinary fruitful market, nanotechnology was a crazy idea in the mind of science fiction writers. In the 1966 movie Fantastic Voyage, a team of researchers is reduced and injected into the blood stream.

Methods

In this aim, we developed, magnetic theranostic platforms that can be loaded with chemotherapeutics and provide targeted and sustained delivery to target tumor cells as well as control over not only the timing but the location of cargo delivery, in order to optimize the drug efficiency.

Results

Our first theranostic platform is a triply stimuli-responsive supramolecular drug delivery system based on cucurbit[7]uril-modified iron-oxide NPs (CB[7]NPs) specific to cancer cells with minimal toxicity to non-cancer models.[2-3] These particles can act simultaneously as heat-mediators for hyperthermia treatment and as delivery vehicles for the chemotherapeutic doxorubicin (Dox).

Our second strategy is based on mesoporous iron-oxide nanoparticles loaded with Dox and coated with a thermo-sensitive polymer (Dox@F108-mNPs).[4] Dox@F108-mNPs are stable in physiological conditions and release slowly their cargo under acidic conditions or suddenly with magnetic heating. The treatment of cancer cells with both Dox@F108-mNPs and magnetically-induced hyperthermia drastically reduced cancer cell viability compared to Dox or Dox@F108-mNPs treatment alone.

Discussion

Both innovative ‘theranostic’ strategies will have the potential to pave the way for treatment of cancer in a highly selective and effective, yet relatively sensitive, manner and will surely result in the personalization of chemotherapy for improved patient outcomes.

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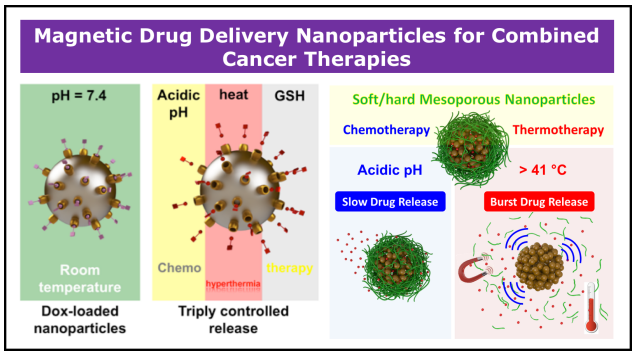

Fbenyettou.png

Biological Interactions of Ultrasmall Nanoparticles: What About the Corona?

Wednesday, 27th September - 17:24 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 362

Dr. Luca Boselli [1], ***Dr. Ester Polo*** [1], ***Dr. Valentina Castagnola*** [1], ***Dr. Hender Lopez Silva*** [1], ***Mr. Francesco Muraca*** [1], ***Prof. Kenneth A. Dawson*** [1]

1. *University College of Dublin*

Despite their potential for biological applications, the demonstrated tendency of nanoparticles (NPs) to accumulate in specific organs, as liver or spleen, in an uncontrolled fashion, still causes a considerable concern.[1]

However, it has been shown that for sizes below 3 nm certain NPs can display non-scalable properties[2] and a different scenario in terms of biological interactions might be disclosed in respect of what is currently described for larger NPs.[4] *In vivo* studies in mice, for example, showed the tendency of gold ultrasmall NPs (USNPs) to exhibit efficient renal clearance and almost no liver accumulation.[5] This might not be due merely to their small core size. Generally, proteins present in the biofluids strongly interact with NPs forming long-lived biomolecular *corona* which, by altering the NPs surface, determine their final fate in the body.[3] Nevertheless, considering that most of the plasma/serum proteins present a hydrodynamic diameter up to 5 times the size of USNPs, can we still speak about biomolecular *corona?*

Investigation of USNPs-proteins complex is very challenging since the protocols normally used for larger NPs are ineffective. We obtained interesting insights on the bio-interactions of 5, 3 and 2 nm gold NPs (with a range of surface functionalisation) by using gel-electrophoresis. These assays, normally used to separate even small peptides, allowed to observe striking differences in the way NPs with 1 nm of size difference can interact with the biological environment. Below a certain size (also strongly depending on the surface chemistry of the particle) the long-lived NP-protein interactions could be nearly eliminate, suggesting that the corona might fluctuate rapidly, possibly leading to quite distinct biological outcomes compared to larger particles.

USNPs represent a new promising tool in nanomedicine, possibly not limited by unwanted organs accumulation but their interaction with biological fluids must be deeply investigated since it can be the key to understand their biodistribution and cell trafficking.

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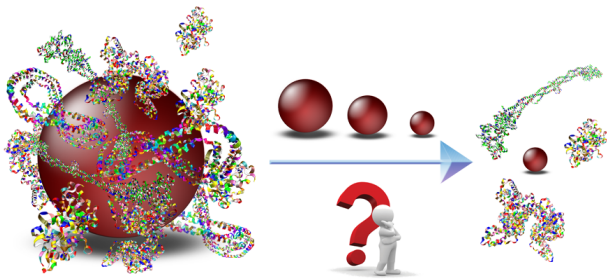

Fig1.png

Development of a multifunctional nanodevice to treat breast cancer

Wednesday, 27th September - 17:41 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 398

Ms. Mª Victoria Cano-cortés [1], Mr. Saúl Navarro Marchal [2], Dr. Juan Jose Diaz Mochon [1], Prof. Juan Antonio Marchal Corrales [2], Prof. Rosario M. Sanchez-Martin [1]

1. *Genyo_University of Granada,* ***2.*** *University of Granada*

Introduction: NanoChemBio team has a robust know-how in the field of nanotechnology. They have prepared a broad portfolio of nanoparticles of different nature that has been broadly used in nanomedicine. They have developed a portfolio of protocols for multifunctionalization of these nanoparticles based on orthogonal protection strategies. These engineered nanoparticles have been successfully conjugated to small molecules, proteins and nucleic acids without altering the properties and activity of the bioactive cargoes.
Aim: To develop an effective, safe and non-toxic nanosystem based on the use of synthetic nanospheres that are multifunctionalized with (i) a drug, (ii) a diagnostic tracker and (iii) a ligand for targeted delivery. These new nanodevices will combine the following characteristics in a single therapeutic agent: pharmacological selectivity, tissue specificity and personalized treatment.
Results and Discussion: (i) Protocols have been successfully developed to generate these theranostic nanoparticles in a reproducible manner. (ii) Efficiency of cellular uptake and cell viability in a panel of breast cancer cell lines have been successfully achieved. (iii) In vivo tumour development and tracking of nanodevices were analysed by NIR fluorescence imaging. Interestingly, the IC50 of standard anticancer drug is higher than the IC50 of nanoparticle-bound drug. From the in vivo studies, there are three main achievements worth highlighting: (1) location of the nanoparticles within the tumour area; (2) tumour size was markedly reduced and (3), in contrast to free drug, nanoparticle-bound drug did not induce any toxicity in the mice.
Conclusions: A prototype of this nanodevice to treat breast cancer and to monitor treatment efficiency together with to determine localization of the tumour focus has been developed. Further studies will be carried out to scale-up the production of this nanodevice and to study in vivo nanotoxicity.
Acknowledgements: The authors thank the Research Results Transfer Office (OTRI) of the University of Granada for support on the technological development of this project. This research was supported by (i) University of Granada - Plan Propio Investigación y Transferencia 2016 -P28: PSETC (PSE/16/003), (ii) Spanish Ministerio de Economía y Competitividad (BIO2016-80519-R) and (iii) Marie Curie Career Integration Grants within 7th European Community Framework Programme (FP7-PEOPLE-2011-CIG-Project Number 294142).

Targeted in vivo imaging of tumour and stromal cells within a complex tumour microenvironment

Wednesday, 27th September - 17:58 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 136

Dr. Francesco Zamberlan [1], ***Dr. Lyudmila Turyanska*** [2], ***Ms. Aleksandra Tchoryk*** [1], ***Dr. Andrew Benest*** [1], ***Ms. Pamela Collier*** [1], ***Dr. Philip Clarke*** [1], ***Dr. Yukio Imamura*** [3], ***Dr. Takashi Jin*** [3], ***Prof. Neil Thomas*** [1], ***Prof. Amalia Patane*** [1], ***Dr. Anna Grabowska*** [1]

1. *University of Nottingham,* ***2.*** *University of Nottingham and University of Lincoln,* ***3.*** *RIKEN Quantitative Biology Center Laboratory for Nano-Bio Probes*

Introduction

There is growing awareness of the importance of the tumour microenvironment (TME) in determining how cancer patients respond to therapy. To optimally screen drugs during pre-clinical drug development in animal models, translate them into the clinic, and determine optimal timing of drug combinations, imaging systems that can monitor individual components of the TME and their response to therapy are required. Recently, quantum dots (QDs)[1,2] have emerged as bioimaging probes and near-infrared (NIR) QDs, emitting in the 'biological window' of wavelengths where tissue and water absorbance is minimal and penetration of light into tissue is maximal, offer numerous advantages compared to conventional fluorophores.

Methods

We have developed PbS QDs emitting in the NIR region and have tailored their surface chemistry to allow for bioconjugation of bioorganic molecules. Contemporaneously, targeting peptides, selected from the current literature, have been tested in complex tumour microenvironments such as 3D spheroids of both colorectal cancer cells with fibroblasts, and a co-culture of cancer cells, fibroblasts and endothelial cells.

Results

We have developed protocols for the optimal synthesis of NIR QDs and studied their in vivo biodistribution (Figure 1). We have also identified peptides that specifically target individual cells in the TME (Figure 2 and Figure 3). Our QDs can be targeted to individual elements of the tumour microenvironment after establishing protocols for the bioconjugation of targeting peptides on their surfaces.

Discussion

We have demonstrated the *in vivo* biocompatibility of the PbS NIR QDs for the first time. We have also shown that the peptide-mediated targeting is effective in complex 3D in vitro models in which cancer cells, fibroblasts and endothelial cells are grown in co-culture, and spheroid models which better represent the complexity of patient tumours and xenografts than standard 2D cancer models.

In the end, QDs carrying these targeting peptides have potential *in vivo* to allow real-time monitoring of the presence of cancer and stromal components during xenograft establishment.

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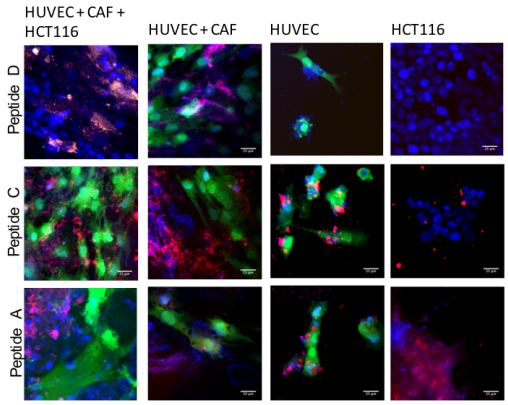


Figure 2 Confocal images of average of Z-projection of peptide screening on a model of endothelial cells (HUVECs) in co-culture with cancer-associated fibroblasts (CAFs) and colorectal cancer cells (HCT116). The HUVECs are eGFP-labelled and shown in green; the peptides are labelled with red-emitting dye (TAMRA); all the cells have been stained with DAPI.

Angiogenesis model confocal images.png

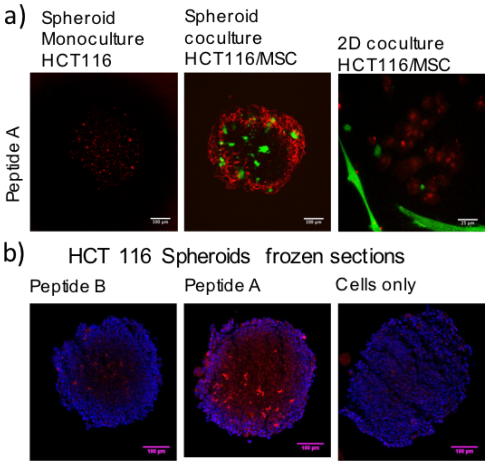


Figure 3 a) Confocal images of maximum Z-projection of peptide screening in 3D cell culture was carried out on a spheroid model of colorectal cancer cells (HCT116, blue), with and without mesenchymal stem cells (MSCs, eGFP-labelled, green). The peptides are labelled with red-emitting dye (TAMRA). **b)** Spheroids' frozen sections and comparison between HCT116 cells (blue stained) only, peptide A and peptide B as negative control for the uptake imaging experiment.

Colorectal cancer model confocal images.png

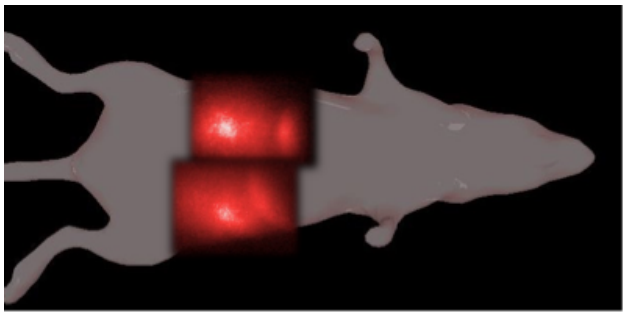


Figure 1 Intensity surface PL images of liver (left) and spleen (right) of a mouse 24 hrs after tail vein injection of QDs.

Mouse pl.png

18 FluoroDeoxyGlucose: Beyond Diagnosis to Use in Novel Therapy

Wednesday, 27th September - 18:15 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 270

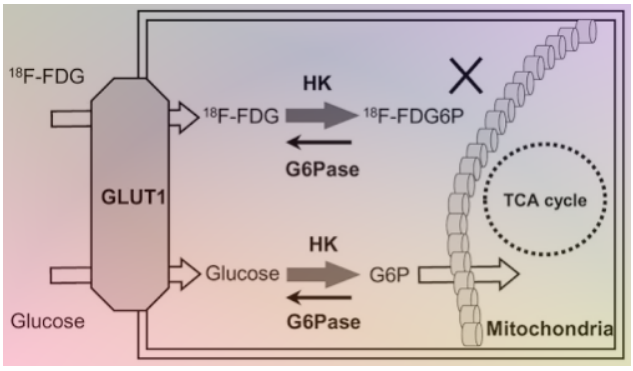
Dr. Indranil Khan [1]

1. Medical College Kolkata

Background: PET CT scan has revolutionized the treatment of several types of cancer especially Lymphoma (Hodgkin's Disease and aggressive Non Hodgkin Lymphoma), Non Small Cell Lung Cancer, Head and Neck Squamous Cell Cancers and various other cancers that express 18-FDG avidity. 18FDG PET CT provides structual and functional imformation and provides more accurate extent of disease spread and localization of malignancy. The high FDG concentration may be used to selectively kill all FDG avid tumor cells.

Methods: 18 Fluorodeoxyglucose is preferentially concentrated in tumor tissue due to high tumor activity (reportedly 200 times more glycolysis than normal tissue cells). Alkylating chemotherapy agents like Cyclophosphamide have high non cell cycle specific tumoricidal property. If we can successfully conjugate an alkylating agent with an 18FDG radio isotope, we can attain high concentrations of the chemotherapy drug within the tumor tissue while normal tissues are selectively spared (less toxicity). Massive preferential killing of tumor cells can be expected in such a situation producing a Complete Response (CR) or a Partial Response (PR) of the tumor tissue be it localized disease or the distant metastasis.

Discussion: The new approach using FDG seems to be promising as it's expected to target both local and metastatic disease. The discussion provided here may be put into preclinical studies for studying the efficacy of this new therapeutic modality before clinical trials may be carried out.



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Targeting Phosphatidylinositol-3-Kinase Signaling with Simultaneous DNA Damage in Cancer Cells by Cholesterol Based Chimeric Nanoparticle.

Wednesday, 27th September - 18:32 - Nanomedicine for cancer diagnosis & therapy - Auditorium - Oral - Abstract ID: 255

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Introduction: Phosphatidylinositol-3-kinase (PI3K) signaling has been a major target as it is found to be mutated and over expressed in many types of cancers. PI3K inhibition alone by small molecules often fails to offer effective therapy because of emergence of drug resistance. Recent studies revealed that inhibiting singling pathways with simultaneous DNA damage is effective to combat cancer. However, targeting PI3K signaling with small molecules in combination with DNA damaging drugs is challenging as it leads to severe side effects due to nonspecific interactions in cancer patients. To overcome this we have synthesized chimeric nanoparticles (CNPs).

Methods: Cholesterol drug conjugates were synthesized and conjugates 3, 4, and 6 (Fig.1) were mixed with phosphatidylcholine and DSPE-PEG and synthesized CNPs by lipid film formation, hydration, and extrusion method. CNPs were characterized for their size, shape, and morphology by dynamic light scattering (DLS), field-emission scanning electron microscopy (FESEM), atomic force microscopy (AFM), and transmission electron microscopy (TEM). Drug loading and release kinetics were assessed by UV- visible spectroscopy. To demonstrate the efficacy of these CNPs in inhibiting tumor cell growth in different cancer cells, different in-vitro cell experiments were performed.

Results and Discussions: CNPs have shown simultaneous loading of PI103, cisplatin and doxorubicin in a controlled ratiometric manner also demonstrated increased release of all three drugs through sustainable manner over 120 h at pH = 5.5 compared to neutral pH. The CNPs showed much improved in vitro cytotoxicity in HeLa, HL60, MCF7, and MDAMB- 231 cancer cells compared to a free drug cocktail at 24 and 48 h also induced apoptosis. Confocal imaging revealed that these CNPs were internalized into cells through endocytosis in a time dependent mode over 6 h in HeLa, MDA-MB-231, and MCF-7 cells. These CNPs exhibited their efficacy by damaging DNA and inhibiting Akt as a downstream modulator of PI3K signaling in HeLa cervical cancer cells. To conclude, these CNPs have the potential to open up new directions in next-generation nanomedicine by targeting multiple oncogenic signaling pathways with simultaneous induction of DNA damage for the augmented therapeutic outcome by reducing off-target toxicity and overcoming drug resistance.

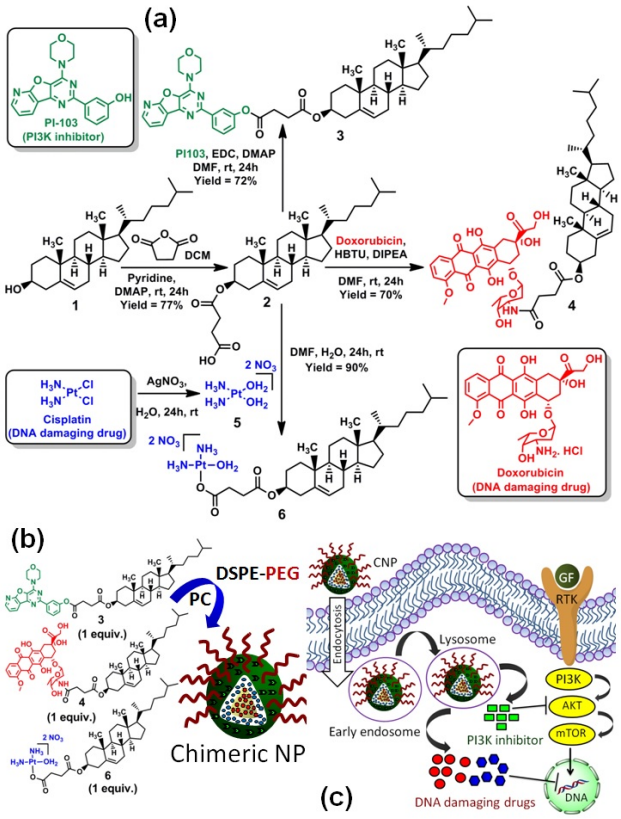
(a)
PI-103
(PI3K inhibitor)
PI103, EDC, DMAP
DMF, rt, 24h
Yield = 72%
3
DCM
Pyridine,
DMAP, rt, 24h
Yield = 77%
1
2
Doxorubicin,
HBTU, DIPEA
DMF, rt, 24h
Yield = 70%
4
DMF, H2O, 24h, rt
Yield = 90%
Cisplatin
(DNA damaging drug)
AgNO3,
H2O, 24h, rt
5
6
Doxorubicin
(DNA damaging drug)
(b)
DSPE-PEG
PC
(1 equiv.)
(1 equiv.)
(1 equiv.)
Chimeric NP
(c)
CNP
GF
RTK
Endocytosis
Lysosome
Early endosome
PI3K
AKT
mTOR
PI3K inhibitor
DNA damaging drugs
DNA

Fig. 1 .png

Authors Index


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Conferences, Events & Workshops