Barcelona
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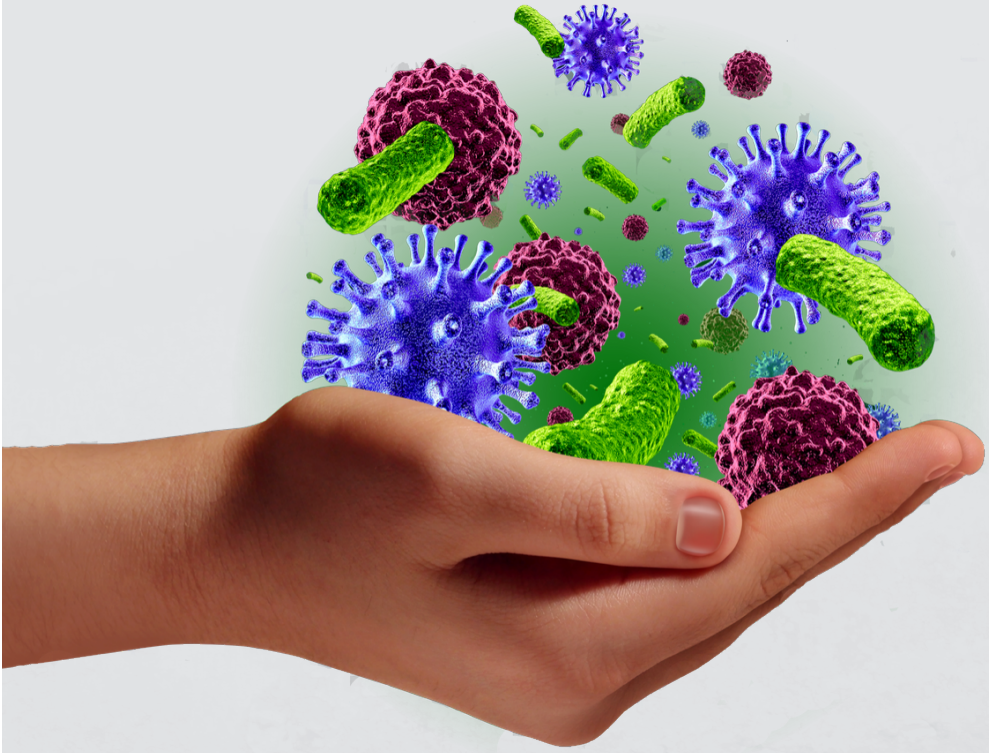

Book of Abstracts

ICONAN 2024

Bridging BASIC BIO-NANO-SCIENCE
and CLINICAL TRANSLATION

Table of Contents

A platform nanotechnology: the creation of a biotech company.

Monday, 15th January - 09:00: Plenary Session 1 (Auditorium) - Oral - Abstract ID: 333

Dr. Francesca Crawford [1]

1. *Somaserve Ltd*

Not all science is amenable to commercialisation. The potential to create a product be it a pharmaceutical, diagnostic, device or anything else is a key determinant in this regard. Drug discovery is a broad church and can range from an individual molecule or class having a specific pharmacological effect to a widely applicable platform technology that can be exploited across multiple indications depending on the chosen payload.

Drug delivery technologies are attractive because they are not a 'single bet' in other words scientific and investment risk are mitigated. Central to building a spin out company is the ability to attract financial support, without money to drive the research and product development little advancement can be made.

PolyNaut, a polymer nanovesicle is a perfect example of an advanced platform technology that has differentiation from other nano delivery systems such as AAVs (adeno associated virus) and lipid NPs. PolyNaut has capacity to encapsulate cargoes ranging from hydrophilic and hydrophobic small molecules, peptides, proteins and antibodies as well as genetic materials. Polynaut also benefits from targeting (phenotypic targeting of selected cell surface receptors), tissue penetration and intracellular shuttling through GOTO technology. Polynaut is capable of crossing the blood brain barrier through transcytosis and with phenotypic targeting delivering cargo intracellularly to selected cells using endocytosis.

Although such wide capability is useful, it can also be a handicap. Building a biotech company based on a drug delivery technology rests firmly on what you select to deliver and thereby the indication. There are fashions and if the area selected does not find favour, the commercialisation will ultimately be slow or not be possible. It is important to showcase sufficient but not excessive capability as all goals need to be perceived and actually achievable both with available financial resource and the team.

ViaNautis Bio was founded in 2018 to exploit polyNaut technology. There have been 2 seed rounds of funding followed by a successful Series A in 2023, raising a total of $28.5m. The business plan has changed over the five years from a service model to a genetic product therapeutic pipeline development company- the art of effecting that change has also been important in growing a successful biotech company. This will be explored further in the plenary session.

Clinical Translation of Graphene & Lessons Learnt

Monday, 15th January - 09:40: Plenary Session 1 (Auditorium) - Oral - Abstract ID: 335

Prof. Kostas Kostarelos [1]

1. *The University of Manchester, Nanomedicine lab, Faculty of Biology, Medicine & Health, Manchester, United Kingdom.*

The use of nanomaterials in medicine has been growing at an unprecedented rate for a variety of therapeutic, diagnostic or combinatory applications. The clinical translation of advanced materials that are being discovered or synthetically engineered is considered by many the critical factor to determine the ´success´ or ´failure´ in nanomedicine. In this talk, our decade-long experience in the clinical translation of graphene two-dimensional nanosheets will be discussed. Recent progress in the development of clinically used graphene oxide will be described, with emphasis on two very different first-in-human clinical investigation studies undertaken recently using graphene-based technologies. Emphasis will be placed on common lessons learnt and whether graphene can serve as a case study on the early-stage clinical translation of advanced nanomaterials.

Multimodal Polypeptide-based Therapeutics bypassing Challenging Biological Barriers

Monday, 15th January - 10:45: Plenary Session 2 (Auditorium) - Oral - Abstract ID: 342

Prof. Maria Vicent [1]

1. *Polymer Therapeutics Lab. Prince Felipe Research Center (CIPF)*

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

In our group, we have overcome the main classical limitations for the synthesis of defined polypeptides using precise controlled reactions followed by an adequate characterization yielding to well-defined polypeptidic architectures by NCA polymerization techniques[2]. In addition, post-polymerization techniques allow us the introduction of a variety of functionalities yielding a set of orthogonal reactive attachment sides[1]. Using these techniques and following a bottom-up strategy we have been able to obtain polypeptides with different architecture (including diblock-co-polymers or star-shaped with the capacity to self-assemble yielding supramolecular nanostructures) with interesting properties regarding tissue[3] , subcellular compartment tropism[4] or even capability of brain delivery[5]. This strategy together with an adequate polymer-drug linker design[3-6] enabled in vitro and in vivo evaluation, revealing a lack of toxicity, an enhanced in vitro cell internalization rate and significantly greater half-life in vivo together with a significant lymph node,[3] mitochondria[4] or brain accumulation[5]. These results allow us to envisage these systems as promising nanocarriers for therapeutic or diagnostic applications.

Acknowledgments:

ERC-CoG-2014-648831 “MyNano”, ERC-PoC- Polymmune, ERC-PoC-PolyBraint, Biomolmacs H2020-MSCA-ITN-2019-850418, PID2019-108806RB-I00, Pol@Mets (MFA/2022/065), Foundation Health La Caixa-HR19/52160021-NanoPanther, HR22-00602-NanoGBA. Part of the equipment employed by GVA and co-financed with FEDER funds.

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Protocells and Therapeutics: Harnessing Biomimicry with Self-Assembled Polymersomes and Bioconjugates

Monday, 15th January - 11:25: Plenary Session 2 (Auditorium) - Oral - Abstract ID: 336

Prof. Sebastien Lecommandoux [1]

1. *University of Bordeaux*

Our scientific approach is based on biomimicry, as we engineer synthetic mimics of natural macromolecules (such as proteins or glycoproteins), and explore their controlled and tunable self-assembly to form self-assembled structures similar to those found in nature (such as virus or cell membranes). In this context, we develop polymer-based self-assembled nanoparticles, mostly polymeric vesicles, also named polymersomes, with high loading content of active pharmaceutical ingredients (e.g., anticancer drugs, peptides, proteins) and targeting ability. Our expertise includes the synthesis of precise, biocompatible polymers such as polypeptides (by chemical synthesis or recombinant DNA technology), polysaccharides, and polypeptide-polysaccharide conjugates.

We present here an overview of the self-assembly of amphiphilic block copolymers developed in our laboratory, focusing polymersomes, and their contribution in nanomedicine. We pay particular attention to block copolymer vesicles based on polysaccharides, polypeptides and proteins and report here an overview on the design of Elastin Like Polypeptides (ELPs) based conjugates and their applications in nanomedicine, biomaterials and artificial cells. We pay special attention to their modification with saccharides [1], polysaccharides [2] and lipids [3], aiming at mimicking both the structure and functionality of glycoproteins and lipoproteins. We developed synthetic strategies for the design of glycosylated polypeptides and polysaccharide-polypeptide biohybrids with controlled placement of sugar functionality. The ability of these systems for different biomedical applications, from drug-delivery to inhibitor, will be presented [4]. In addition, the design of a new class of lipoproteins based on ELPs with unique thermo-responsive character will be proposed. These biosynthetic lipoproteins can self-assemble into lipopolymersomes, with tunable membrane permeability, opening avenues in drug delivery and artificial cell design [3]. Finally, our most recent advances in the design of complex, compartmentalized and functional artificial cells will be presented. Such a system is a first step towards the challenge of structural cell mimicry and functionality, and could act in the future as an autonomous artificial cell capable of detecting and healing in situ any biological deregulation [5-7].

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Innovative drug-loaded and hybrid-coated nanoparticles for multiple myeloma treatment

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 297

Mr. Nicolò Maria Percivalle [1], ***Dr. Marco Carofiglio*** [2], ***Ms. Marzia Conte*** [1], ***Prof. Valentina Cauda*** [1]

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Introduction

Despite the progresses made in recent years, the development of effective tools against severe pathologies such as cancers is proving to be extremely challenging, since the physico-chemical characteristics of the therapeutic agents and their behavior in physiological conditions and biological environment have to be considered, as well as their potential drawbacks. Multiple myeloma, in particular, is a tumor for which relapses and disease progressions are common among affected patients. In this work, an innovative nanoconstruct to treat such disease is proposed, exploiting drug-loaded, iron-doped zinc oxide nanocrystals coated by a biomimetic shell composed of a lipid bilayer made from extracellular vesicles combined with synthetic lipids.

Methods

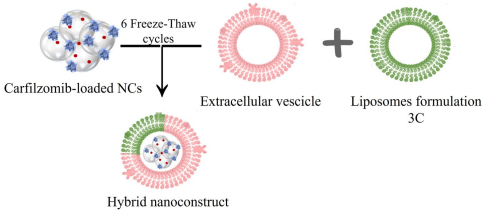
Iron-doped zinc oxide nanocrystals (ZnO-Fe NCs) were firstly synthesized and functionalized, before being coated with a mix of synthetic lipids and extracellular vesicles derived from healthy B-lymphocytes. The obtained nanoconstruct was then characterized and administered to two different multiple myeloma cell lines to assess its cytotoxicity compared to the administration of naked nanocrystals. Preliminary *in vitro* tests were also performed to determine the efficacy of the drug-loading process on the NCs surface.

Results

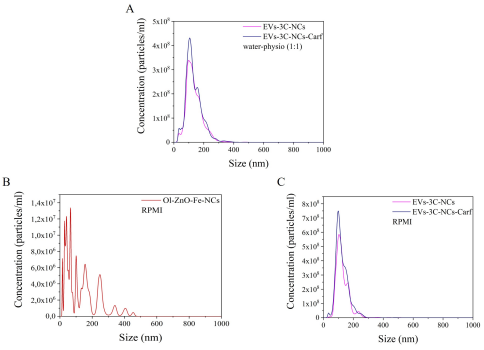
NTA measurements demonstrate that the nanoconstruct is highly stable in both water-physiological solution (1:1) and in complete cell culture medium, in which instead naked nanocrystals tend to aggregate. Once administered to the two different multiple myeloma cell lines (AMO-1 and KMS28), the coated NCs proved to be extremely safe in terms of cytotoxicity up to the maximum concentration tested (40 μg/ml), while naked NCs resulted cytotoxic already at low concentrations. Furthermore, preliminary tests concerning the drug loading process confirm the therapeutic efficacy of the nano-vehiculation of the drug.

Discussion

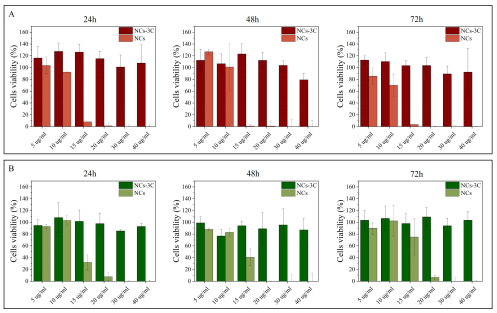
The promising obtained results make this innovative nanoconstruct an exceptionally interesting starting point for the treatment of multiple myeloma, since its therapeutic capabilities can be greatly enhanced by tailored adjustments, such as the functionalization of the lipidic shell for targeting purposes and the combination of the administration of the nanoconstruct with the application of external stimuli (i.e. ultrasounds) for synergistic treatments, while the intrinsic properties of the NCs allow to exploit the nanoconstruct as a contrast agent for diagnostic purposes.



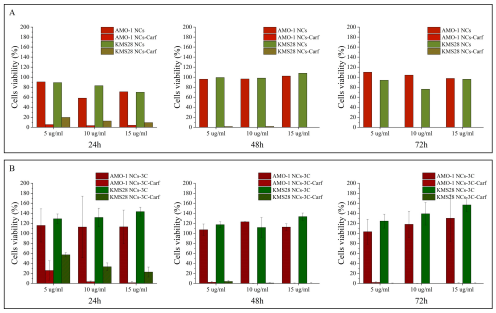
Schematic rapresentation of the nanoconstruct development.jpg



Nta measurements.jpg



Cytotoxicity of coated and naked ncs.jpg



Cytotoxicity of coated and naked ncs with and without drug.jpg

Chitosan-stabilized metallic nanoparticles as nanosystems to support proton radiotherapy of cancer - in vitro studies

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 295

Dr. Bartosz Klębowski [1], ***Dr. Joanna Depciuch-Czarny*** [1], ***Prof. Magdalena Parlińska-Wojtan*** [1]

1. *Institute of Nuclear Physics Polish Academy of Sciences*

The demand for new ways of treating cancer is extremely important due to the annual increase in the incidence of this disease. Traditional methods such as surgery often turn out to be unhelpful, e.g. when the tumor is located in a hard-to-reach place. Non-surgical methods also fail due to systemic toxicity (chemotherapy) or causing skin burns and secondary cancers (photon radiotherapy). An innovative approach, intensively researched in recent years, is the application of precise proton radiotherapy (PT) additionally supported with metallic nanoparticles (NPs).

Our research attempted to enhance the effect of irradiating cancer cells with a proton beam using the nanoradiosensititzers. These radiosensitizers - in our case, metallic NPs based on palladium (Pd NPs) and gold (Au NPs) - increase the effectiveness of PT by generating reactivity of oxygen species (ROS) in irradiated cells. Moreover, the NPs were introduced into the cells at such a concentration that they did not cause a significant decrease in cell survival, but only generated a toxic effect after irradiation.

For this purpose, ultrasmall Pd NPs and Au NPs stabilized with chitosan were synthesized. For comparison, NPs stabilized with classic (non-sugar-based) reducing agents, were also obtained. *In vitro* studies were performed on two glioma cell lines: U118 and LN229. Biological assays, such as MTS test, clonogenic assay and ROS measurements were used to evaluate the cytotoxicity of NPs and effectiveness of NPs-assisted PT. The holotomographic microscopy technique was used to determine the dynamics of NPs penetration into cells.

The results showed that NPs have a promising radiosensitizing potential, which was especially visible in the case of Pd NPs. Importantly, it has been shown that these NPs in low concentrations do not cause themselves cytotoxic effects on cells. Interestingly, chitosan-modified NPs turned out to be more effective than those synthesized using classic reducing agents. This is due to the significant biocompatibility of chitosan, which masks the toxicity of NPs, enabling their more effective cellular uptake. This was confirmed by holotomographic microscopy, which showed faster penetration of chitosan-modified NPs into the glioma cells.

This research was funded by Polish National Science Centre, grant number UMO-2020/37/N/ST5/02414.

Bacteriophage Encapsulation for Treating Infections in Cystic Fibrosis Patients

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 87

Dr. Lorena García Hevia [1], ***Mr. Alexis Dorta*** [1]

1. *IDIVAL - University of Cantabria*

Cystic fibrosis (CF) is a genetic disorder characterized by chronic lung infections, often involving antibiotic-resistant bacteria, which present significant challenges in clinical management. Bacteriophage therapy, an emerging field, offers a promising alternative to traditional antibiotics. Bacteriophages are naturally occurring viruses with the unique ability to infect and replicate within bacteria, making them ideal candidates for combating bacterial infections while minimizing the risk of harming human cells.

Nevertheless, challenges remain in optimizing the dosing and pharmacological properties of bacteriophages for therapeutic use. These challenges can potentially be addressed through the encapsulation of phages using nanotechnology. By producing nanometer-sized particles capable of encapsulating and protecting the phages, providing stability to prevent degradation, facilitating targeted delivery, and ensuring consistent and controlled release, we aim to enhance their efficacy and maximize their potential as a therapeutic intervention.

Here, we show some preliminary results of this novel an interesting project.

Engineering Dynamic Lipid Vesicles with Programmable Lifetime

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 237

Mr. Akhil Venugopal [1]***, Prof. lorena Ruiz*** [2]***, Dr. Mohit Kumar*** [3]***, Prof. Giuseppe Battaglia*** [4]

1. *1Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain). 2 Biomedicine Doctorate Programme, University of Barcelona, Spain,* ***2.*** *1Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain).,* ***3.*** *1Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain).3Department of Organic Chemistry, University of Barcelona, Spain,* ***4.*** *1Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona .4Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.*

Metabolism plays a crucial role in living systems by consuming chemical energy to form highly dynamic self-assembled structures through synthesis and breakdown of molecules [1]. These highly dynamic structures formed under out-of-equilibrium (OOE) conditions define the hallmark features of life such as adaptivity and spatiotemporal control [2]. Here, we present the formation of dynamic self-assembled lipid vesicles under OOE conditions resulting in the formation of transient lipid vesicles i.e., vesicles with a programmable lifetime. To demonstrate the temporal self-assembly of lipid vesicles under OOE conditions we have utilized the dynamic imine bond formation and subsequent lipase-catalysed ester hydrolysis reaction between a hydrophilic phospholipid head group mimic and a hydrophobic amino ester lipid tail mimic. The imine formation reaction between the amino-ester fuel and the aldehyde head led to the formation of an amphiphilic lipid structure which eventually self-assembled into vesicles whereas the ester hydrolysis by the lipase enzyme resulted in the disassembly of vesicles. Detailed analysis through various spectroscopic and microscopic studies confirmed the continuous formation and degradation of lipid vesicles over time as long as the amino-ester fuel lasts. Moreover, by varying the concentration of the lipase enzyme we could attain a temporal control over the lifetime of lipid vesicles. We also demonstrated that these dynamic vesicles can be kept in sustained non-equilibrium steady states (NESS) by a constant supply of fuel. Finally, we have shown that these lipid vesicles under non-equilibrium conditions can be used for drug release by encapsulating nile red as a model hydrophobic drug, where the release of encapsulated drug can be controlled by the kinetics of the enzymatic degradation reaction. We aim to utilize these lipid vesicles as an adaptive interface for nanomedicine in targeted drug delivery.

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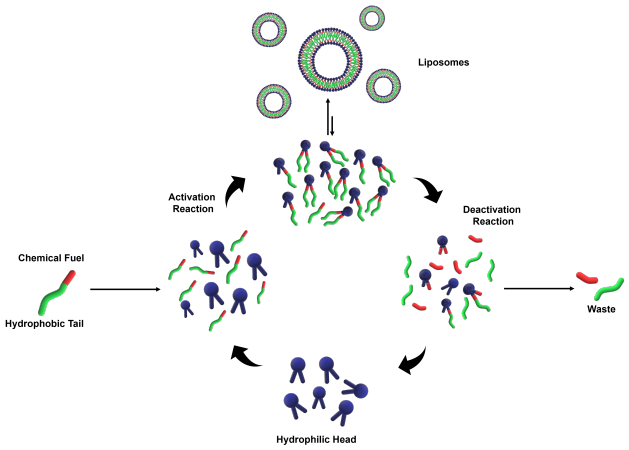

Schematic showing the out of equilibrium self assembly of lipid vesicles.png

On the surface charge of PEG-PLA Polymersomes and micelles: Implications for Biomedical Applications

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 270

Mr. Peter Pfeifer [1], ***Mr. Marco Basile*** [2], ***Dr. Subhadip Ghosh*** [2], ***Mr. Jose Muñoz-López*** [2], ***Prof. Giuseppe Battaglia*** [1], ***Dr. Yangwei Deng*** [3], ***Dr. Denis Checchin*** [3], ***Dr. Aleksandar Ilchev*** [3], ***Dr. Virginia M Gouveia*** [3]

1. *Institute for Bioengineering of Catalonia,* ***2.*** *Institute for Bioengineering of Catalonia (IBEC),* ***3.*** *Vianautis Bio Ltd. Cambridge UK*

Polymeric particles are effective drug carriers in nanomedicine and targeted-delivery. They offer numerous benefits to delivery systems, including controlled release, prolonged circulation time, selective cell recognition, enhanced transcytosis and reduced immunogenicity. Therefore, it's important to choose the right polymers when constructing nanometric carriers, such as micelles or polymersomes. One of the most popular polymer combinations used for making amphiphilic copolymers is poly(ethylene glycol)-poly(lactic acid) (PEG-PLA). This copolymer is well established with both blocks approved in several nations as safe-to-use excipients. The whole copolymer is approved as a micellar drug delivery system for the treatment of several cancers and commercialised as Genexol-PM. However, despite the extensive research on PEG-PLA structures, a crucial detail needs to be addressed in the scientific community: the surface characteristics of these particles. According to ζ-potential studies, depending on the synthetic route and purification method, the self-assembled nanoscopic units possess a significant negative surface charge or develop a strong negative charge within a few days. The presence of a charged surface in biological systems can lead to numerous disadvantageous effects, including non-specific binding, adhesion and undesired immune responses, jeopardising the use of this copolymer in demanding applications such as targeted-drug-delivery or precision medicine.

Our studies suggest that negative surface charge of PEG-PLA vesicles can be attributed to carboxylic-groups derived from both the presence of free PLA-blocks resulting from synthesis and the hydrolysis of the ester-bond between the PEG and PLA units, leading to the shedding of the PEG brush. Therefore, we summarise our efforts to understand this phenomenon and propose solutions to address this issue.

To understand the consequences of the negative surface charge, we conducted a series of protein corona experiments to evaluate unwanted adhesion under serological conditions. We monitored the evolution of the accumulation of negative charge through ζ-potential measurements to gain insight into the kinetics of ester-bond hydrolysis in the self-assembled system. Finally, we revisit possible synthetic routes and purification methods to avoid generating free-standing PLA chains during fabrication. We also share our results in attempting to achieve a reinforced attachment of the two polymeric blocks, preventing ester-bond hydrolysis from a molecular design perspective.

Innovative strategy of highly permeable and highly deliverable nanomedicine based on gold nanoparticles

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 290

Ms. Miao Feng [1]

1. *Center for Hybrid Nanostructures (CHyN), University of Hamburg*

The therapeutic and diagnostic effects of nanomedicines are mainly depending on their delivery efficiency to targeted sites[1]. In the case of nanoparticles (NPs)-based drugs, they usually involve a particulate delivery vehicle and a pharmaceutically active compound, which is to be delivered to and released at the target site[2]. Small NPs may penetrate into deeper location, but they are usually difficult to retain in tissues or tumors. In contrast, big NPs with large sizes (~100 nm) have poor penetrating properties, but are easily trapped around tumor blood vessels and had long-term blood circulation[3]. Thus, designing a hybrid NPs would benefit the penetration effect of nanomedicines, upon reaching the tissue, the NPs will degrade and release smaller NPs into the targeted area. Herein, we demonstrate this concept by a simple protocol of polymer-mediated gold nanocluster (Au NCs) self-assembly into AuNC@PLAG of ca. 100 nm diameter. UV-vis and time-resolved fluorescence measurements have provided insight into the quantum yield enhancement of AuNC@PLAG, up to 4.2 times that of free AuNCs. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to measure cellular endocytosis and exocytosis of Au concentration. The in vitro studies in Hela cells with the high exposure concentration (25 μg/mL, Au ion) has indicated strongly enhanced endocytosis of AuNC@PLAG comparing to free Au NCs, which has increased by 700 %. Furthermore, with the intervention of proliferation blocker, the results of 120 hours of cellular exocytosis showed that the efficiency of degradable AuNC@PLAG was significantly higher than that of colloid gold nanoparticles.

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Nano-Ghost: an innovative drug delivery platform for healing cardiac tissue.

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 319

Ms. Anastasia Brandis [1], ***Dr. Marcelle Machluf*** [1]

1. *Technion*

Introduction:

Cardiac injuries, like myocardial infarction (MI), drive the secondary pathology with advanced heart failure. In this process, inflammation is a primary component of accelerated cardiovascular disease and subsequent fatal events. It is essential for the patient to target the area at risk and stop inflammation in time not allowing it to cause significant damage to the heart, prevent ischemic reperfusion injury, and stop remodeling. Among all regenerative therapies for treating post-MI tissue mesenchymal stem cell (MSC) therapy is the most prospective one. Preclinical animal studies of MSC have shown their ability to target the injured host tissue, suppress inflammation, and reduce infarct size while enhancing cardiac repair.

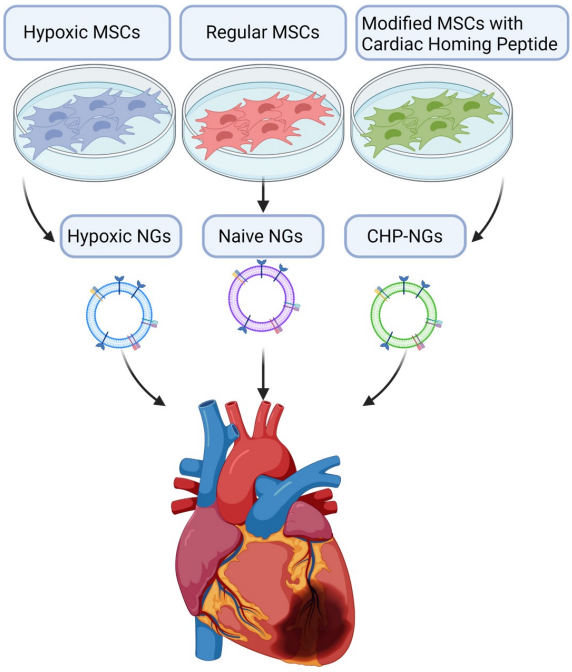
Methods and results:

Our laboratory has developed unique membrane-based nanovesicles produced from the plasma membranes of MSCs, termed Nano-Ghosts (NGs). During their production, these nanoparticles preserve the original features of MCS which help them to integrate into the site of inflammation. Therefore, in order to enhance the infarct-targeting properties of NGs, we decided to modify MSCs prior to their production. In the first approach, we transfected MSCs with a plasmid encoding a cardiac homing peptide (CHP) known for its affinity for left ventricular cardiomyocytes, which is most often the area of infarction. In the next approach, we cultured MSCs under hypoxic conditions (2% O_2) stimulating the expression of migration receptors on their surface such as CXCR4, c-Met, and CD105.

Subsequently, Nano-Ghosts (NGs) were prepared utilizing either modified (MSC-CHP) or conditioned (hypoxic MSC) cells. We confirmed the presence of targeted moiety or migration receptors on the surface of NGs and studied their ability to target cardiac cells. Comparative analysis revealed that both CHP-NGs and Hypoxic NGs exhibited heightened selectivity towards H9C2 rat cardiomyocytes and human iPSC-derived cardiomyocytes in contrast to unmodified NGs.

Conclusion:

NGs were effectively engineered through pre-modification of their source cells before production. Modified NGs demonstrated higher selectivity and targeting potential compared to naïve NGs.



Research concept.jpg

A CXCR4-targeted nanoconjugate for precise drug delivery: Proof of Concept in an Acute Myeloid Leukemia Mouse Model

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 31

Ms. Annabel Garcia León [1], ***Dr. Julián I. Mendoza*** [2], ***Ms. Ariana Rueda Matas*** [1], ***Mr. Luis Carlos Navas*** [2], ***Ms. Vanessa Huaca*** [1], ***Dr. Ugutz Unzueta*** [2], ***Dr. Jorge Sierra*** [3], ***Dr. Josep F. Nomdedéu*** [3], ***Dr. Esther Vazquez*** [4], ***Dr. Antonio Villaverde*** [4], ***Dr. Ramon Mangues*** [1], ***Dr. Isolda Casanova*** [1]

1. *Biomedical Research Institute Sant Pau (IIB Sant Pau); Josep Carreras Leukaemia Research Institute; CIBER de Bioingeniería, Biomateriales i Nanomedicina (CIBER BBN),* ***2.*** *Biomedical Research Institute Sant Pau (IIB Sant Pau); CIBER de Bioingeniería, Biomateriales i Nanomedicina (CIBER BBN),* ***3.*** *Josep Carreras Leukaemia Research Institute; Departament de Hematologia del Hospital de la Santa Creu i Sant Pau,* ***4.*** *CIBER de Bioingeniería, Biomateriales i Nanomedicina (CIBER BBN); Departament de Genètica i Microbiologia de la Universitat de Barcelona; Institut de Biotecnologia i de Biomedicina de la Universitat de Barcelona*

Introduction:
Nanomedicine holds potential in cancer therapy by enhancing drug properties and enabling precise delivery through protein-based nanocarriers. Our team has developed a humanized nanoconjugate for targeting neoplasms characterized by an overexpression of the chemokine receptor CXCR4, which is crucial in cancer cell dissemination and drug resistance development. In this study, we assessed the antineoplastic effect of a CXCR4-targeted nanoconjugate obtained by site-directed drug conjugation, T22-HSNBT-H6-MC-VC-PAB-MMAE (T22-MMAE), both *in vitro* with CXCR4+ Acute Myeloid Leukemia cell lines and *in vivo* using a disseminated AML mouse model.

Methods:
Nanoconjugate production - Protein nanostructures were designed using *in silico* methods, synthesized in *E. coli*, and subsequently purified using IMAC affinity chromatography. T22-MMAE nanoconjugate was generated by covalent binding of maleimidocaproyl functionalized MMAE molecules to a thiol group exposed by an engineered cysteine residue within the humanized T22-HSNBT-H6 nanocarrier. ***In vitro and in vivo assays*** – CXCR4 expression in AML cell lines and BM primary samples were measured by immunohistochemistry and flow cytometry. T22-MMAE cytotoxicity was evaluated using XTT cell proliferation assay kit in AML cell lines and CellTiter-Glo™
Luminescent Cell Viability Assay in bone marrow (BM) samples. A disseminated AML animal model generated by iv injection of bioluminescent THP1 cells was utilized to assess the anticancer effect of the nanoconjugate in NSG mice.

Results:
T22-MMAE showed a potent cytotoxic effect in $CXCR4^+$ AML cell lines, showing a dose-response relationship. Additionally, the nanoconjugate demonstrated a potent antitumor activity in AML patient samples with high CXCR4 expression, without effect in BM samples with low CXCR4 expression. Finally, this potent therapeutic effect was observed in an AML mouse model, where it blocked AML dissemination and led to an increased mouse survival time, without systemic toxicity.

Discussion:
The overexpression of CXCR4 on cancer cells promotes disease progression through pro-survival pathways and chemoresistance. Overexpression of CXCR4, in around 50% of AML patients, correlates with reduced survival, increased chemoresistance, and higher relapse rate. Thus, targeting CXCR4 could be a promising approach for treating refractory or relapsed AML cases that are resistant to chemotherapy. Our results highlight the potential

of T22-MMAE in the treatment of poor prognostic $CXCR4^{+}$ AML.

Evaluating the formation of Protein corona on nanoparticles with different surface chemistry

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 90

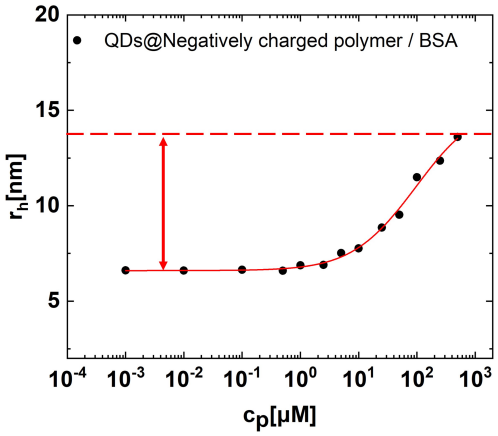
Ms. Chenxi Yang [1], ***Dr. Florian Schulz*** [2], ***Prof. Wolfgang Parak*** [1]

1. *Center for Hybrid Nanostructures (CHyN), University of Hamburg,* ***2.*** *University of Hamburg*

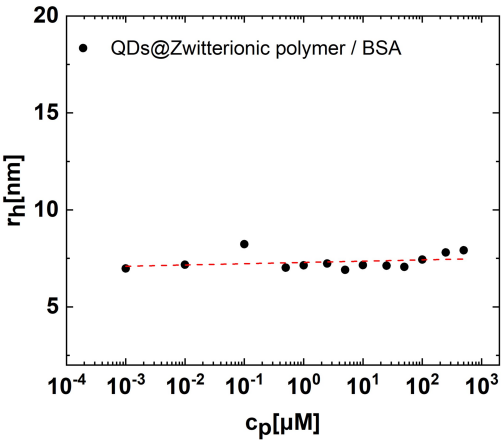
The in vitro research of nanoparticles mainly focuses on functional design, such as improving the efficiency and stability of targeted delivery, but often ignores the interaction of nanoparticles with biomolecules such as proteins after entering the physiological environment. When nanoparticles are exposed to biological fluids, they will soon become coated with proteins and other biomolecules, a phenomenon known as the 'protein corona.' These adsorbed proteins will further replace the nanoparticles' engineered surfaces and alter their biological interactions. Subsequently, the protein corona will have an impact on particle delivery, degradation, and biological applications. The necessity of a comprehensive study of the interaction between different nanoparticles and proteins is highly pronounced in order to more fully assess the merits and demerits of nanoparticles in biological applications and improve the functionalization of nanoparticles.

In this study, we considered the effects of both the properties of the proteins themselves and the chemical modifications on the surface of the nanoparticles on the formation of the protein corona. We designed and synthesized a series of polymers, coating them around the surface of CdSe-CdS quantum dots. Additionally, we selected model proteins with different properties, such as isoelectric point. Through different combinations of nanoparticles and proteins, we studied the patterns of protein corona formation.

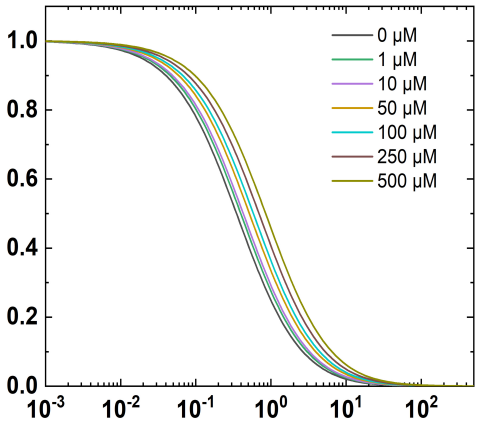
Due to the subtle changes in particle size during the protein corona formation process, some common detection methods, such as dynamic light scattering (DLS) and transmission electron microscopy (TEM), are not sensitive enough to capture these changes. Therefore, we used fluorescence correlation spectroscopy (FCS) to study the adsorption of different types of proteins onto autofluorescent CdSe/CdS quantum dots (QDs) based nanoparticles, which are coated by various surface layers exhibiting different chemical functional groups, thereby endowing the NPs with different physicochemical properties. By using FCS, the change in hydrodynamic radius with protein concentration can be recorded and analyzed, providing us with a better understanding of the interaction between nanoparticles and proteins. This study will provide insights into the structure and functionality of the protein corona that can be leveraged for the development of nanomaterials with controlled biological interactions.



Qds negatively charged polymer.jpg



Qds zwitterionic polymer.jpg



Normalized fluorescence intensity autocorrelation curves.jpg

Combinatorial screening of biscarbamate ionizable lipids identifies a low reactogenicity lipid for lipid nanoparticle mRNA delivery

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 127

Ms. Heleen Lauwers [1]

1. *Ghent University*

Introduction

Messenger RNA (mRNA) holds promise as a therapeutic modality for various diseases. A critical aspect of mRNA drugs is delivering intact mRNA into the cytoplasm of cells. In its native, unformulated form, mRNA cannot achieve this goal. However, encapsulating mRNA into lipid nanoparticles (LNPs) can overcome this issue. LNPs are internalized by target cells and routed into endosomal vesicles, where they must destabilize the endosomal phospholipid membrane to release the mRNA into the cell cytoplasm. These processes are significantly influenced by the ionizable lipid present in the LNP formulation. Here, we report on the synthesis and evaluation of a library of ionizable lipids which contain a biscarbamate motif.

Methods

The ionizable bicarbamate lipids (IBLs) were synthesized using a simple, column purification-limited and scalable strategy based on three customizable building blocks: a dialkyl chain, a homobifunctional activated carbonate ester linker and an ionizable head group (Figure 1A). LNPs were formulated by mixing an aqueous solution of RNA with an ethanolic solution containing an IBL, phospholipid, polyethylene glycol lipid and cholesterol. IBL mRNA LNPs were screened for transfecting enhanced Green Fluorescent Protein (eGFP)-encoding mRNA *in vitro*. The biodistribution of mRNA translation, upon intramuscular administration, was tested *in vivo* in mice using reporter protein and transgenic mouse systems. Innate immune activation by IBL LNP was investigated by testing reactogenicity, systemic cytokine levels, immune cell infiltration and activation at the injected muscle, draining lymph node and spleen.

Results and discussion

Two generations of IBLs were successfully synthesized and demonstrated to be suitable for formulating mRNA into stable LNPs. The evaluation of their mRNA transfection capacity *in vitro*, using eGFP as a reporter protein, identified S-Ac7-DOG as a lead IBL (Figure 1B, 2). Upon intramuscular vaccination, S-Ac7-DOG LNPs induced robust local transfection of mRNA, encoding Firefly luciferase (Figure 3). S-Ac7-DOG LNP, encapsulating mRNA encoding Cre-recombinase, exhibited a distinct biodistribution of mRNA at the cellular level in Ai14 transgenic mice when compared to the benchmark MC3 LNP. This biodistribution involved higher transfection of immune cells in draining lymph nodes and spleen, while demonstrating reduced reactogenicity and diminished innate immune activation (Figure 4).

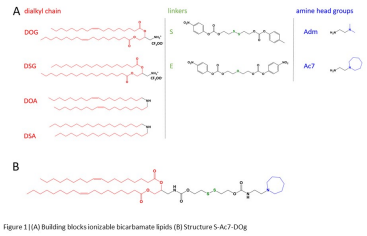


Figure 1 | (A) Building blocks ionizable bicarbamate lipids (B) Structure S-Ac7-DOg

Figure 1.jpg

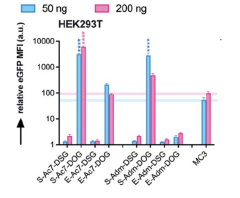


Figure 2 | Flow cytometry analysis of *in vitro* eGFP transfection in HEK293T cells by IBL LNP containing eGFP-encoding mRNA. Cells were treated for 24h with a 50 and 200 ng, respectively, mRNA dose (n=3; mean ± SD). For guidance transfection efficiencies of MC3 LNP are marked by a line. Statistical analysis was performed by one-way ANOVA. Only IBL LNP outperforming MC3 LNP were annotated. ****:p<0,0001)

Figure 2.jpg

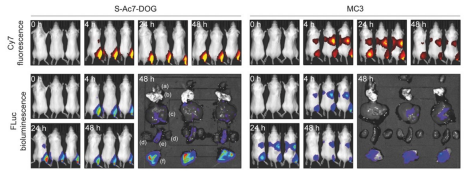


Figure 3| *In vivo* mRNA transfection analysis on tissue level. C57BL/6 mice were injected with Cy7-PE labeled LNP at a 5 µg mRNA dose (mRNA encoding for luciferase) in the quadricep of the lower right leg. IVIS images of Cy7 fluorescence and Fluc bioluminescence. Organ annotation: (a) heart (b) lungs (c) liver (d) kidneys (e) spleen (f) muscle.

Figure 3.jpg

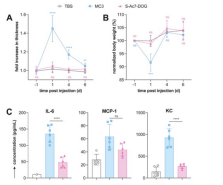


Figure 4| Reactogenicity in response to intramuscular LNP injection. WT C57BL/6 mice were injected with LNPs at a 5 µg mRNA (mRNA encoding for E7 mRNA) dose in the quadricep of the lower right leg. Effect of LNP injection on (A) injection site swelling and (B) body weight (n= 6, mean ± SD) (C) Cytokine levels in blood measured 6h post injection by multiplex analysis (n=6, mean ± SD). Statistical analysis by one-way ANOVA with Tukey's multiple comparison test. In panels A and B the color of the annotation corresponds to the color code of the LNP and compares LNP to TBS (n.s, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001.)

Figure 4.jpg

The development of multifunctional lipid nanocomposites in reprogramming M2 macrophage polarization and inducing apoptosis for an enhancement in anticancer effects

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 135

Dr. Kamonlatth Rodponthukwaji [1], Dr. Ladawan Khowawisetsut [2], Dr. Nathachit Limjunyawong [1], Dr. Sith Sathornsumetee [3], Dr. Chatchawan Srisawat [4], Dr. Primana Punnakitikashem [4]

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Cancer has been one of the deadliest diseases, causing a high rate of mortality worldwide. Despite several traditional therapies, unsatisfactory outcomes remain a significant challenge. In the tumor microenvironment (TME), prominent protumor macrophages (M2) create an immunosuppressive TME. Reversing M2 into tumoricidal macrophages or M1 can thus be a promising strategy to inhibit tumor proliferation. Resiquimod (R848), a toll-like receptor 7/8 agonist, has reportedly shown potential for repolarizing M2 into M1 macrophages. Additionally, programmed cell death, in terms of cancer cell apoptosis induced by polyinosinic–polycytidylic acid (PIC), can also play a vital role in anticancer activity. Therefore, a therapeutic system with the capability to reprogram M2 macrophages, accompanied by the induction of apoptosis in cancer cells, could provide an effective cancer treatment.

Herein, we have developed a therapeutic platform with multifunctionality. A lipid-based delivery system containing R848 and PIC (LNP-R848-PIC) was successfully constructed. All fabricated nanoparticles were characterized using a zetasizer, UV-Vis spectrophotometer, transmission electron microscopy (TEM), and gel retardation assay. The increased hydrodynamic size and reduced charge of LNP-R848-PIC compared to unloaded LNP confirmed the successful loading of therapeutic agents. The gel retardation assay also indicated the optimal PIC:LNP loading ratio. Subsequently, the role of individual therapeutic agents, PIC and R848, in biological effects was investigated. The presence of PIC enabled cancer cell death through the apoptosis pathway, as confirmed by the increase in caspase 3/7 activity compared to the groups without PIC incorporation. M1 polarization was strongly induced by the presence of R848, as evidenced by the expression of CD80 (M1 surface marker) and the suppression of CD206 (M2 surface marker) from flow cytometry analysis. The upregulation of TNF and IL6 genes and the release of proinflammatory cytokines (TNF-α, and IL-6) was assessed. Co-culturing M2 and MDA-MB-231 in a transwell system (Figure 1A) was performed to confirm the multifunctionality of LNP-R848-PIC. The mortality of cancer cells was significantly enhanced by combining the effects of proinflammatory cytokines secreted from activated M2 and the induction of apoptosis with the treatment of LNP-R848-PIC (Figure 1B). Accordingly, the fabricated platform with multifunctionality may serve as a potent delivery system for cancer treatment.

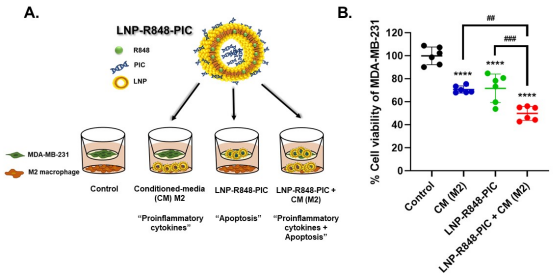

Figure 1 a. schematic of co-culturing of m2 macrophage and mda-mb-231 in transwell system. b. cell viability of mda-mb-231 after treated with lnp-r848-pic..jpg

Antibody functionalized nanogels with high loading of anesthetic nanocrystals for the treatment of osteoarthritis

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 149

Ms. Cristina Remirez de Ganuza [1], ***Ms. Marta Sainz*** [2], ***Dr. Manuel Arruebo*** [1], ***Dr. Gracia Mendoza*** [3]

1. *Affiliation 1. Department of Chemical Engineering. Aragon Institute of Nanoscience and Materials (INMA), CSIC-University of Zaragoza, Campus Río Ebro-Edificio I+D. Affiliation 2. Aragon Health Research Institute (IIS Aragon),* ***2.*** *Department of Chemical Engineering. Aragon Institute of Nanoscience and Materials (INMA), CSIC-University of Zaragoza, Campus Río Ebro-Edificio I+D, 50018 Zaragoza, Spain,* ***3.*** *Affiliation 1. Aragon Health Research Institute (IIS Aragon), Zaragoza. Affiliation 2. Department of Pharmacology and Physiology, Forensic and Legal Medicine, Veterinary Faculty, University of Zaragoza, Zaragoza.*

Osteoarthritis (OA) is a chronic joint disease that affects millions of people worldwide and has no cure. With this purpose, we decided to explore the use of bupivacaine, a widely used local analgesic, to relieve pain associated with OA. Furthermore, nanotechnology has arisen in the last decades as a promising field of study for drug delivery applications. In this context, bupivacaine encapsulation in poly(ethylene glycol) methyl ether methacrylate (OEGMA)-based (POEGMA) nanogels functionalized with an antibody targeting inflammation, would allow a controlled and targeted release compared to the local administration of the free drug.

POEGMA nanogels loaded with bupivacaine nanocrystals were synthesized as described before by Alejo *et al.* and functionalized with an antibody against glycoproteins upregulated during inflammation. Three different antibody quantities were tested and the functionalization was confirmed using the Bradford Assay. To assess the resulting nanogels morphology, size and electrokinetic potential, Dynamic Light Scattering (DLS) and transmission electron microscopy (TEM) were used. The cytotoxicity of the nanogels in the macrophage J774A.1 cell line was evaluated by the Blue Cell Viability Assay.

Antibody functionalization does not affect NPs morphology, as corroborated by TEM images (Fig 1). The particle size distribution histogram obtained by analyzing different TEM images shows a narrow distribution, resulting in a mean diameter value of 109 ± 10 nm. As colloidal water-based suspensions were used, size distribution was also analyzed by DLS observing that the particle size slightly increased as the synthesis steps proceeded but the nanosystems were always smaller than 200 nm, with the polydispersity index around 0.1, corroborating the monodispersity of the systems, thus, the functionalization does not cause particle agglomeration or irreversible aggregation. Bradford assay of the supernatant confirms the functionalization with the antibody. Furthermore, cell viability of the immunonanogels showed percentages higher than 70% in all concentrations, fulfilling the recommendations of the ISO 10993-5, and encapsulation of bupivacaine in the nanogels decreases the cytotoxicity of the free drug (Fig 2).

Bupivacaine-loaded nanogels were successfully functionalized and demonstrated minimal toxicity in vitro in J774A.1 macrophage cultures. This system may allow its use in different biomedical applications for the management of inflamed OA joints.

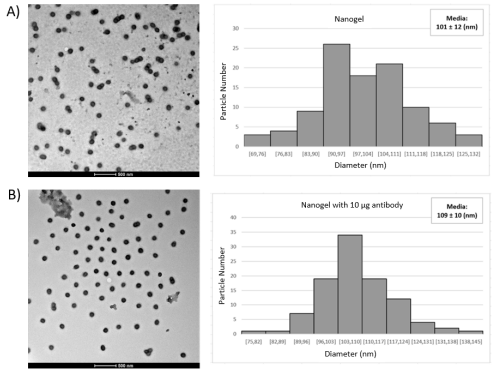

Figure 1. POEGMA nanogel morphology and size distribution histograms. *A) Nanogel before functionalization. B) Nanogel functionalized with 10 µg of antibody.*

Figure1.png

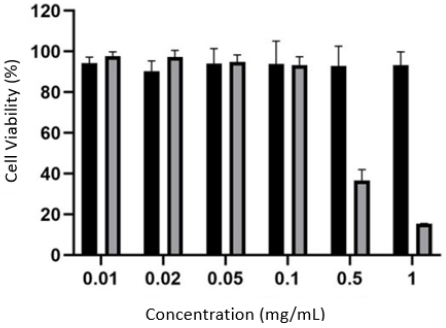

Figure 2. Cytotoxicity of the nanogels functionalized with 10 µg of antibody (black) and free bupivacaine (grey) in the murine macrophage J774 cell line.

Figure2.png

Advancing beyond PEG: Exploring alternative shielding in lipid nanoparticles

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 155

Dr. Silvia Alonso de Castro [1], ***Ms. Paula Martínez Colomina*** [1], ***Ms. Verónica Serrano*** [1], ***Dr. Sergio Esteban*** [1], ***Ms. Inés García*** [1], ***Dr. Lidia Herrera*** [1], ***Dr. Josep Garcia Garcia*** [1], ***Dr. Aroa Duro-Castaño*** [1], ***Dr. Vicent J. Nebot*** [1]

1. *Curapath S.L.*

The integration of polyethylene glycol (PEG) in drug formulations has been widely employed in pharmaceuticals due to its capability enhancing solubility and extending systemic circulation times.[1] Remarkably, PEGylated lipids are commonly applied in the formulation of lipid nanoparticles (LNPs), the most successful nucleic acid delivery system. However, the increasing reports of PEG-associated immunogenicity present a significant challenge, leading to potential hypersensitivity reactions and diminishing the therapeutic efficacy of PEGylated compounds. [2-3]

To overcome this, at Curapath we have developed bioinspired alternatives to PEG based on polypeptides and polypeptoids, which are designed to retain the beneficial properties of PEGylation, such as extended systemic circulation and reduced opsonization, while minimizing potential immunogenicity. We present here nonionic amphiphilic lipid-polymers as alternative to PEG. Our alternatives, when formulated in LNPs lead to stable formulations, higher transfection *in vitro* and comparable *in viv*o performance to that of Benchmark PEG-formulations.

References

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Development of a novel hydrogel-coated micro-needle platform for the diagnosis of skin cancer

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 160

Mr. Connor Daniels [1]

1. *Imperial College London, Department of Bioengineering*

Introduction:

Melanoma is the 5th most diagnosed cancer in the UK and USA. Despite accounting for only 1% of skin cancers, melanoma is responsible for 90% of skin cancer fatalities. Incidence rates have risen rapidly worldwide, with a 140% increase since the 1990s in the UK. Current diagnosis typically begins by a highly subjective and inaccurate visual inspection in primary care followed by an invasive skin biopsy in secondary care. With less than 10% of biopsies being positive for melanoma, the current diagnostic pathway for skin cancers is highly inefficient, leaving patients with unnecessary scaring and burden health systems with unneeded expenditure.

Methods:

Novel hydrogel-coated polymer-based microneedle patches were designed, engineered, and characterised which are capable of sampling interstitial skin fluid (ISF) in a rapid and painless manner. Functionalised with bespoke Peptide Nucleic Acid (PNA) molecular probes, the modified hydrogels were capable of capturing tumour-derived or tumour-specific cell-free nucleic acid biomarkers (most specifically microRNAs or miRs) within ISF through sequence-specific hybridisation. PNA probes were subsequently functionalised with target-responsive redox probes with the aim to achieve on-patch, electrochemical miR detection for rapid testing at the point of care.

Results

To date, a small library of hydrogel-PNA conjugates have been synthesised and characterised (both physically and chemically). Their ability to selectively sample and capture nucleic acid biomarkers through in situ hybridisation to the PNA probes was demonstrated in vitro. An electrochemically active redox probe was successfully designed, synthesised, and characterised. The ability of the redox probe head to detect miR hybridisation to the PNA capture probe (through intercalation between PNA:RNA base pairs) was also investigated in vitro.

Conclusion

Having demonstrated the potential of our microneedle-based nanotechnology to non-invasively sample skin ISF and to capture non-invasively miR biomarkers within it in a sequence-specific manner, this proof-of-concept study sets the foundation for a minimally invasive device that can diagnose skin cancer at the POC (point-of-care), reducing the need for painful and often unnecessary skin biopsies.

Conjugation of aptamers and proteins to lithium niobate harmonic nanoparticles for targeted cancer bioimaging

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 240

Mrs. Alessandra Spada [1], ***Mr. Adrian Gheata*** [1], ***Prof. Sandrine Gerber*** [1]

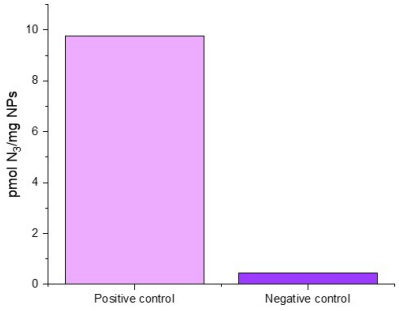
1. *Group for Functionalized Biomaterials, Institute of Chemical Sciences and Engineering, SCI-SB-SG, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*

Nowadays, cancer is a major health burden and second leading cause of death in industrialized countries. Cancer mortality can be reduced through early stage detection of tumor development and appropriate selective treatment. Nanomedicine has emerged as an appealing strategy to support early diagnosis and targeted tumor therapy. Harmonic nanoparticles (HNPs) are known for their efficient non-linear optical response by generation of second and third harmonic signals under ultrafast laser irradiation, which makes them versatile probes for bioimaging applications. HNPs can be functionalized with i) contrast agents for multimodal imaging; ii) photocaged cargos for controlled drug delivery and iii) targeting ligands for active targeting of cancer cells.

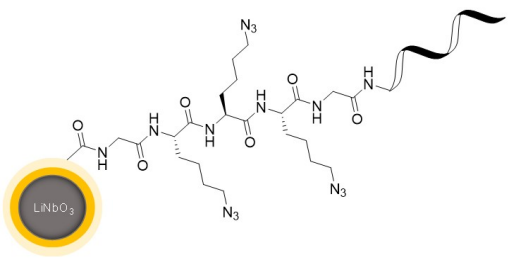
We herein present a functionalization strategy based on the silanization of $LiNbO_3$ (LNO) HNPs through water-in-oil microemulsion. Subsequent surface covering with peptide mimics equipped with reactive functionalities, including azides and carboxylic acids, followed by post-conjugation to targeting ligands were investigated. Functionalized NPs were characterized through dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FT-IR) and scanning transmission electron microscopy (STEM). The density of surface azido groups accessible to azide to alkyne [3+2] cycloaddition was evaluated through fluorescent labelling. Then, cysteine-bearing anti-EGFR nanobodies were coupled to the NPs through the use of clickable spacers previously synthesized.

The intermediate peptide-coated NPs showed spherical morphology and resulted in monodisperse suspensions in aqueous buffers, with hydrodynamic diameters below 200 nm and polydispersity indexes (PDIs) below 0.14. The density of reactive azido groups was estimated at 10 pmol per mg of NPs. Despite their promising antifouling properties evidenced with model proteins, anti-EGFR nanobodies-coated NPs showed essentially non-specific electrostatic protein adsorption rather than covalent linkage. Furthermore, the NPs showed aggregation and significant increase in size. Alternative functionalization strategies using anti-EGFR DNA aptamers are therefore currently investigated.

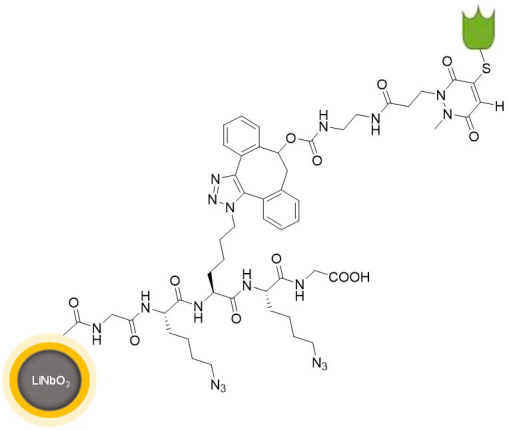
The long-term perspective of the project is to use multifunctional HNPs as theranostic nanoplatforms. Potentially, these nanocarriers, simultaneously decorated with targeting ligands, anti-cancer drugs and imaging probes, could combine early detection and *in vivo* cancer treatment.



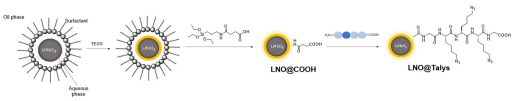
Density reactivefunctionalities.jpg



Antiegfraptamer-nps.jpg



Antiegfrnanobodies-nps.jpg



Functionalizazion procedure.jpg

Drug-loaded Urease-powered nanomotors for the potential treatment of bladder cancer

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 262

Ms. kristin fichna [1], ***Dr. Valerio Di Carlo*** [1], ***Ms. Ines Marcias*** [1], ***Dr. David Esporrin Ubieto*** [1], ***Dr. Maria Crespo*** [1], ***Prof. Samuel Sánchez*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST)„* ***2.*** *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Catalan Institute for Research and Advanced Studies (ICREA)*

In recent years, enormous research efforts have been made to minimize the side effects of drugs and to increase their therapeutic efficiency in the treatment of cancer. Bladder cancer, for example, is the ninth most common cancer worldwide for which current therapies prolong patient survival, but also show high relapse rates, making it urgent to improve existing therapies. Using the catalytic reaction of enzymes that consume bioavailable fuels to propel micro- and nanoparticles (nanomotors) has expanded their potential applicability in nanomedicine and might provide a platform to overcome current challenges in drug delivery. Here, we present urease-powered nanomotors based on mesoporous silica nanoparticles (MSNP) loaded with clinically relevant drugs (Mitomycin, Erdafitinib) for the potential treatment of bladder cancer. The procedure of MSNP synthesis to obtain homogeneous particle size distributions and ensure proper pore opening for subsequent drug loading has been optimized. Furthermore, swarming behaviour in ionic and proteinaceous media has been tested in presence of different concentrations of urea (0 mM up to 300 mM). In addition, spectral flow cytometry as a novel tool to analyze particle delivery efficiency has been carried out with mouse bladder carcinoma cells (MB-49) after incubation (1 h, 4 h) with active (MSNP-Urease) and passive (MSNP-BSA) FITC-labelled nanoparticles at different urea concentrations. When the nanoparticles were incubated with MB-49 cells, active nanomotors showed a 3.2-fold increase of the delivery efficiency in presence of 100 mM urea compared to passive nanoparticles after only 1 h of incubation. The drug loading results, biocompatibility tests and enhanced delivery efficiency of active nanomotors to MB-49 cells may proof their potential to be used in future nanomedical applications for the treatment of bladder cancer.

The potential of nanomedicine in obesity: crosslinked polymeric micelles targeting CPT1A in brain cells to modulate feeding

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 267

Mr. Jesus García-Chica [1], Dr. West Kristian Paraiso [1], Mr. Carlos Palacin [1], Ms. Ana Cristina Reguera [1], Dr. Sebastian Zagmutt [1], Mr. Shigeto Fukushima [2], Dr. Núria Casals [1], Prof. Kazunori Kataoka [2], Dr. Xavier Ariza [3], Dr. Sabina Quader [2], Dr. Rosalía Rodriguez Rodriguez [1]

1. *Universitat Internacional de Catalunya,* ***2.*** *Innovation Center of Nanomedicine,* ***3.*** *Universitat de Barcelona*

The alarming global increase of neuron-related diseases such as neurodegeneration, obesity and related complications suggest an urgent need for new therapeutic strategies. One promising approach involves the inhibition of carnitine palmitoyl-transferase 1 A (CPT1A) in neurons, a critical protein involved in fatty acid oxidation (FAO). Notably, inhibiting hypothalamic CPT1A has been established as a means to reduce food intake and body weight through the modulation of the AgRP-POMC neuronal circuit. However, this target is difficult to reach in vivo with the current drugs. In the present investigation, we propose the development of an advanced core-crosslinked poly-ion complex (PIC) micelle that can stably encapsulate the CPT1A inhibitor C75-CoA for in vivo modulation of feeding and energy expenditure. We are currently decorating the PIC micelles with the ligand calcitonin to selective targeting of AgRP-POMC neurons in the hypothalamus.

We synthesized a triblock co-polymer with polyethylene glycol (PEG) as a hydrophilic shell forming block, a poly(L-aspartamide) (PAsp) middle block functionalized with an aromatic aldehyde moiety PAsp(Aldehyde)), and a poly(L-lysine) (PLys) cationic block designed to form a PIC micelle with (±)-C75-CoA. A PEG-PAsp diblock co-polymer functionalized with hydrazide groups (PEG-PAsp(Hydrazide)) was added to the micelle to crosslink the aldehyde-containing PEG-PAsp(Aldehyde)-PLys through hydrazone formation, forming monodisperse PIC micelles of around 40–45 nm in size.

In vitro characterization in hypothalamic neuronal cell lines showed micelles' ability to modulate FAO and increase drug uptake compared to the free form and non-crosslinked micelles. Moreover, intracerebroventricular administration of these micelles in mice exhibited a significant reduction in food intake and body weight compared to the free drug. Additionally, PIC micelle encapsulating the CPT1A inhibitor effectively modulate the expression of hunger-related neuropeptides, enhancing the satiating effects when compared to free C75-CoA. Notably, the PIC micelle showed the ability to activate specific nuclei within the hypothalamus, as evidenced by c-Fos immunostaining. These findings underscore the potential of this nanomedicine in exploring the landscape of neuron-related disorders such as obesity. Our inclusion of salmon calcitonin to target AgRP-POMC neurons represents a significant step towards the development of innovative and effective strategies for addressing the obesity epidemic.

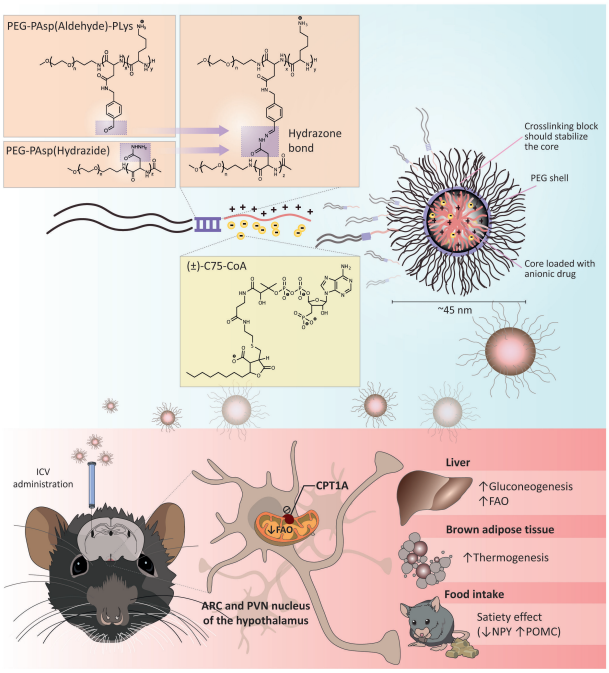


Figure 1 version 11oct 1.jpg

Versatile biocompatible silica nanoparticles for cancer theragnosis

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 271

Dr. Esperanza Medina-Gutiérrez [1], ***Ms. Marina Llenas Martínez*** [1], ***Mr. Ruben Garcia Fontarosa*** [1], ***Dr. Raúl Herance Camacho*** [2], ***Dr. Gerard Tobías Rossell*** [1]

1. *Institut de Ciència dels Materials de Barcelona (ICMAB-CSIC),* ***2.*** *Vall d'Hebron Institut de Recerca (VHIR)*

Cancer accounted for almost 10.0 million deaths worldwide in 2020, being prostate cancer (PC) the most common in men. Despite its good prognosis at early stages, its outlook is significantly worsened when metastases develop. Moreover, available therapies entail disadvantages, such as common adverse effects, leading to a poor quality of life. With this aim of improving the diagnosis and treatment of this disease, mesoporous silica nanoparticles (MSN) loaded with fluorophores and externally functionalized are being explored as theragnostic nanocarriers in vitro and in vivo.

In vitro experiments were performed on cancer cell lines, and included internalization by flow cytometry and confocal microscopy and viability MTT assay. Liver quenching of fluorophores was assessed in vitro. In vivo, MSN biodistribution and toxicity were first assessed after intravenous administration on immunocompetent mice, being euthanized at different time points. A subcutaneous PC model was employed to test MSN retention by tumor cells. Tumor fluorescence was assessed up to 48 h. At endpoint, organs were collected for ex vivo histopathological studies.

Cancer cell lines shown proper internalization of MSN and a lack of cytotoxicity at high concentration (Fig. 1). Liver did not show a quenching effect on MSN in vitro. MSN were well tolerated in vivo, and organs did not show any toxicity signs. Ex vivo organ fluorescence evinced a transient accumulation of MSN in lungs. No remarkable signal was detected in any other organ, despite sample heterogeneity, meaning a probable lack of acute non-specific systemic toxicity. When intratumorally administered, MSN were retained in tumor up to 48h and did not reach other organs (Fig. 2).

While more exhaustive experiments need to be performed, these results indicate the potential of our MSN as nanocarriers. Finally, and given the functional versatility of MSN, we are setting the bases for MSN loading with therapeutic or diagnostic agents and modification of their surface motifs with targeting molecules to ensure highly precise diagnosis and personalized therapies for cancer patients.

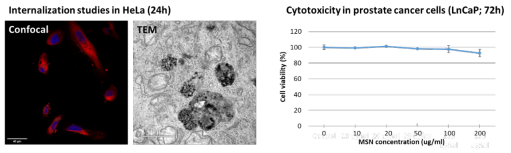


Figure 1.png

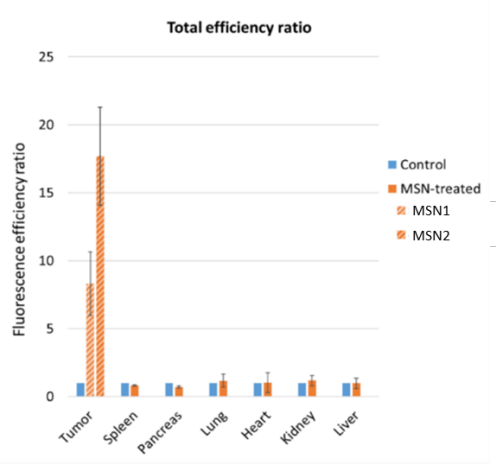


Figure 2.png

Live cell imaging of LRP1 trafficking

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 239

Mr. Alessandro Ronzoni [1], ***Mr. Víctor Mejías Pérez*** [1], ***Dr. Joana Fort*** [2], ***Dr. Cátia Lopes*** [3], ***Dr. Iris Batalha*** [1], ***Dr. Manuel Palacin*** [2], ***Prof. Giuseppe Battaglia*** [4]

1. *Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC),* ***2.*** *Institute for Research in Biomedicine (IRB Barcelona),* ***3.*** *Institute for Bioengineering of Catalonia (IBEC),* ***4.*** *Institute for Bioengineering of Catalonia*

LRP1 is a member of the LRP family, a group of receptor proteins involved in lipid homeostasis, cell migration, proliferation, and differentiation. LRP1 is involved in neurodegenerative disorders and cancer; thus, it is crucial to understand its structure and interactions with ligands (1).

Part of my research project employs molecular biology techniques to engineer human LRP1 DNA, express it in various cell lines, and perform imaging on live cells.

Working with a plasmid coding for the sequence of full-length human LRP1, I generated a fusion protein with the red fluorescent protein “mCherry XL.” The obtained DNA sequence, called “LRP1-mCherry XL”, codes for LRP1 fused to mCherry XL at its C-terminal cytosolic domain, and the features present in the vector DNA sequence make it suitable for expression in human cell lines.

I am using this plasmid to transfect HEK293T and HBMEC cells and achieve fusion protein expression. With this tool, I perform confocal imaging, observing a successful presentation of the protein and its localization on the cell membrane. So far, I have completed multi-channel acquisition, observing the cell nuclei, the cell membrane, and the LRP1-mCherry XL protein. The obtained data consists of 2D images, 3D reconstructions, and videos.

Especially the video files show how the protein is not statically expressed on the membrane but translocates within the cell. Starting from this data, we decided to co-express it with syndapin2, already reported to mediate the transcytosis of Amyloid-β together with LRP1 (2). We aim to have both proteins fused with fluorophores, enabling us to observe the behaviors of the two proteins, their colocalization, and the dynamic of the tubulation process. Once obtained this data, we aim to introduce labeled Amyloid-β in the experiment and simultaneously observe all three elements during the transport process.

This project aims to collect data regarding the behavior and dynamics of LRP1 during the trafficking process; as a side project, I am expressing and purifying LRP1 to study its structure. With the data from these two experimental lines, we hope to contribute to understanding this protein’s characteristics, its role in intracellular transport, and its mechanisms of interaction.

A dose-controlled comparative assessment of the differential pulmonary immune response induced by instilled or inhaled nanoparticles

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 307

Mr. Penghang Chen [1], ***Dr. Pramod Kumar*** [1], ***Mr. David kutschke*** [2], ***Dr. Carola Voss*** [1], ***Dr. Tobias Stoeger*** [1], ***Dr. Lin Yang*** [1], ***Dr. Otmar Schmid*** [1]

1. *Comprehensive Pneumology Center (CPC-M) / Institute of Lung Health and Immunity (LHI), Member of the German Center for Lung Research (DZL), Helmholtz Munich,* ***2.*** *Comprehensive Pneumology Center (CPC-M) and Institute of Lung Health and Immunity (LHI), Member of the German Center for Lung Research (DZL), Helmholtz Munich, Germany*

Carbon nanoparticles (soot), as prevalent air pollutant, are known to induce not only pulmonary inflammation but also have the potential to lead to lung fibrosis and carcinogenesis upon prolonged inhalation. To investigate the biological effects of inhaled carbon nanoparticles in preclinical animal models, researchers often employ the technologically simple, yet non-physiologic intratracheal instillation (IT) of bulk nanosuspensions rather than aerosol inhalation (IH) as exposure method.

Albeit IT exposure yields significantly more bronchial and patchy deposition compared to the uniform alveolar deposition obtained with IH, current knowledge regarding effects of pulmonary particle distribution on toxicological and pathological impact is insufficient. In this study, we establish a method for delivering equivalent nanoparticle doses into murine lungs via two distinct exposure of IT and IH and assess their differential effects on immune cell responses in the lung.

The total pulmonary dose of carbon nanoparticles (here: Printex90) was measured by light absorbance (850 nm) after enzymatic digestion of lung tissue. Highly efficient, dose-controlled delivery of aerosolized Printex90 and fluorescence (resin) nanoparticles was performed by mechanical ventilation (IH exposure). Bronchial and alveolar deposition fractions of fluorescent (resin) nanoparticles were assessed from light sheet fluorescence microscopy images of non-dissected lungs. This enables us to deliver an equivalent dose of Printex90 via IT and IH within a relatively short exposure period (<20 min). At 24 hours after exposure the lungs were lavaged and assessed for differential cytology; lung tissue was also harvested for more in-depth analysis of acute immune responses, which is beyond the scope of the current presentation.

Pulmonary application of 15 µg of Printex90 corresponds to an alveolar dose of 12 µg and 14 µg for IT and IH exposure, respectively. These similar alveolar dose levels are consistent with similar levels of neutrophil influx (ca. 15%) indicating a moderate inflammatory response in the lung. More detailed analysis of differential cytology, immune response, and disease mechanisms will be investigated. Overall, we demonstrate that comparative toxicological studies on the effect of nanoparticle distribution due to different pulmonary exposure routes (IT and IH) can be performed for equivalent bronchial and alveolar dose levels.

Modulating macrophage polarization with magnetic nanoparticles containing toll-like receptor agonist: an alternative approach to enhance anticancer treatment

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 48

Dr. Kamonlatth Rodponthukwaji [1], ***Dr. Nathachit Limjunyawong*** [1], ***Dr. Ladawan Khowawisetsut*** [2], ***Dr. Chatchawan Srisawat*** [3], ***Dr. Sith Sathornsumetee*** [4], ***Dr. Primana Punnakitikashem*** [3]

1. *Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University,* ***2.*** *Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University,* ***3.*** *Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University,* ***4.*** *Department of Medicine (Neurology), Faculty of Medicine Siriraj Hospital, Mahidol University*

Developing alternative cancer treatments has always been a critical challenge to overcome side effects caused by traditional chemotherapy. In tumor microenvironment (TME), protumor macrophages, known as M2, play a critical role in creating immunosuppressive TME, contributing to several pro-tumorigenic outcomes. Reversing M2 into tumoricidal macrophages (M1) phenotype presents an alternative strategy to inhibit tumor proliferation. Among various stimuli, resiquimod (R848), a toll-like receptor 7/8 agonist, has demonstrated potential in repolarizing M2 macrophages into M1 phenotypes. However, the systemic delivery of R848 faces obstacles due to its insolubility and toxicity. Therefore, utilization of nanotechnology as a therapeutic carrier can reduce the dosage and rapid distribution effect, leading to more effective therapeutic outcomes . With unique magnetic properties, magnetic nanoparticles have also drawn attention as they can simultaneously be exploited as contrasting agents for cancer diagnosis.

Herein, we constructed a nanocomposite of carboxymethyl dextran-coated magnetic nanoparticles loaded with R848 (MNP-Dex-R848) (Fig.1A). The negatively charged MNP-Dex-R848 exhibited a larger hydrodynamic size than the control MNP-Dex, while the surface charge remained unchanged (Fig.1B) . We confirmed the success of incorporate R848 into the MNP-Dex structure using UV-Vis spectroscopy and Fourier-transform infrared spectroscopy (FTIR). Additionally, X-ray diffraction (XRD) was used to identify the magnetic characteristics . magnetic characters of the nanoparticles, demonstrating that the incorporation of R848 did not affect the magnetic characteristics. Thus, we have successfully synthesized the R848-loaded magnetic nanocomposites with the desired physical properties. Subsequently, we evaluated the immunomodulatory properties of these MNP-Dex-R848 nanoparticles on THP1-derived macrophages . The treated MNP-Dex-R848 to macrophages showed no toxicity, confirming the biocompatibility of the nanocomposites. To determine the capability in reprogramming the M2 macrophages, surface marker analysis was performed using flow cytometry at 72 h. The results demonstrated a higher percentage of CD80 (M1 surface marker) positive cells and a considerable decrease in CD206 (M2 marker) compared to the untreated group. These findings suggested that our nanosystem had the potential to reprogramming macrophages. In conclusion, our synthesized MNP-Dex-R848 nanocomposites hold promise as a potential candidate in a theragnostic nanosystem for cancer management.

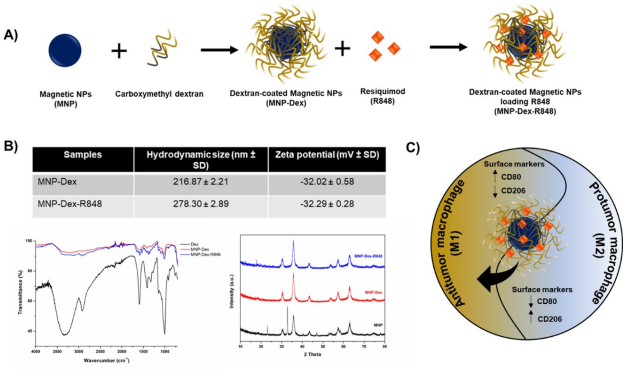


Figure 1 a. schematic representation of material synthesis b. materials characterization c. macrophage polarization from m2 to m1 by nanocomposites.png.jpg

Development of novel nanoemulsions with hydrophilic and lipophilic surfaces for nose to brain delivery of multiple antiseizure compounds

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 175

Ms. Jawza Almutairi [1], ***Dr. Hossam Tayeb*** [1], ***Dr. Haythum Tayeb*** [1], ***Prof. Badrah Alghamdi*** [1], ***Prof. Hussam Murad*** [1]

1. *King Abdulaziz University*

Introduction:

Status epilepticus (SE) is a neurologic emergency condition characterized by abnormally prolonged seizure activity. It is associated with a poor prognosis with longer delay in treatment. Therefore, expeditious treatment of SE is crucial. The conventional management of SE involves the administration of multiple antiseizure medications to the systematic circulation by intravenous route, which is possible only in hospital settings due to the necessity of venous access. The delivery of pharmacological agents by the intranasal route directly to the brain is a new area of investigation.

- In this work, multi-compartment water-in-oil-in-water nanoemulsion (W/O/W-NE) is formulated to simultaneously encapsulate levetiracetam and valproate in the water phase, aiming for intranasal in vivo applications.

Methods

Water-in-oil nanoemulsion (W/O-NE) internal core is made of 30% water (to solubilize water-soluble drugs) and stabilized by 30% Imwitor 600 (polyglyceryl-3 polyricinoleate), while the outer layer is composed of 40% Miglyol 812. W/O-NE was then utilized as an oil phase for preparing the W/O/W-NE, which was stabilized with a surfactant and cosurfactant, 7% Imwitor 375 and 7% Tween 80, respectively and final concentration of 10% Miglyol 812. Creating W/O-NE and W/O/W-NE is done by emulsification using an ultransoic processor, then the size of the nanoemulsion droplets and the polydispersity indexes (PDIs) are determined by Zetasizer.

Results

The droplet size and dispersity of drug-loaded W/O NEs were found to be 18.5 ± 0.44 nm with a PDI of 0.058. Having a W/O-NE droplet size below 20 nm facilitates the development of a multi-compartment W/O/W-NE. W/O/W-NEs have demonstrated average droplet sizes of 77.70 ±0.55 nm with a PDI of 0.158. Achieving nanodroplet sizes <100 nm is a crucial factor for intranasal drug delivery.

W/O NE and W/O/W-NE formulations retained their physiochemical characteristics and were stable for 90 days at low temperature (4°C), room temperature and body temperature (37°C). W/O/W-NE has also shown stability against a high salt concentration solution, phosphate buffered saline (PBS), which mimics physiological conditions.

Discussion

The formulated nanoemulsion exhibits optimal size, dispersity, and stability, indicating its potential as a drug delivery system for anti-seizure drugs, offering a rapid and efficient alternative to intravenous administration to treat SE.

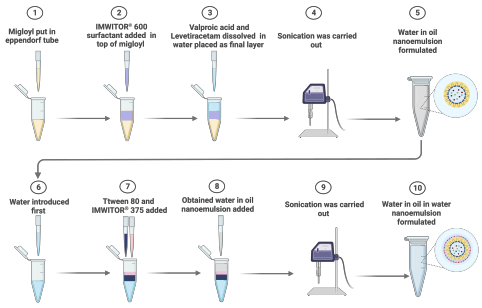
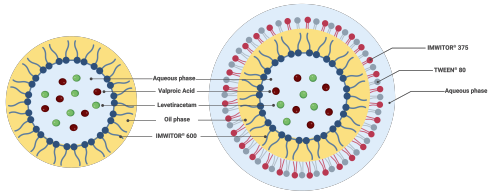
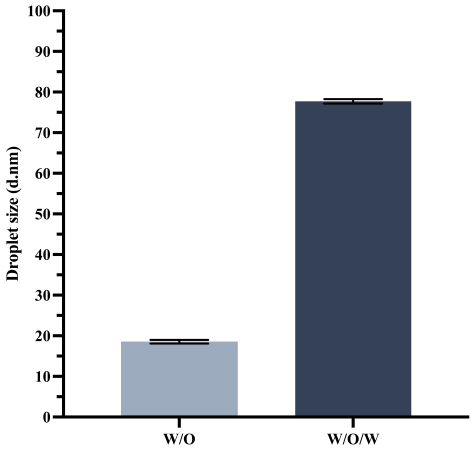


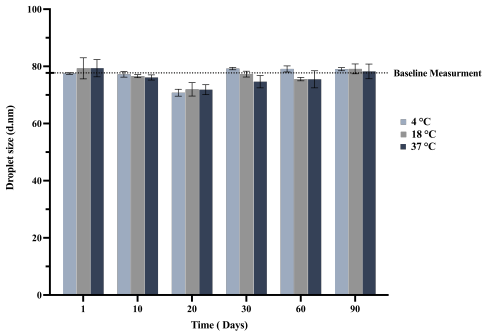
Diagram of nanoemulsion formulation process.png



Structure of w o and w o w nanoemulsion.png



Average droplet size d.nm of water in oil nanoemulsion and water in oil in water nanoemulsion.png



The impact of varying storage temperatures on the size of w o w-nes.png

Tuneable Artemisia absinthium loaded nanoemulsions for antimicrobial applications

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 243

Mrs. Nojod Hasaballah [1], ***Dr. Shareefa AlGhamdi*** [1], ***Prof. Adeel Chaudhary*** [2], ***Dr. Hossam Tayeb*** [3]

1. *Biochemistry Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia,* ***2.*** *Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia,* ***3.*** *Nanomedicine Unit, Center of Innovation in Personalized Medicine, King Abdulaziz University, Saudi Arabia*

Biosurfactants stabilised-nanoemulsions (NEs) are promising antimicrobial formulations comprising functional and tuneable interfaces. NEs are featured with high loading capacity, safety, controlled release, and enhanced biointeractions. Peptide surfactants, amphiphilic biomolecules, lower interfacial tension (IFT) due to their rapid adsorption kinetics at oil/water interfaces, producing stable NEs with small droplet size [1]. AM1, a novel designer peptide, rapidly adsorbs at oil-in-water interfaces and lowers IFT. AM1 can form stable interfacial film at the interface due to its metals binding affinity. AM1 was mainly utilised to stabilise nanodroplets during emulsification, however, AM1 is affected in high strength ionic conditions, causing emulsion instability [2]. Herein, we aimed to enhance the stability and biological activity of AM-1-NE through bioconjugation techniques. AM1 peptide was functionalised with polyethylene glycol (PEG) having different conformations but similar molecular mass to improve the physical stability and biointeraction of AM1-NEs. AM1-PEG conjugates were synthesized and investigated for their interfacial activity at Artemisia absinthium essential oil (Art-EO)-loaded-Mig812/water interface. Art-EO-loaded AM1-NEs were stabilised using AM1-PEG conjugates following emulsification. The oil phase of PEGylated AM1-NEs (P-AM1-NEs) comprises Miglyol 812 loaded with Art-EO, that has antimicrobial properties and extracted from Saudi Arabian plants by hydrodistillation method. P-AM1-NEs was utilised to enhance water solubility, safety, and antimicrobial activity of Art-EO against gram positive (*S.aureus*) and gram negative (*E.coli*) bacteria. The physical stability of Art-EO-loaded AM1-NE were evaluated using dynamic light scattering for changes in droplet size. Negatively charged NEs, stabilised mainly using natural lipid surfactants, were also produced to study the effect of charge on the antibacterial activities in comparison with the positively charged P-AM1-NEs. Antimicrobial activities were studied based on microdilution method to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Both positively and negatively charged NEs have demonstrated physical stability, monodispersity and droplet sizes range between 100-150 nm. P-AM1-NEs lowered the required MIC and MBC against the selected bacteria by at least 4 folds when compared to negatively charged NEs. Long-term functional and physical stability of NEs will be investigated. In summary, this study shows the need for understanding the mechanism of interactions between nanomaterials surfaces and biological targets.

Locked Nucleic Acid (LNA) modified DNAzymes for the specific recognition of double-stranded DNA

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 29

Mr. Muhannad Ateiah [1], ***Mr. Aleksandr A. Bushaev*** [1], ***Ms. Maria S. Rubel*** [1], ***Prof. Dmitry M. Kolpashchikov*** [2]

1. *Laboratory of Solution Chemistry of Advanced Materials and Technologies, ITMO university, Saint Petersburg, Russia* , ***2.*** *Chemistry Department, University of Central Florida, Orlando, United States of America*

Introduction:In spite of the tremendous developments in nucleic acid chemistry and DNA nanotechnology, sequence-specific recognition of dsDNA under near physiological conditions remains an ongoing challenge. The discovery of CRISPR/Cas advanced the field of dsDNA recognition; however, it depends on protein enzymes and suffers from low specificity. Is it possible to bind and cleave dsDNA using a protein-free oligonucleotide-based nanostructures? The protein-independent probe could have a simplified intracellular delivery and be more compatible with chemical modification. Combined with RNA and DNA cleaving deoxyribozymes (DNAzymes), such probes could become a basis for protein free nucleases, a useful tool for gene editing and therapy. To this end we designed and tested a structure called Invasion Bubble (IB).

Methods:We designed and tested several IB constructs which are: Fully LNA modified IB, IB that contains a stretches of 3 LNA bases followed by DNA, IB that contains alteration between LNA and DNA in its sequence, and unmodified IB. Different SNPs were introduced either in the target sequence or in the IB sequence.

Results & Discussion:Double strand invasion of duplex DNA can be energetically favorable if both DNA strands are targeted simultaneously. This encouraged us to design a nanostructure based on LNA modified DNAzymes, called IB. The IB is equipped with the catalytic core of RNA/cleaving 10-22 deoxyribozyme which is split between Dza and Dzb. Therefore, when the IB is stabilized, the catalytic core is formed and becomes active to subsequently cleaves a specifically designed fluorophore and quencher labeled reporter substrate. We tested a series of different LNA modified and unmodified IB to examine the effect of position and number of LNA substitutions on the IB stabilization. We found that the alteration in IB sequence between LNA and regular DNA generated the best invasion efficiency, and the fluorescent signal is 3 times higher above the background. The IB is also highly selective since it was able to detect and differentiate between different SNPs. This results encourages us to further test the IB in terms of nd-FISH.

Acknowledgment:The project is funded by Ministry of education and science of the Russian federation No FSER-2022-0009 and Priority 2030 program.

Synthesis of gemcitabine conjugated graphene oxide as a multifunctional theranostic nanoprob

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 92

Mr. Rıza Acar [1], ***Mrs. Zeynep Yarar*** [2], ***Mrs. Ozge Kozgus Guldu*** [2], ***Prof. Emin Ilker Medine*** [2]

1. *Department of Biotechnology, Graduate School of Natural and Applied Sciences, Ege University, Bornova-Izmir,* ***2.*** *Department of Nuclear Applications, Institute of Nuclear Sciences, Ege University, Bornova-Izmir*

There are many different types of brain tumors. However, diagnosis and treatment rates are low. The blood-brain barrier plays a major role in this. Its existence acts as a barrier that causes most brain-targeted drug delivery systems to not achieve therapeutic efficacy. For this reason, the researchers are focusing on new types of carrier systems for the targeted diagnosis and treatment of the tumor region. Nanoparticles are considered to be an efficient drug delivery that can mediate penetration of drugs into the brain and they protect therapeutic agents by encapsulating them and delivering them to the target site. Recently, nano-graphene derivatives have come to the fore as nanocarriers in cancer diagnosis and treatment researches in medicine and biomedical fields due to their specific properties. Graphene oxide (GO) is considered as a potential nanocarrier system in cancer studies due to its pH sensitivity, large surface area and tunable surface chemistry. In this study, ^{131}I-labelled GO was synthesised as a specific multifunctional theranostic agent for brain tumors. The anticancer drug (Gemcitabine, Gem) (68.7%) and target molecule (Folic acid, FA) (59.5%) were conjugated by surface modification of the synthesized GO nanoparticles. The cytotoxic and uptake effects of the synthesized nanoparticles (GO-Gem-FA) on U-87 MG and SH-SY5Y brain cells were investigated at the *in vitro* level. IC_{50} values of 11.85 µg/mL and 36.76 µg/mL were found, respectively. At the same time, an increase in cellular uptake of ^{131}I radiolabelled nanoparticles was observed in both cell lines over time. Folate receptor expression is known to be higher on human glioblastoma and neuroblastoma cell types than on normal cells. Therefore, targeted drug carrier nanoparticle systems target the folate receptor, which is expressed at higher than normal levels in cancer cells. In this study, ^{131}I-GO-Gem-FA nanoparticles were shown to have targeted therapeutic properties and it is believed that it will contribute to the literature and the field of nuclear medicine with its theranostic ability.

Colloidal Silver Nanoclusters as Antimicrobials

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 100

Ms. India Cook [1]

1. *University of Southampton*

In this study, we report the synthesis and antimicrobial activity of atomically precise silver nanoclusters (AgNCs). The AgNCs are composed of 44 silver atoms are synthesised using a wet-chemistry, one-pot synthesis method.

The antimicrobial activity of the AgNCs was evaluated against a panel of 13 bacterial species, including those listed as Global Priority Pathogens (GPPs) by the Word Health Organisation (WHO) due to antimicrobial resistance and risk to population health. The results showed that the AgNCs exhibited strong antimicrobial activity against some species including N. gonorrhoeae, A. baumannii and P. aeruginosa, with minimum bactericidal concentrations (MBCs) less than 100 μM. However, AgNCs demonstrated low antimicrobial efficacy against S. aureus and E. feacalis. The AgNCs were also found to be non-toxic to human cells.

These results suggest that these atomically precise AgNCs have clinical potential as novel antimicrobial agents, effective against a narrow range of bacterial species. The high potency and low toxicity of AgNCs make them a promising new class of antimicrobial agents for use in the fight against the rise in antimicrobial resistance.

Real time in line size monitoring with the commercially available DLS system: Accuracy and Repeatability assessment

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 344

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 344

Dr. Hanna Anop [1]

1. *Cordouan Technologies*

Poster of the exhibitor **CORDOUAN Technologies**

How to overcome the fundamental challenges in bioimaging and nanomedicine

Monday, 15th January - 14:30: Nanomedicine mechanisms of actions - I (Auditorium) - Oral - Abstract ID: 55

Prof. Hak Soo Choi [1]

1. *Harvard Medical School*

Two fundamental and unsolved problems facing bioimaging and nanomedicine are nonspecific uptake of intravenously administered diagnostic and/or therapeutic agents by normal tissues and organs, and incomplete elimination of unbound targeted agents from the body. To solve these problems, we have synthesized a series of indocyanine contrast agents that varied systematically in net charge, conformational shape, hydrophilicity/lipophilicity, and charge distribution. Using 3D molecular modeling and optical fluorescence imaging, we have defined the relationship among the key independent variables that dictate biodistribution and tissue-specific targeting such as lung and sentinel lymph nodes (*Nat Biotechnol.* 2010), human prostate cancers (*Nat Nanotechnol.* 2010), and human melanomas (*Nat Biotechnol.* 2013). Recently, we have developed a new pharmacophore design strategy "structure-inherent targeting," where tissue-specific targeting is engineered directly into the non-resonant structure of a near-infrared fluorophore, thus creating the most compact possible optical contrast agent for bioimaging and nanomedicine (*Nat Med.* 2015). The biodistribution and targeting of these compounds vary with dependence on their unique physicochemical descriptors and cellular receptors, which permit 1) selective binding to the target tissue/organ, 2) visualization of cancer specifically and selectively, and 3) provide curing options such as image-guided surgery or photon-induced therapy. Our study solves two fundamental problems associated with bioimaging and nanomedicine and lays the foundation for additional targeted agents with optimal optical and *in vivo* performance.

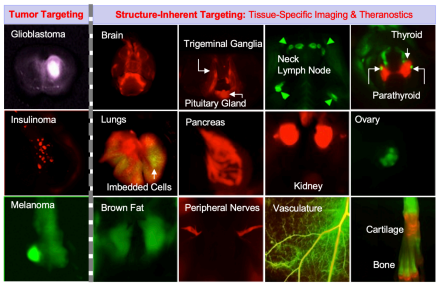


Figure 1. Structure-inherent targeting concept used for the design of tissue-specific imaging and theranostics.

Structure-inherent targeting.png

Correlative super resolution and electron microscopy methods for nanomedicine

Monday, 15th January - 14:47: Nanomedicine mechanisms of actions - I (Auditorium) - Oral - Abstract ID: 139

Dr. Silvia Pujals [1], ***Dr. Teodora Andrian*** [2], ***Dr. Lorenzo Albertazzi*** [3]

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Correlative light and electron microscopy (CLEM) entails a group of multimodal imaging techniques that are combined to pinpoint to the location of fluorescently labelled molecules in their ultrastructural context. Correlative super resolution and electron microscopy is one CLEM modality in which super resolution microscopy is used instead of conventional fluorescence microscopy techniques. Single-molecule localization microscopy (SMLM) is one of the super resolution microscopy families, offering excellent resolution (5–25 nm), multi-colour imaging and quantification capability with single-particle precision.[1,2] Thus, the improved resolution of SMLM leads to a nanoscale localization precision of the specific fluorescent labels in the ultrastructural reference space provided by EM.

Super-resCLEM methods have been mainly applied to biological samples; here we introduce it for synthetic materials. The decoration of nanoparticles with functional moieties is a key strategy to achieve cell targeting in nanomedicine. The interplay between size and ligand number is crucial for the formulation performance and needs to be properly characterized to understand nanoparticle structure–activity relations. However, there is a lack of methods able to measure both size and ligand number at the same time and at the single particle level. We address this issue by introducing a super-resCLEM method, specifically by combining one type of SMLM (DNA-PAINT) with TEM.[3]

Moreover, we are now applying super-resCLEM methods to learn about the trafficking of different nanomaterials inside cancer cells.

FIGURE 1. Super-resCLEM of a PLGA-PEG nanoparticle: functional ligands are counted by DNA-PAINT, while TEM provides the morphology and size.

Acknowledgements

The authors thank Lidia Delgado Valderrama and Maria Yolanda Muela Castro from the Electron Cryomicroscopy Unit from CCiTUB.

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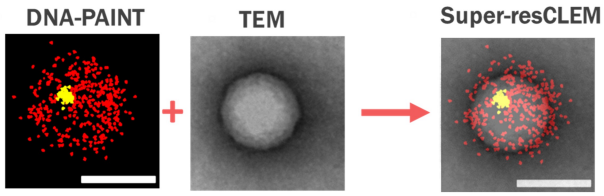


Imagen1.jpg

Integrating phenotypic targeting in physiologically-based pharmacokinetics modeling

Monday, 15th January - 15:04: Nanomedicine mechanisms of actions - I (Auditorium) - Oral - Abstract ID: 184

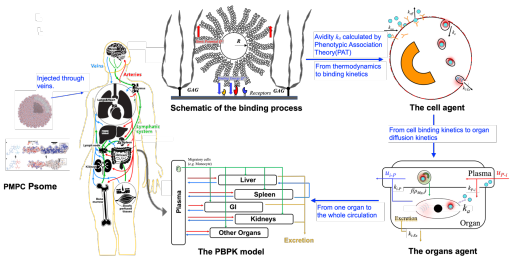
Mr. Zhendong Xie [1], ***Prof. Giuseppe Battaglia*** [2], ***Ms. Silvia Acosta Gutiérrez*** [3], ***Prof. Xiaohe Tian*** [4], ***Prof. lorena Ruiz*** [5], ***Prof. Giancarlo Franzese*** [6]

1. Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain)., 2. Institute for Bioengineering of Catalonia, 3. Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain), 4. West China Hospital of Sichuan University, 5. Institute for Bioengineering of Catalonia (IBEC), 6. University of Barcelona, Department of Condensed Matter, Faculty of Physics,

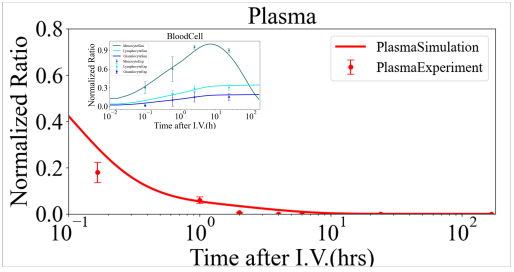
Selective drugging, also known as the “magic bullet,” is the concept that drugs can target specific molecules, cells, or targets while minimising interactions with other parts of the body. Nanoparticles (NPs) with functioned ligands target cells with a certain range of receptors due to the multivalent effect. To develop precision drugs, it is crucial to understand how NPs are distributed in different organs and interact with different cell types in vivo. Our focus is on investigating the distribution of poly(2-(methacryloyloxy)ethylphosphorylcholine)-poly(2-(diisopropyl-amino)ethyl methacrylate) (PMPC-PDPA) polymersome. We use the PMPC polymersome’s interactions with different receptors to target specific cell groups based on phenotypic association theory (PAT), a statistical model based on the description between nanocarriers and cell phenotype (receptor density and glycocalyx).

We integrate phenotypic targeting in physiologically-based pharmacokinetics modeling (PBPK) to mimic the distribution of NPs in organs in silico to identify the most selective combination of parameters for precision drugs. The PBPK is built based on the circulation system, anatomy data, and cell protein atlas to predict the distribution of NPs among different organs, considering the advection among various biological fluids, diffusion of NPs in different organs, and NPs’ interaction with different cells. A non-Langmiur differential rate equation (NLDRE) is applied to extrapolate the PMPC-cell interaction kinetics based on single-cell level uptake experiments. The association constant/affinity kA/j is derived from the PAT to reveal the selectivity of NPs to different cells. We propose that the difference in kA/j results in a larger distribution and cell targeting discrimination.

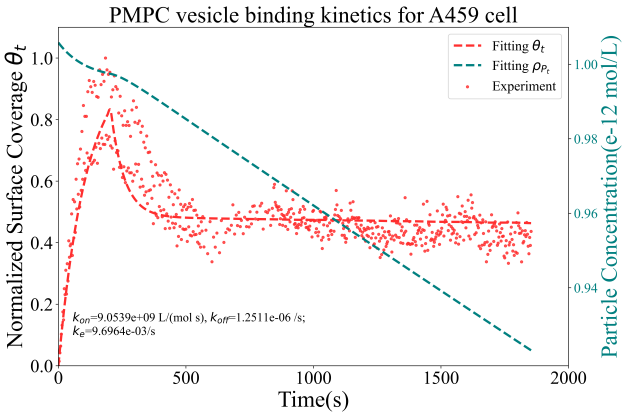
Through experiments in vivo, we obtained information about drug distribution among different organs, the selectivity of NPs to different cells, and some undetectable parameters such as glycocalyx density. Based on these parameters, we change the injected dose, the NP radius, and the polymerization of the PMPC ligand to simulate the distribution of PMPC in silico and develop a better administration strategy.



Chematic.png



Plamsaandbloodcells.png



Fittingpmpc vesicle binding kinetics for a459 cell.png

Predicting cellular uptake of nanoparticles with machine learning

Monday, 15th January - 15:21: Nanomedicine mechanisms of actions - I (Auditorium) - Oral - Abstract ID: 75

Ms. Aparna Loecher [1], Dr. Michael Bruyns-Haylett [2], Dr. Pedro Ballester [1], Prof. Salvador Borros [2], Dr. Nuria Oliva [2]

1. *Imperial College London, Department of Bioengineering,* ***2.*** *Institut Químic de Sarrià (IQS), Universitat Ramon Llull (URL)*

Introduction:

Oligonucleotide therapies have promising applications in tissue engineering and regeneration, but instability and limited intracellular delivery have hampered their successful clinical translation. Poly(β-amino ester)s (pBAEs) are nucleic acid delivery vectors that enable clinical translation. However, formulations need to be optimised for each cell line in a trial-and-error fashion, requiring time and resources. Machine learning (ML) is an ideal in silico screening tool to learn the non-linearities of complex data sets, like material and cellular parameters dictating nanoparticle uptake. We tested 60 pBAE formulations in 4 cell lines as a proof-of-concept that ML is a suitable tool to predict cellular internalisation (Figure 1A).

Methods:

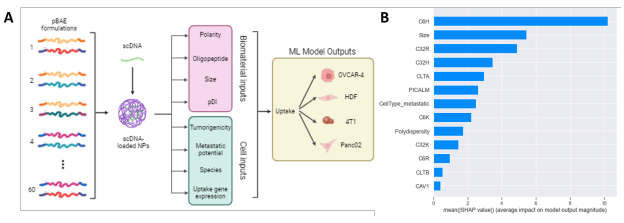
OVCAR-4, Panc02, 4T1 and HDFs were transfected with 60 pBAE nanoparticle formulations containing fluorescently labelled oligonucleotide. Gene expression related to endocytosis was extracted from microarray data (GEO). Normalised data was split into training, validation and test sets in an 80:10:10 ratio. The model with the best performance was analysed using SHapley Additive exPlanations to interpret the model and gain an understanding into the important features.

Results and discussion:

Gradient-boosted trees (GBT) was the best performing ML model (MAE=10.57%). SHAP analysis revealed that nanoparticle size, terminal histidines and arginines, and expression of CLTA and PICALM played a key role in defining uptake (Figure 1B). Partial dependence plots, which show the interaction of two variables on the predicted output, revealed that the presence of lysines leads to higher uptake in cells with lower expression of CLTA, while arginines trigger higher uptake in cells expressing high levels of CLTA. This suggests that arginine pBAEs potentially use clathrin-mediated endocytosis, while lysine pBAEs can employ alternative cellular entry pathways.

Conclusions:

This study demonstrates that ML is a key tool to gain understanding of the complex non-linearities underlying pBAE cellular uptake. Polyplex size, backbone chemistry and terminal oligopeptides play distinct roles in cellular uptake, which often display divergent behaviour in different cell types. CLTA and PICALM seem to play a key role controlling cellular trafficking as a function mainly of the terminal oligopeptides. This work is a step towards a model to predict transfection efficiency in new cell lines.



Panel abstract.png

Machine learning-guided high throughput nanoparticle design

Monday, 15th January - 15:38: Nanomedicine mechanisms of actions - I (Auditorium) - Oral - Abstract ID: 245

Ms. Ana Ortiz-Perez [1], ***Mr. Derek van Tilborg*** [1], ***Dr. Roy van der Meel*** [1], ***Dr. Francesca Grisoni*** [1], ***Dr. Lorenzo Albertazzi*** [1]

1. *Eindhoven University of Technology*

Introduction

From cancer immunotherapies to novel vaccines, nanomedicines have great potential to revolutionize the future of medicine. Nanomedicines can be engineered from many materials, and their properties and biological function can be tailored based on their composition. Multicomponent nanoparticles such as polymeric nanoparticles can be made of a multitude of building blocks. The numerous combinations of these components can yield to hundreds to thousands of possible nanoparticle formulations. With such vast design space, high-throughput methodologies for formulation, screening and decision making are needed to efficiently design nanocarriers and explore structure-activity relationships.

Methods

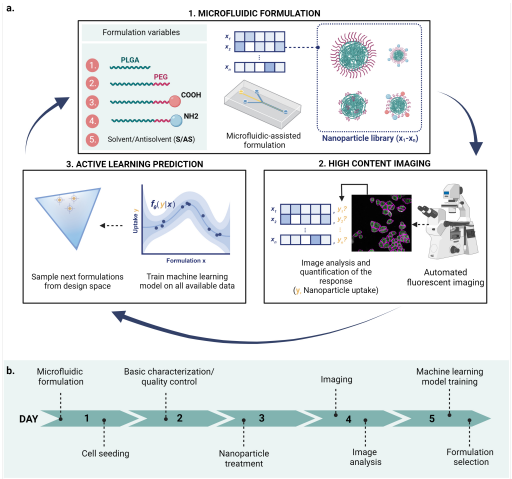
In this work we aim to combine in a single workflow three key technologies to accelerate the exploration of nanoparticle design: (1) microfluidic-assisted formulation, (2) high-content screening (HCS) and (3) active learning. A hydrodynamic flow focusing (HFF) device was used for microfluidic-assisted nanoprecipitation of a library of PLGA-PEG nanoparticles. The fluorescently-labelled nanoparticles were then screened for uptake in an automated fashion (i.e., HCS). A machine learning model was trained to predict (response: uptake) based on formulation variables. An active learning approach was leveraged to iteratively grow the dataset in subsets of 10 particles per cycle, to (1) improve the model (exploration) and (2) optimize response (exploitation).

Results

As a case study, we explored the design of non-targeted PLGA-PEG nanoparticles to optimize their uptake in MDA-MB-468 breast cancer cells, by varying 4 polymer components and one process variable. Starting from a starting dataset of < 30 nanoparticles, our approach led to an increase in nanoparticle uptake from ~5-fold to ~15-fold after two machine learning-guided design iterations. We further tested the ability of the final model to predict low-uptake and high-uptake PLGA-PEG nanoparticles, showing that the model could learn relations between composition patterns and biological activity (uptake).

Discussion/Conclusion

This workflow combining microfluidics, HSC and ML was successfully used to optimize a biological response in a rapid and efficient way. The versatility of these key technologies allows it to expand this way of ‘exploring nanoparticle design’ to multiple nanoparticle compositions and biological responses beyond cellular uptake. Moreover, this approach can guide hypothesis formulation to unravel novel structure-activity relationships, powering nanoparticle development.



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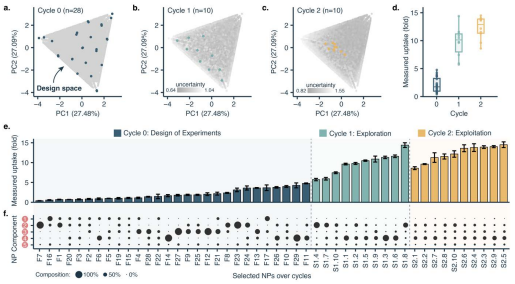
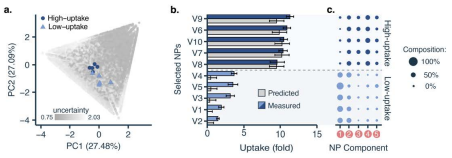


Ortiz-perez results1.jpg



Ortiz-perez results2.jpg

Engineering multivalent protein nanoconjugates for targeted drug delivery in CXCR4+ tumours.

Monday, 15th January - 14:30: Nano-oncology - I (Room 507) - Oral - Abstract ID: 6

Dr. Ugutz Unzueta [1], ***Dr. Maria Virtudes Céspedes*** [1], ***Dr. Isolda Casanova*** [1], ***Dr. Ramon Eritja*** [2], ***Dr. Ramon Mangues*** [1], ***Dr. Esther Vazquez*** [3], ***Dr. Antonio Villaverde*** [3]

1. *Biomedical Research Institute Sant Pau (IIB Sant Pau),* ***2.*** *Institute for Advanced Chemistry of Catalonia (IQAC),* ***3.*** *Universitat Autonoma de Barcelona (UAB)*

Nanomedicine seeks for efficient targeting tools in order to avoid the severe side effects associated to the off-target accumulation of cytotoxic drugs in chemical oncotherapies. Related to that, although drug targeting is currently mainly explored through antibody-drug-conjugates they still do not reach the desired clinical performance as low percentage of the injected material is localized into target cells [1]. Here, we have engineered and recombinantly produced nanostructured protein materials that show excellent properties as nanocarriers for targeted drug delivery. This is done by recruiting in a single polypeptide a specific tumour-homing ligand as well as a histidine-rich architectonic tag that induce the self-assembling of the protein building block into regular size nanoparticles through specific cross-molecular interactions involving divalent cations and the histidine imidazole rings [2]. This confers nanoscale size that avoid renal filtration and allows the multivalent presentation of the targeting moiety, conferring tumour super-selectivity.

Using this technology, we have successfully developed a tumour-targeted multivalent nanocarrier of around 12 nm decorated with the peptide T22, a ligand of the tumoral marker CXCR4 that is overexpressed in more that 20 different solid and hematologic tumours [3]. Thus, intravenous administration of T22-GFP-H6 nanoparticles exhibited excellent biodistribution to CXCR4+ cells with more than 85% of administered material accumulated in tumour tissue [4,5]. Then, the covalent binding of antitumoral drugs (FdU, AraC or MMAE) to the targeting nanocarrier (T22-GFP-H6) produced a set of different nanoconjugates that demonstrated to selectively eliminate CXCR4+ cancer stem cells upon repetitive intravenous administration of the nanodrugs leading to the regression of the tumour progression and the blockage of its dissemination into different mouse models of solid (colorectal cancer) or hematologic (leukemia and lymphoma) tumours in absence of systemic toxicity [6-8].

Hence, the precise manipulation of divalent cations for the nano-biochemical control of multivalent protein nanocarrier materials and derived tumour-targeted nanoconjugates appear as a powerful approach for the creation of innovative smart nanomedicines against cancer.

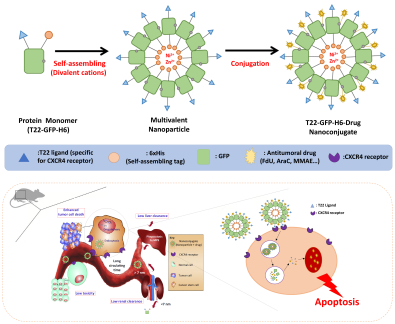


Figure.png

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Full bibliography.png

Slow and sustained delivery of Gemcitabine and Curcumin using biodegradable polymer gel lead to enhanced anti-tumor efficacy against pancreatic cancer

Monday, 15th January - 14:47: Nano-oncology - I (Room 507) - Oral - Abstract ID: 23

Ms. Archana Kumari [1], ***Dr. Arijit Mal*** [1], ***Prof. Rajdip Bandyopadhyaya*** [1], ***Dr. Abhijit De*** [2]

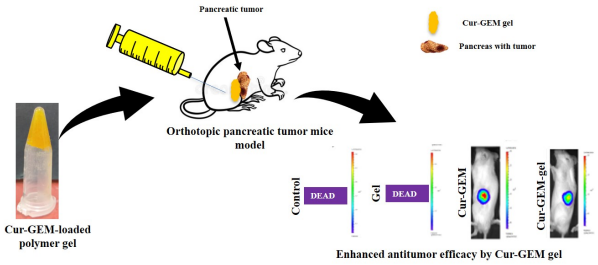
1. *Colloids & nanomaterials Lab., Department of Chemical Engineering, Indian institute of technology Bombay,* ***2.*** *Molecular Functional Imaging Lab., ACTREC, TMC, Kharghar, Navi Mumbai*

Introduction: Gemcitabine (GEM) is a stand-alone chemotherapeutic drug reported to increase the survival of pancreatic cancer (PC) patients. However, main challenges in systemic therapy of GEM have been, its: (i) Inability to reach PC cells because of dense stroma, (ii) low plasma half-life and low bioavailability, (iii) its cytotoxicity to healthy cells and resistance to PC cells. Additionally, (iv) low dose of GEM treatment leads to development of metastasis and drug resistance in PC. To overcome these challenges, we developed biodegradable gel-based localized and sustained delivery of GEM for PC management (shown in Figure 1). We have also combined curcumin (Cur) with GEM to potentiate the anticancer activity of GEM. GEM being highly hydrophilic, we used chitosan for developing the gel. Further, to improve the mechanical properties, we combined polyvinyl alcohol (PVA) with chitosan (Cur-GEM-gel).

Method: Cur-GEM-gel was made by using a polyol salt and crosslinker. Gelation time was measured by rheology at 37 °C. Cell viability was assessed on 2D and 3D cell model of PC cells with different combinations of Cur and GEM and an equivalent amount of Cur-GEM loaded gel for 5 days. Pre-clinical antitumor efficacy was carried out in late-stage orthotopic PC model imitating the clinical scenario.

Results: The gel displays a desirable, controlled release of both the drugs in acidic pH (pH of pancreatic tumor microenvironment is acidic, i.e., 5.5) after 6 days, with only 30% release of GEM and 40% release of Cur. Cur-GEM loaded in gel shows more killing than free drug, given individually or in combination, in both 2D and 3D cell models. Cur-GEM combination inhibits PC cell proliferation and also shows 11-fold more DNA double-strand break in comparison to free drugs. Cur-GEM combination gel showed higher antitumor efficacy in orthotopic PC mice model.

Discussion: Continuous and sustained release of drugs not only kills PC cells but also alters the lipid profile. Cur-GEM showed 1.5-fold high antitumor effect in mice when loaded in gel.



Dual drug-loaded polymer gel for pancreatic cancer treatment.jpg

Investigating the relevance of H-ferritin nanocages in improving tumor-targeted delivery of Indocyanine Green: combined analysis involving UHPLC-MS/MS and fluorescence determinations on murine tissue homogenates

Monday, 15th January - 15:04: Nano-oncology - I (Room 507) - Oral - Abstract ID: 215

Dr. Marta Sevieri [1], ***Dr. Cristina Sottani*** [2], ***Dr. Arianna Bonizzi*** [3], ***Dr. Arianna Chesi*** [1], ***Dr. Leopoldo Sitia*** [1], ***Dr. Saverio Robustelli*** [2], ***Dr. Elena Grignani*** [2], ***Prof. Fabio Corsi*** [1], ***Dr. Serena Mazzucchelli*** [1]

1. *Department of Biomedical and Clinical Sciences, Università degli studi di Milano,* ***2.*** *Environmental Research Center, Istituti Clinici Scientifici Maugeri IRCCS, 27100 Pavia,* ***3.*** *Breast Unit, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Ital*

Introduction: Recently, we proposed the use of ferritin nanocages (HFn) as delivery systems of ICG for fluorescence image-guided surgery (FGS) applications in oncology. HFn stand out among other nanocarriers thanks to their capability to specifically target cancer tissues and reduce side effects with a strong impact in many biomedical applications related to cancer treatment and diagnosis. HFn binds to human cells interacting with the transferrin receptor 1 which is highly expressed on cancer cells, being effective as tumor targeted delivery vector. Previous *in vivo* studies in which HFn nanocages were loaded with ICG (HFn-ICG) demonstrated their suitability as nanotracers for the identification of the primary tumor mass in comparison to the free dye [1]. However, the relevance of the encapsulation in HFn in determining an improved tumor accumulation of ICG deserves further investigation.

Methods: With the aim of deciphering the role of HFn nanocages in improving tumor-targeted delivery of ICG, we took advantage of a combined analysis involving ultrahigh performance liquid chromatography - tandem mass spectrometry (UHPLC-MS/MS) on murine tissue homogenates [2], matched with fluorescence intensities determinations by *ex vivo* optical imaging (Figure 1). Organs homogenates were obtained from 4T1-tumor bearing BALB/c mice randomly divided in different groups (20 minutes, 1h, 2h, 6h, 24h, 48h e 72h) and intravenously injected with ICG or HFn−ICG at a concentration of 3.8 mg/Kg.

Results and discussion:

The quantification of ICG performed with UHPLC-MS/MS combined with fluorescence detection, confirmed the superior delivery of ICG thanks to HFn nanocage. Indeed, the higher fluorescence signal observed at the tumor was associated with a higher amount of ICG in mice injected with HFn-ICG (Figure 2) at each timepoint. This pointed out that the higher visualization of the tumor in terms of fluorescent signal is not only due to a protective and stabilizing effect on the fluorescence given by the nanoformulation, but mainly attributable to a specific tumor targeted delivery. In addition, we confirmed that HFn-ICG does not alter the biodistribution of ICG in the organs responsible for its metabolism or in off-target organs, further supporting its employment as delivery system of ICG in FGS applications in oncology.

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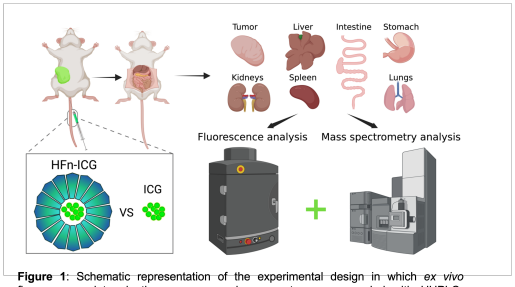

Figure 1: Schematic representation of the experimental design in which *ex vivo* fluorescence determinations on organs homogenates were coupled with UHPLC-MS/MS analysis.

Figure 1.png

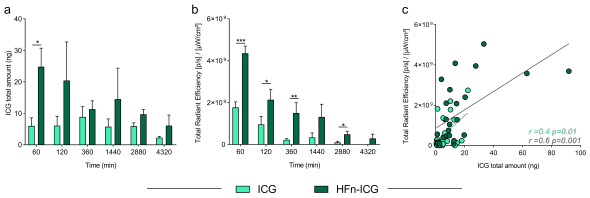

Figure 2: Comparison between ICG concentration determined in tumor homogenates by UHPLC-MS/MS and fluorescence detected at the tumor of mice injected with ICG or HFn-ICG during the biodistribution study (60, 120,360,1400, 2880, 4320 minutes). a) Bar chart reporting the total amount of ICG (ng) measured in tumor homogenates; b) Total fluorescence values determined in tumors. Student t-test * 0.02<p<0.04, **p=0.004; ***p=0.001; c) Scatter plots of the correlation coefficient between the total amount of ICG and total fluorescence detected at the tumor for ICG (r=0.4, p=0.01) and for HFn-ICG (r=0.6, p=0.001).

Figure 2.png

Developing cisplatin-cross-linked DNA nanostructures for cancer therapy

Monday, 15th January - 15:21: Nano-oncology - I (Room 507) - Oral - Abstract ID: 324

Dr. Leo Sala [1], ***Dr. Jaroslav Kočišek*** [1]

1. *J. Heyrovsky Institute of Physical Chemistry*

Self-assembled DNA nanostructures such as DNA origami offer promising scaffolds for building multifunctional nanomaterials. A single nanostructure can be decorated with various agents, e.g., therapeutic drugs, metallic nanoparticles, and fluorescent probes, at addressable sites [1]. Various efforts have been made to optimize such nanostructures for medical applications because of opportunities for multiple functionalities especially in concurrent drug delivery, imaging, and disease diagnostics [2]. However, additional reinforcement is often necessary to ensure the stability of drug carriers for in vitro and in vivo applications. We have exploited the use of cisplatin to impart both its DNA-cross-linking and cytotoxic properties to synthesize more robust chemotherapeutic DNA nanostructures [3]. Cisplatin loading was characterized by ICP-MS and STEM-EDS while stability and morphological changes were evaluated through AGE and AFM identifying the optimal loading at ~1000 cisplatin molecules per nanostructure. Using the MTT assay, we observed cytotoxic behavior in FaDu cells of nanomolar concentrations of cisplatin-cross-linked DNA origami nanostructures in the form of 2D nanotriangles and 3D nanoblocks. Preliminary results on gamma-ray-irradiation of FaDu cells exposed to cisplatin-cross-linked DNA nanostructures will also be presented as we explore the potential use of these nanostructures for concomitant chemoradiotherapy. Cisplatin-cross-linked 2D DNA nanotriangles functionalized with a near-infrared dye can also be tracked in mice using fluorescence imaging. Our results show the potential of cisplatin-cross-linked DNA nanostructures for chemo/radiotherapy and bioimaging as carriers for drugs, radiosensitizers, and fluorescent probes and identified various points for development to optimize them for biomedical applications.

We acknowledge the support from Czech Science Foundation grant no. 24-11503S.

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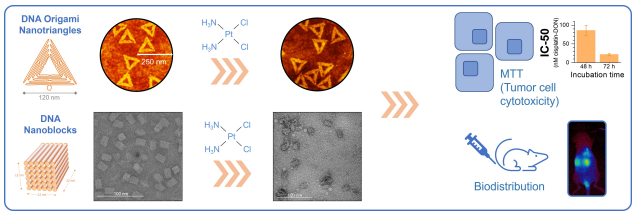


Sala cisplatindnaorigami.jpg

Towards Translation of Nanoparticle-Mediated Histotripsy for the Treatment of Breast Cancer

Monday, 15th January - 15:38: Nano-oncology - I (Room 507) - Oral - Abstract ID: 247

Ms. Sarah Hall [1], ***Mr. Waleed Mustafa*** [2], ***Mr. Khan Imran*** [3], ***Dr. Jeremy Brown*** [4], ***Dr. Irving C Allen*** [3], ***Dr. Yasemin Yuksel Durmaz*** [2], ***Dr. Eli Vlaisavljevich*** [1]

1. *Virginia Polytechnic Institute and State University, Department of Biomedical Engineering & Mechanics, Blacksburg, VA,* ***2.*** *Istanbul Medipol University, School of Engineering and Natural Sciences, Department of Biomedical Engineering, Istanbul,* ***3.*** *Virginia-Maryland College of Veterinary Medicine, Department of Biomedical Sciences and Pathobiology, Blacksburg, VA,* ***4.*** *Dalhousie University, School of Biomedical Engineering, Department of Electrical and Computer Engineering, Nova Scotia*

Nanoparticle-mediated histotripsy (NMH) is a non-invasive, non-thermal, targeted ablation method that combines acoustically active nanoparticles with short-duration (<20 us) focused ultrasound pulses. The nanoparticles act as cavitation nuclei, which seed and sustain cavitation during treatment. NMH has the potential to treat multi-focal or difficult-to-image tumors, such as micrometastases. This study investigates the feasibility of NMH using perfluorohexane-filled nanocone clusters (NCCs) for the treatment of breast cancer using *in vitro* and *in vivo* models.

A 6.3MHz transducer was used to treat 4T1 breast cancer cells in collagen gels (**Figure 1A**). Cells treated with NMH were cultured with NCCs ($1x10^{-5}$ mL PFH/mL media) during treatment and controls were cultured with media. Cells were treated at 12 cycles, 250Hz pulse repetition frequency (PRF), and pressures <20MPa. Fluorescence microscopy and live-dead staining were used to quantify ablation. A 1MHz transducer was used to ablate orthotopically implanted 4T1 breast tumors in BALB/c mice (**Figure 1B**). Experimental mice (n=7) were intra-tumorally injected with NCCs ($5x10^{-5}$ mL PFH/mL PBS) before treatment; control mice (n=6) did not receive an injection. Mice were treated at 15MPa and 500Hz PRF. Cavitation was confirmed with ultrasound imaging during treatment. Ablation was evaluated using histological analysis.

In the *in vitro* model, the results showed precise ablation of 4T1 cells at sub-histotripsy threshold pressures (<25MPa) in the presence of NCCs and the controls (no NCCs) showed no ablation as depicted in **Figure 2**. Cavitation bubble clouds were observed with ultrasound imaging in the breast tumors of mice injected with NCCs whereas bubble clouds were not observed for the control group (no injection of NCCs) during treatment as shown in **Figure 2A-B**. Histological analysis confirmed ablation regions within the breast tumors of the experimental group and no ablation in the control group as shown in **Figure 2C-D**. The results of this study demonstrate the potential of NMH as a non-invasive ablation method for breast cancer *in vitro* and *in vivo*. Ongoing studies are expanding on this work to develop targeted, tissue-selective ablation of metastatic breast cancer using targeted and dual-function NCCs.

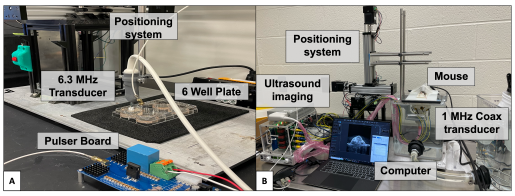


Figure 1. A) NMH treatment setup for *in vitro* model. B) NMH treatment setup for *in vivo* model.

Fig 1 - nmh treatment setups.png

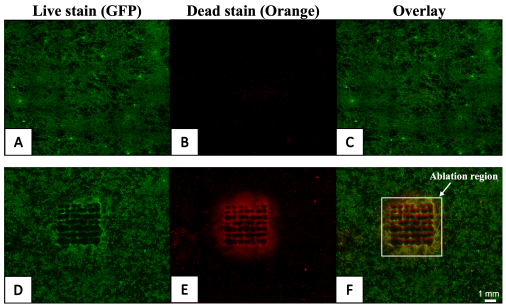


Figure 2. NMH treatment at 20 MPa, 12 cycles, PRF = 250 Hz, and a dosage of 500 ppt. Fluorescence images were taken at 5X magnification. A-C) Control group with PBS only. D-F) NMH group with PBS + NCCs. F) Treatment zone (in square) shows a clearly defined region of ablation.

Fig 2 - nmh treatment in vitro.png

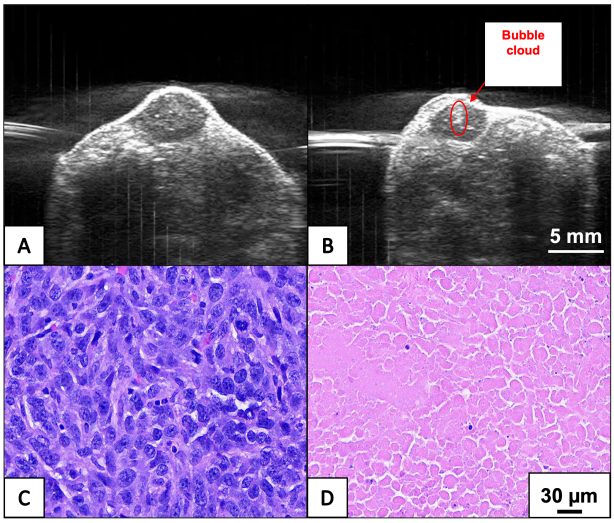


Figure 3. NMH treatment at 15 MPa, PRF = 500 Hz, and a dosage of 500 ppt. A,B) US images of a breast tumor during treatment. C,D) Histological analysis after treatment. B,D) Ablation was observed for mice injected with NCCs. A,C) No ablation was observed for control group not injected with NCCs.

Fig 3 - nmh treatment in vivo.png

An inverted cell culture model to study magnetic hyperthermia for the treatment of colorectal cancer

Monday, 15th January - 15:55: Nano-oncology - I (Room 507) - Oral - Abstract ID: 211

Ms. Yuming Zhang [1], ***Mr. Shaquib Rahman Ansari*** [1], ***Ms. Qianying Chen*** [1], ***Dr. Alexandra Teleki*** [1]

1. *Department of Pharmacy, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide. Superparamagnetic iron oxide nanoparticles (SPIONs) induced magnetic hyperthermia therapy presents a promising alternative for CRC treatment, offering advantages such as deep tumor penetration, local tumor heating, and targeted tumor destruction. This project focuses on developing SPIONs with high heating efficiency for oral delivery to colon cancer lesions. To assess the hyperthermia effects in a biosimilar environment, we introduced an inverted cell culture model that can overcome the issue of excessive nanoparticle sedimentation observed in traditional upright cell configurations. Such sedimentation can significantly influence nanoparticle-cell interactions and magnetic hyperthermia performance, leading to localized overheating which is unlikely to occur in real colon tumor environments.

Methods

Silica-coated $Mn_{0.6}Zn_{0.4}Fe_2O_4$ was synthesized using a flame aerosol reactor, followed by surface conjugation with methoxy polyethylene glycol (5kDa-mPEG) to improve nanoparticle suspension stability in biological media. The heating efficiency of mPEG-$Mn_{0.6}Zn_{0.4}Fe_2O_4$ was characterized in various biosimilar colonic tumor environments (colonic mucus, colonic fluid and tumor tissue phantom) using an alternating magnetic field at 14 mT. The nanoparticle uptake and hyperthermia outcomes of CRC cell lines Caco-2 and SW480 were studied using the inverted and upright cell culture configurations (Figure 1).

Results

There is no significant difference in the heating efficiency of mPEG-$Mn_{0.6}Zn_{0.4}Fe_2O_4$ in different biosimilar colonic environments, which indicates that the local environment does not impact the heating efficiency of the nanoparticles at the applied magnetic field strength (Figure 2). This finding increases the clinic potential of mPEG-$Mn_{0.6}Zn_{0.4}Fe_2O_4$, as a sustained particle heating performance will ensure consistent clinical outcomes. We observed substantial nanoparticle sedimentation in the upright culture configuration which results in difficulty in the complete removal of the surface-bound SPIONs when quantifying nanoparticle cell uptake, subsequently leading false-positive uptake results. Such drawbacks were eliminated when using the inverted culture configuration.

Discussion

mPEG-SPIONs with high heating efficiency for CRC treatment were successfully developed. The inverted cell culture is a more realistic model for studying SPION-cell interactions when aiming at oral delivery of SPIONs to cancer sites, as it minimizes false positive results arising from nanoparticle sedimentation.

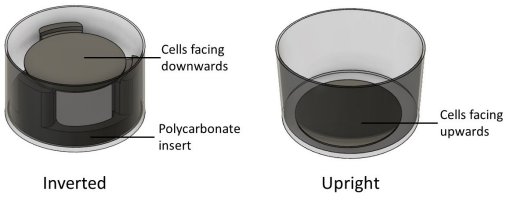


Figure 1.jpg

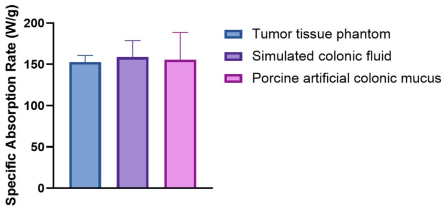


Figure 2.jpg

Biomimetic photonic sensors for immunotherapy evaluation

Monday, 15th January - 14:30: Nanosensors (Room 608) - Oral - Abstract ID: 18

Dr. Maria Soler [1], ***Ms. Razia Batool*** [1], ***Prof. Laura Lechuga*** [1]

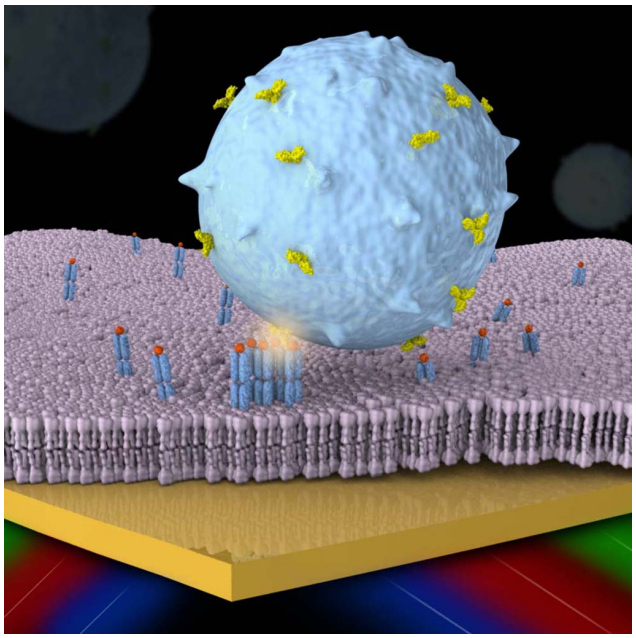
1. *Nanobiosensors and Bioanalytical Applications Group (NanoB2A), Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC, BIST, and CIBER-BBN*

In the era of precision medicine, immunotherapies have emerged as a new hope to treat and cure serious diseases like cancer, viral infections, or autoimmune disorders. These therapies harness our own immune system to combat pathogens and malignancies with remarkable efficacy while minimizing adverse effects on the patient. However, the complexity of current manufacturing and assessment protocols for immunotherapies development are greatly hampering its global implementation, with unattainable costs and administration delays. To address this bottleneck, we aim at introducing innovative analytical technologies that allow for rapid, accurate, and reliable study of immune mechanisms, such as cell interaction or secretion activity, and therefore accelerate and simplify the production and laboratory evaluation of these novel treatments.

We have developed a biomimetic optical technology based on nanoplasmonic sensing capable of monitoring cell interactions in real time and a label-free format. Our biosensor exhibits an excellent sensitivity and specificity, employing low-volume samples (150 mL) with relatively low concentration of cells (detection limits around 10^2-10^3 cells/mL) and without the need of external fluorescent tags. Through the formation of an artificial cell membrane, we can easily tailor the type and number of receptors and ligands to be evaluated, minimizing the time-consuming cell culturing procedures and offering a nature-like platform for reliable cell biology studies. Furthermore, our nanobiotechnology system is integrated in a compact and user-friendly device providing rapid analysis (15 min/assay) at the point of need.

We have demonstrated our methodology for the evaluation of immunotherapies for infections and cancer. In the first case, we introduced our technology for the rapid screening of therapeutic monoclonal antibodies (mAb) as early anti-viral treatment for COVID-19, obtaining accurate neutralization data with low-moderate viral titers. In the case of cancer, we applied the biomimetic plasmonic sensor for the affinity and kinetic analysis of bioengineered tumor infiltrating lymphocytes (TIL) to be used in melanoma treatment, and we are currently working on the evaluation of different checkpoint inhibitor therapies.

We believe that the large versatility and unique capabilities of our platform has the potential to support biomedical laboratories in the pursuit of efficient, accessible, and timely personalized medicine.



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Exploiting the antithrombin biomolecular corona of extracellular vesicles for augmented antithrombin deficiency diagnostics

Monday, 15th January - 14:47: Nanosensors (Room 608) - Oral - Abstract ID: 27

Prof. Annalisa Radeghieri [1], ***Dr. Andrea Zendrini*** [2], ***Ms. Silvia Alacqua*** [1], ***Dr. Giuliana Martini*** [3], ***Prof. Paolo Bergese*** [1]

1. *Department of Molecular and Translational Medicine, University of Brescia,* ***2.*** *Center for Colloid and Surface Science (CSGI), Florence,* ***3.*** *Clinical Chemistry Laboratory, Spedali Civili Hospital, Brescia, Italy*

Introduction:

In the last few years it has been evidenced biological fluids are nanostructured, featuring populations of extracellular nanoparticles (ENPs) secreted by cells in the extracellular space, ranging from lipoproteins to extracellular vesicles (EVs)– and use them as (nano) lines of communication to regulate/mediate key physio-pathological processes.
One of the latest findings in the field is that ENPs enrich on their surface a cargo of proteins and biomolecules, partly recruited in body fluids after their release. The nature of such "biomolecular corona"(BC), which has been accounted so far for synthetic nanoparticles, holds the promise to disclose new ENP properties and functions. Although the field is encouraging, the mechanisms and specificity driving protein recruitment onto the ENP surface, are still unknown. Advancing knowledge in this direction could open for the BC exploitation in early disease etiology, identification and stratification.
This seminar will rationally introduce ENPs and BC concept, discussing our latest results concerning the identification of the serpin family member Antithrombin as a component of the BC of plasma EVs and related functional and diagnostic implications.
Methods: We separated and fully characterized EVs from plasma of healthy or AT deficient (ATD) patients through differential ultracentrifugation followed by sucrose density gradient. We next combined dot blot analysis, WB, 2D electrophoresis and enzymatic assays to reveal the nature of AT adsorption onto EVs.
Results: We show AT is effectively present on EVs bearing an exofacial topology. Physiosorbed EV-AT still retains activity, as evidenced by evaluating the formation of the TAT complex *in vitro*. Being AT a glycosylated protein we show for the first time by 2D-SDS PAGE, a selective adsorption of AT glycoforms onto EVs, indicating glycan involvement in the recruiting process. Finally, differences in AT glycoform enrichment in the BC of EVs from healthy and ATD subjects are also shown.
Discussion: this work evidences plasma EVs act as scavengers for specific AT glycoforms, suggesting EV-BC might be highly considered to unravel hidden roles of AT in the coagulation process and to develop novel diagnostic tests and therapeutics for ATD management.

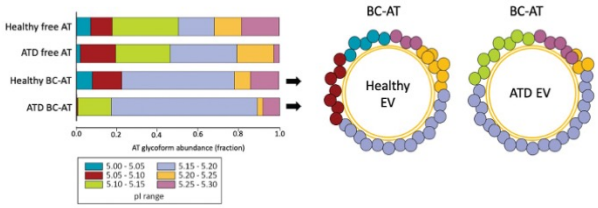

Figure 1: Antithrombin deficiency (ATD), an example of the diagnostic potentiality of the EV-BC. Partition (densitometric profiles, obtained from antithrombin (AT) 2D WB profiles, left, and schematics, right) of the relative amount of AT glycoforms free in plasma and enriched in the EV-BC of healthy subjects and ATD-affected patients (representative of a subject pool). pI range: isoelectric point range. From Radeghieri, A. and P. Bergese (2023). "The biomolecular corona of extracellular nanoparticles holds new promises for advancing clinical molecular diagnostics." Expert Rev Mol Diagn **23**(6): 471-474.

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DNA biodetection assay based on Whispering Gallery Mode Energy Transfer

Monday, 15th January - 15:04: Nanosensors (Room 608) - Oral - Abstract ID: 102

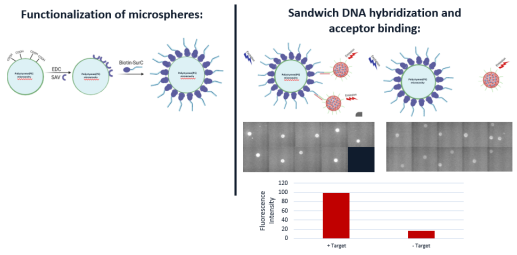
Ms. Nour Alkastntini [1], ***Mr. Thomas PONS*** [1], ***Mr. subha Jana*** [1], ***Mr. Andrey klymchenko*** [2], ***Mr. Andreas Reisch*** [2], ***Mrs. Elisabete Cruz da silva*** [2], ***Mrs. Nina Melnychuk*** [1], ***Mrs. Xiangzhen Xu*** [1]

1. *LPEM-ESPCI Paris,* ***2.*** *Laboratoire de Bioimagerie et Pathologies, Université de Strasbourg*

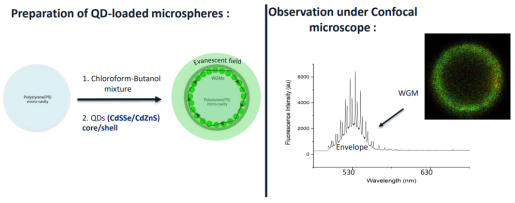
Fluorescence-based biosensors have emerged in a response to demand for specific and simple detection in biomedical diagnostics. Förster resonance energy transfer (FRET) based assays present multiple advantages such as rapid, easy to use and highly specific analysis. However, this technique has a strong dependence on the donor-acceptor distance (~ 10 nm) and has a limit of detection usually lying in the nM range. To overcome this limitation, we introduce Whispering Gallery Mode Energy Transfer, a **highly sensitive** biosensing platform based on energy transfer between polymeric microcavities loaded with semiconductor quantum dots as energy donors and dye-loaded polymeric nanoparticles (dyeNP) as energy acceptors. First, quantum dots are introduced inside the microcavities by using a one step infusion technique, then a part of their emission couples efficiently to Whispering Gallery Modes (WGMs) within the microcavities. This generates an evanescent field at the surface of the microcavities, which spread up to a few tens to hundreds of nm above the cavity surface, enabling a wide range of distance for energy transfer to surface-bound acceptors. Moreover, the high-quality factor of the WGMs and their large surface enable highly efficient energy transfer.

Here, we develop an assay to detect specific Survivin DNA sequences, a cancer biomarker. The microcavities are functionalized with streptavidin to immobilize a Survivin capture (SurC) sequence, and are incubated with dyeNP functionalized with a second Survivin capture probe. The presence of the Survivin target in solution initiate an assembly of the dyeNP at the microcavity surface due to a sandwich DNA hybridization.

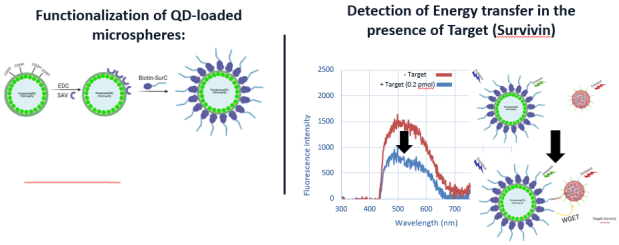
The high absorption cross-section and brightness of the dyeNP as energy acceptors ensures optimal energy transfer efficiency. This translates into a reduction in the emission signal from QD-excited WGM donors compared to the situation in absence of Survivin targets.



Results-2.png



Results-1.png



Results-3.png

Multiplex Detection of Cancer Protein Biomarkers Using SERS-Based Immunoassay on a Natural Diatomite Strip

Monday, 15th January - 15:21: Nanosensors (Room 608) - Oral - Abstract ID: 59

Mr. Mehmet Kahraman [1], ***Ms. Ayse Mine Saridag*** [1], ***Mrs. Isik Didem Karagoz*** [1]

1. *Gaziantep University*

Detection of cancer-related circulating biomarkers in body fluids has tremendous prevalence for cancer screening, diagnosis of cancer in its early stages, monitoring of cancer development, and evaluation of the response to therapy. Surface-enhanced Raman scattering (SERS) is an emerging analytical technique used for the characterization of biological and non-biological structures. It is known that nanostructures having plasmonic properties increase Raman intensity. Thus, the fabrication of plasmonic nanostructures with different plasmonic properties is a significant research interest for the SERS community. Recently, there has been great interest in photonic biosilica (diatomite) due to the additional enhancement of SERS. It is well known that the overlapping of the guided-mode resonances (GMRs) properties of diatomite and the plasmonic properties of nanostructures increases SERS activity. In this study, a diatomite-based SERS active strip is prepared by coating diatomite with AgNPs using the layer-by-layer assembly method. The SERS-active platform surfaces are modified with specific antibodies for HER2, PSA, and MUC4 cancer proteins. Raman tags are prepared to obtain a Raman signal in the presence of each cancer protein when the sandwiches occur. After the sandwich assay, Raman map images are generated based on the chosen peaks on the SERS spectra. The results demonstrated that the diatomite-based SERS active strip shows high sensitivity and selectivity for detecting biomarkers related to breast cancer, prostate cancer, and pancreatic cancer. The use of photonic biosilica in combination with plasmonic nanostructures provides a promising approach for developing ultrasensitive and multiplexed SERS-based biosensors for cancer diagnosis and monitoring.

SERS-based bacterial biosensors with multiplexing capabilities

Monday, 15th January - 15:38: Nanosensors (Room 608) - Oral - Abstract ID: 97

Ms. Lara Costas-Ríos [1], ***Dr. Daniel García-Lojo*** [1], ***Mr. Artai Toba-Pérez*** [1], ***Prof. Jorge Pérez-Juste*** [1], ***Dr. Isabel Pastoriza-Santos*** [1], ***Dr. Gustavo Bodelón*** [1]

1. *CINBIO, Universidade de Vigo, Campus Universitario As Lagoas Marcosende, Vigo 36310, Spain*

The progress in synthetic biology has improved our capability to fabricate robust bacterial biosensors as programmable living devices for diagnostic applications and environmental monitoring. However, current bacterial biosensors mostly rely on bioluminescence, colorimetric, or fluorescence as output signals, which have low sensitivity and poor multiplexing capabilities. Surface-enhanced Raman scattering (SERS) is a highly-sensitivity analytical tool with outstanding multiplexing capabilities, which has been successfully applied for the *in situ* detection and imaging of secreted bacterial metabolites [1], [2]. In this work, we report the development of a new class of bacterial biosensors based on SERS. To this aim, bacteria strains were engineered to express different Raman-active reporters in response to the presence of specific target analytes (Figure 1). The unambiguous identification of the selected Raman reporters and their limit of detection demonstrates the great potential of the SERS-based bacterial biosensors for multiplex detection developed herein.

This work was supported by the Ministerio de Ciencia, Innovación y Universidades; reference PID2019-109669RB-I00. And European Union Reference 965018 Biocellphe.

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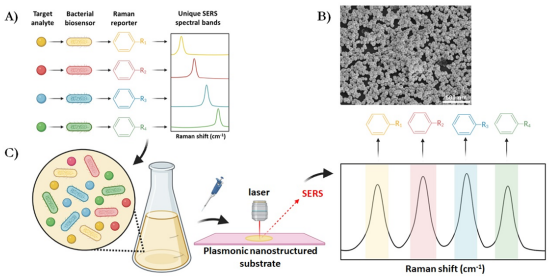

Figure 1: A) Scheme showing bacterial biosensors which respond to specific target analytes, each one producing a Raman reporter with unique SERS fingerprints. B) Scanning electron microscope image of the nanostructured plasmonic substrate employed in this work. C) Scheme of multiplexed detection of different target analytes by SERS. The recorded spectra show the characteristic fingerprint signal from each Raman reporter expressed by the bacterial cells upon sensing their cognate target analytes.

Aim.png

Pushing the detection limits in lateral flow immunoassays through the digital SERS approach: Ultralow detection of SARS-CoV2 N-protein in real samples

Monday, 15th January - 15:55: Nanosensors (Room 608) - Oral - Abstract ID: 129

Ms. Lara González Cabaleiro [1], ***Dr. Lorena Vázquez Iglesias*** [1], ***Dr. Carlos Fernández Lodeiro*** [1], ***Prof. Jorge Pérez Juste*** [1], ***Dr. Isabel Pastoriza Santos*** [1]

1. *CINBIO, Universidade de Vigo, Departamento de Química Física, Campus Universitario As Lagoas, Marcosende, 36310 Vigo, Spain Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO, 36310 Vigo, Spain*

The SARS-CoV2 epidemic has highlighted the need for early diagnosis, where point-of-care (PoC) facilities are critical to prevent the spread of the disease. Herein, we have combined indirect SERS technology with LFIA (lateral flow immunoassay) to develop an ultrasensitive test for PoC applications. SERS-based LFIA will allow us to overcome some drawbacks of traditional colorimetric LFIA such as low sensitivity and/or limit of detection/quantification [1]. This test has been designed to specifically detect SARS-CoV2 nucleoprotein (N protein) from the virus in real samples. This ultrasensitive PoC test requires SERS tags. Thus core-shell Au@Ag nanoparticles (Au@Ag NPs) [2] codified with Rhodamine B ITC (RBITC), as Raman reporter molecule, and bioconjugated with antibodies against N protein have been obtained. Next, the performance of the SERS-LFIA for N protein detection has been evaluated optimizing several parameters (concentration of particles, running buffer and SERS measurements). The results revealed that SERS-LFIA improves the limit of detection by several orders of magnitude in comparison to the colorimetric one. Additionally, applying a digital protocol for SERS analysis [3], it is possible to correlate between digital counts (or positive events) and the analyte concentration was obtained at the single-particle SERS regime. Moreover, SERS-LFIA also allows the correlation between the N protein concentration with the viral load data from real samples which are based on healthy nasal swabs with spiked of SARS-CoV2 inactivated virus. The viral loads will be given as digital droplet polymerase chain reaction (ddPCR) values, instead of the most commonly used cycle threshold (Ct) value [4] , which enables to obtain the viral level as copies of ribonucleic acid (RNA) per sample volume. This reinforces the ultrasensitive detection of the virus in the samples analysed since the existence of false positive/negative results are practically non-existence.

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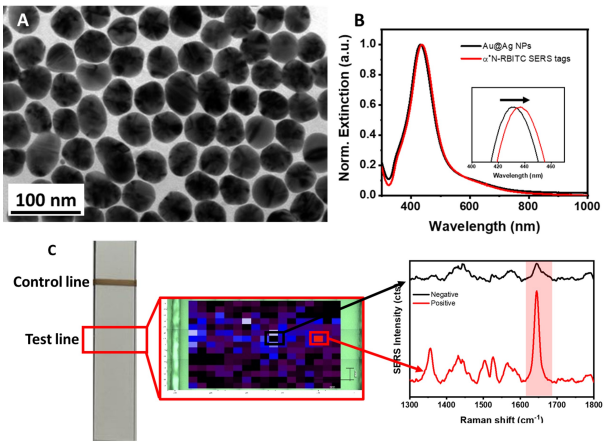

Figure 1. (A) Representative TEM image of spherical Au@Ag core-shell nanoparticles (Au@Ag NPs). (B) Normalized extinction spectra of Au@Ag NPs before (black) and after functionalization with RBITC and anti-N-protein (red line). The inset clearly shows the red shift in the plasmon band peak after the codification of Au@Ag NPs. C) From left to right, lateral flow strip after running a concentration of 2.17 pM of N protein, SERS signal to baseline mapping acquired from 1620 cm^{-1} to 1660 cm^{-1} which is the characteristic peak of α-N-protein-RBITC SERS tags, and SERS spectra of a positive and negative event considering as threshold three times the standard deviation of the background.

Image1.jpg

Embryonic stem cell-derived human lung organoids for nanosafety testing

Monday, 15th January - 16:50: Nanomedicine mechanisms of actions - II (Auditorium) - Oral - Abstract ID: 279

Dr. Rahaf Issa [1], Dr. Neus Lozano Valdés [2], Prof. Kostas Kostarelos [1], Dr. Sandra Vranic [1]

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Lung organoids are 3D tissue-engineered mini-lungs that display structure and functions consistent with their *in vivo* counterparts. These complex models have been used in a variety of applications, from studying lung development to disease modelling, but studies have yet to interrogate their suitability for nanomaterial testing. Here, we report the step-wise differentiation of human embryonic stem cells into a multilineage mature lung organoid, and determine their potential as a tool for the toxicity and safety assessment of carbon-based nanomaterials and micro and nanoplastics. After 50-days of differentiation, the lung organoids exhibit the six major proximal (goblet, basal, club, ciliated) and distal (AECI/II) epithelial cell types of the adult lung. The organoids contain functional cells, evidenced by active ciliary beating and surfactant/mucin production and deposition.
Next, we optimised a microinjection protocol for nanomaterial cargo delivery to the organoid lumen. As proof-of-principle, the organoids were exposed to graphene oxide (GO) or Mitsui-7 carbon nanotubes (CNTs) for up to 7 days. In agreement with established *in vivo* data, we demonstrate that MWCNT, but not GO, elicit adverse effects on lung organoids, leading to a pro-fibrotic phenotype. By comparing the impact of two well studied carbon-based NMs, we validated lung organoids as a tool for predicting pulmonary NM-driven responses.
Finally, we enriched the established lung organoid model with the immune component – human embryonic stem cell-derived macrophages. We optimized the culturing conditions and validated the suitability of the model for nanosafety testing by using the polystyrene beads and polyethylene terephthalate (PET) fibres.
In summary, organoids recapitulate the key features of our complex pulmonary epithelium (such as cell type composition, 3D organisation and architecture). Such systems hold great potential to not only reduce *in vivo* testing but also replace the current use of simple *in vitro* pulmonary models for toxicology studies.

Computational study of the low-density lipoprotein receptor-related protein 1 (LRP1) structure and dynamics

Monday, 15th January - 17:07: Nanomedicine mechanisms of actions - II (Auditorium) - Oral - Abstract ID: 292

Mr. Gian Marco Tuveri [1], ***Dr. Silvia Acosta Gutierrez*** [2], ***Prof. Giancarlo Franzese*** [3], ***Prof. lorena Ruiz*** [4], ***Prof. Giuseppe Battaglia*** [5]

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The brain is the most energy-expensive organ in humans, consuming around 20% of the body's metabolic resting rate. At the same time, the molecular balance that leads to biochemical reactions in the brain makes it extremely delicate to alterations from the outside environment, that is, the blood circulation. Evolution led our bodies to develop a special wall between the neuronal environment and the blood flux, the so-called Blood-Brain Barrier BBB. This barrier is composed of endothelial cells that tightly wrap the capillaries and apply strict control over the molecules that enter and exit the brain. In this control, the membrane proteins called receptors play a fundamental role by binding to the molecular agents and activating the inwards/outwards transport mechanism. The present research focuses on the structure and function of a specific receptor, the low-density lipoprotein receptor-related protein 1, LRP1. LRP1 comprises 4544 amino acids, around 1200 of which involved in three long and flexible structures that contain coordinated calcium ions. These three structures are believed to have an active role in ligand binding activity [1][2] and to activate a peculiar and very efficient transport mechanism [3]. No structure of LRP1 is currently available. Recently, a membrane protein closely related to it, LRP2, has been resolved [5]. The information from this structure supplies an experimental insight on the tertiary and quaternary structures of LRP1. LRP2 structure has been used as a template in the formulation of a new structure for LRP1, using the homology modelling method. The new model shows how LRP1 can assume a coiled conformation and form homodimers thanks to specific amino acids, conserved among LRP1 and LRP2. Furthermore, the investigation approaches the problem using atomistic molecular dynamics MD simulations. The MD results allow us to speculate on the structural characterization of LRP1 and the time evolution of its flexible domains. The behaviour of these domains agrees with the expected behaviour of the coiled conformation of LRP1 at neutral pH. Without external constraints, flexible domains tend to coil to the same end-to-end distance observed in the dimerized structure, implying their natural tendency to converge to a favourable spatial configuration.

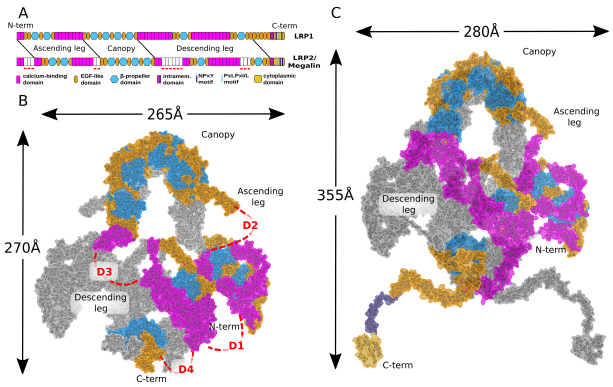

Figure 1. A. LRP1 and LRP2 are composed of the same structural units. The repetition of these units is also conserved in the two receptors. It appears only shifted by the presence of five extra EGF-like domains in LRP1, close to the C-terminal, while LRP2 possesses five extra calcium-binding domains on the N-terminal. The white units, underlined in red in the LRP2 sequence, indicate the missing domains in the LRP2 structure obtained by *Beenken et al*. B. Structure of LRP2 extracted from mice kidney and imaged with Cryo-TEM (*Beenken et al.*). LRP2 shows a clear quaternary structure composed of the two monomers, one coloured (foreground) following the scheme in A., and the other in grey. LRP2 appears to be bent in correspondence with the four propellers in blue (canopy) and presents two extremes: the ascending leg (from the N-terminal to the canopy) and the descending leg (from the canopy to the C-terminal). The missing domains were not included in the published structure for the low resolution of atomic positions, indicating high flexibility; here, they are substituted by a red dotted line. C. LRP1 structure obtained by homology modelling from the LRP2 in B. The structural features, like the intermonomer interaction sites are conserved. Moreover, we modelled the missing domains and a possible conformation of the intramembrane and cytoplasmatic domains.

Lrp1-2 comp caption.png

Super-resolution microscopy to monitor the protein release from PLGA nanocarriers

Monday, 15th January - 17:24: Nanomedicine mechanisms of actions - II (Auditorium) - Oral - Abstract ID: 186

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Introduction

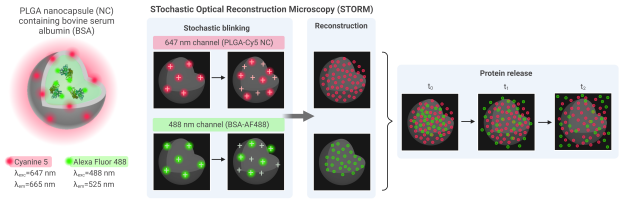
In recent years, poly(D,L-lactic-co-glycolic) acid (PLGA) has been extensively used in nanomedicine since it is a biocompatible and biodegradable polymer. PLGA nanocarriers in the form of nanocapsules (NCs) can incorporate one or various therapeutic agents in their interior. Water-in-oil-in-water emulsion methods are best suited to encapsulate hydrophilic drugs like proteins. The protein release from these nanocarriers has been studied using conventional techniques. However, gaining knowledge of the detailed release profile and kinetics is of utmost importance for the development of feasible nanoparticle-based therapies. Here we introduce the use of super-resolution microscopy for detailed imaging of the protein release from PLGA nanocarriers.

Methods

A double emulsion-solvent evaporation method is used to produce PLGA NCs containing bovine serum albumin (BSA) as a model protein. PLGA NCs are labelled with Cyanine 5 (Cy5) and BSA with Alexa Fluor 488 (AF488) to be imaged by STochastic Optical Reconstruction Microscopy (STORM). STORM is a single molecule localization microscopy technique with an excellent spatial resolution (down to 20 nm). Direct STORM images are acquired using a Nikon N-STORM system in which PLGA-Cy5 NCs are imaged by a 647 nm laser and BSA-AF488 molecules are imaged by a 488 nm laser. TetraSpeck microspheres are used as fiducial markers and imaged by a 561 nm laser. For data treatment, the NIS Elements Nikon software is used to generate lists of localizations of the blinking dyes, which are then analyzed by a MATLAB script to quantify the amount of protein present in the nanocarriers and to examine the release of the protein from the NCs.

Results and discussion

We will show how STORM enables the imaging of protein inside PLGA NCS and its release from PLGA NCs with nanometric accuracy. BSA-AF488 quantification at different time points (from 0 days to 30 days) reveals the escape of protein away from PLGA-Cy5 NCs. The degradation of the polymeric structure is also studied. We advocate that this methodology could be applicable to a wide variety of nanocarriers complementing the existing approaches for protein release studies.



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AI-powered 3D imaging reveals innovative insights into pulmonary nanoparticle delivery and lung geometry in health and disease

Monday, 15th January - 17:41: Nanomedicine mechanisms of actions - II (Auditorium) - Oral - Abstract ID: 207

Dr. Lin Yang [1], ***Dr. Pramod Kumar*** [1], ***Dr. Qiongliang Liu*** [1], ***Mr. Penghang Chen*** [1], ***Ms. Chenxi Li*** [1], ***Mr. Carsten Lüth*** [2], ***Mr. Lars Krämer*** [2], ***Ms. Christina Gabriel*** [1], ***Dr. Aicha Jeridi*** [1], ***Dr. Marie Piraud*** [3], ***Dr. Tobias Stoeger*** [1], ***Dr. Claudia Staab-Weijnitz*** [1], ***Dr. Jäger Paul*** [2], ***Prof. Markus Rehberg*** [1], ***Dr. Fabian Isensee*** [2], ***Dr. Otmar Schmid*** [1]

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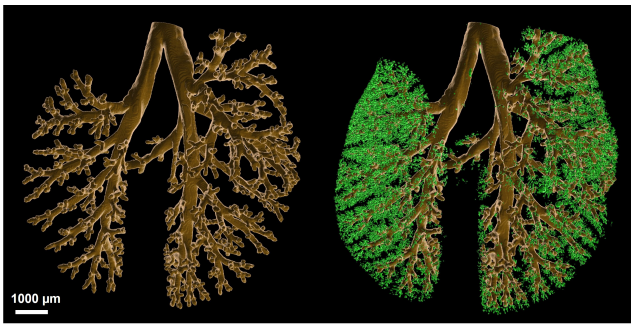
Pulmonary nanoparticle (NP) delivery plays a crucial role in localized therapy and assessing the health implications of inhaled substances. However, a comprehensive understanding of the multiple interconnected biological networks of the lung, spatially-resolved pulmonary substance delivery, and biokinetics of nanoparticles (NPs) - as surrogate for nano-drug carriers, along with NP-induced innate immunity in the healthy and diseased lungs, remains elusive. We employ advanced 3D imaging and deep learning pipelines to reveal the intricate structural organization of the lung, NP-aerosol deposition profiles, and aspects of cellular immunity under homeostatic and fibrotic conditions in mice.

Pulmonary NP delivery, including intratracheal instillation and ventilator-assisted aerosol delivery (3 µm droplets of a fluorescent NP suspension), is conducted in both healthy and bleomycin-induced fibrotic mice. Whole lung samples were collected at various time points from 0 hours to 14 days after NP application. Employing tissue clearing and light sheet fluorescence imaging, we comprehensively assess multiple lung anatomical networks and the biodistribution of NPs at cellular level. In particular, we established AI and deep learning pipelines (convolutional neural networks) to achieve precise segmentation and reconstruction of the entire bronchial airway tree, blood vessels, and lymphatic trees.

Leveraging AI-driven quantitative morphological analysis, we observe significant alterations in multiple lung networks during lung fibrogenesis, which subsequently exhibit recovery during the resolution phase. Spatially resolved NP delivery characteristics, including inter- and intra-acinar distribution patterns and regional NP dosages, show distinct features in the lung fibrosis model. Notably, NPs exhibited limited deposition to highly diseased areas, such as regions with fibrotic foci. Furthermore, phagocytosis and the migration of tissue-resident macrophages (TRMs), responsible for acinar NP transport, are compromised under diseased conditions.

In conclusion, this study offers impartial evidence of alterations in multiple structural networks of the lung, in aerosol-based NP deposition profiles and in NP translocation in a pulmonary fibrosis mouse model. Our openly-shared, diverse 3D murine lung network models are expected to enhance the power of future studies in lung disease and disease-informed targeted pulmonary drug delivery.



Lung airway and nanoparticles2.jpg

4D bioprinted dynamic cardiopulmonary in vitro models

Monday, 15th January - 17:58: Nanomedicine mechanisms of actions - II (Auditorium) - Oral - Abstract ID: 329

Mrs. Uxue Aizarna Lopetegui [1], ***Mrs. Ane Urigoitia Asua*** [1], ***Dr. Malou Henriksen Lacey*** [2], ***Dr. Dorleta Jimenez de Aberasturi*** [3]

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Dynamic 4D-printed in vitro cardiopulmonary models presenting changes and responses as the human tissues do, are excellent tools for studying the fundamental mechanisms governing native tissue functions and pathophysiology, and to perform drug testing studies. However, the exact reproduction of such forces in a realistic manner is highly challenging. In this line, we have explored how nanoparticles (NPs) can serve as multifaceted tools to develop such dynamism in 3D in vitro models and facilitate their characterization using multimodal imaging techniques. We have demonstrated that by incorporating photo-responsive plasmonic NPs embedded within a thermoresponsive ink, highly controllable cyclic expansion-contraction changes can be achieved. Considering this, we have therefore developed a dynamic 3D printed pulmonary artery model that recreates the physical forces to which cells are exposed during arterial pulsation. The external hybrid stimuli responsive layer including the NPs mimics the arterial wall, while the more internal layers are based on extracellular matrix-derived bioinks containing the relevant human derived cells. All inks have been characterized in terms of chemical and mechanical properties, printability, and biocompatibility, after which 3D bioprinting has been used to build the vascular tissue model. We have observed that the cyclic forces induce the expression of mesenchymal activation gene signatures associated with the YAP/TAZ pathway, which is a central mechanotransduction network. Our work highlights the importance of recreating the dynamic aspects of the human body, in vitro cell models and how NPs can help to recreate them.
Developing these advanced models implies improvements in cell engineering techniques, in material designs, as well as advanced imaging tools to accurately characterize them. To address this, we are also exploring the use of hybrid NPs included into the model which can also act as contrast agents for correlative imaging techniques.

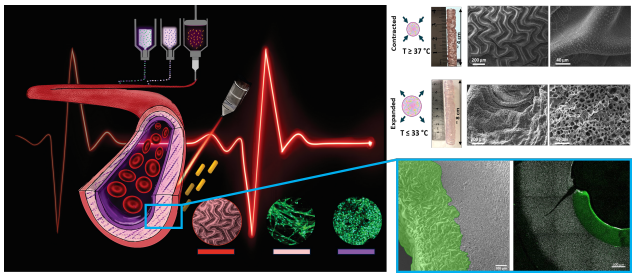


Image artery.png

Addressing Replication Challenges in Nanoscience Research: The Curious Case of Nanoparticles Endosomal Escape

Monday, 15th January - 18:15: Nanomedicine mechanisms of actions - II (Auditorium) - Oral - Abstract ID: 19

Dr. Mustafa ElGharib [1], ***Dr. Maha Said*** [1], ***Prof. Raphaël Levy*** [1]

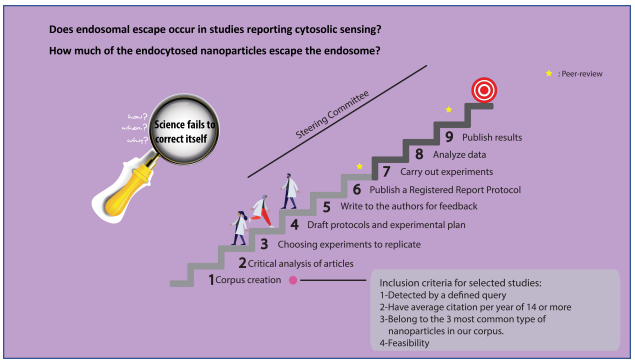
1. *Université Sorbonne Paris Nord*

The 12th-century metaphor ‘’standing on the shoulders of giants” implies that we see further and accomplish more thanks to the wisdom and discoveries of great minds who paved the way for our work. To know which theories are a good base to stand on, scientists have to repeat experiments. Thus, replication is a critical component of scientific research.

In nanoscience, replication is particularly challenging due to the intricate nature of the systems at the interface between disciplines. We propose to address an ongoing topic of nanoscience debate through a formal replication effort. We will focus on the issue of nanoparticle endosomal escape, which is of great importance to many proposed applications, by replicating some articles that report intracellular sensing of specific analyte(s) in the cytosol.

Here, we will be presenting the key steps of this reproducibility project involving the choice of the first articles and the initiatives that we are taking to encourage other scientists, including the authors of those articles, to participate in this replication effort. Prior to any experimental work, we will publish pre-registration reports detailing our plans and protocols, and we will encourage the community to comment on those reports. We adopt an open science approach and will share our data and conclusions whether they confirm or not the initial findings.

We hope that this work will enable us to clarify contested issues in the field of nanobiotechnology and will help the community really stand on the shoulders of giants and not on a nanobubble.



Replication steps.png

FerOX: doxorubicin loaded ferritin nanocages reduce toxicity against T-lymphocytes in breast cancer treatment

Monday, 15th January - 16:50: Nano-oncology - II (Room 507) - Oral - Abstract ID: 254

Dr. Serena Mazzucchelli [1], ***Dr. Marta Sevieri*** [1], ***Dr. Francesco Andreata*** [2], ***Dr. Francesco Mainini*** [1], ***Dr. Lorena Signati*** [1], ***Dr. Francesca Piccotti*** [3], ***Dr. Marta Truffi*** [3], ***Dr. Arianna Bonizzi*** [3], ***Dr. Leopoldo Sitia*** [1], ***Dr. Carlo Morasso*** [3], ***Dr. Barbara Tagliaferri*** [3], ***Prof. Fabio Corsi*** [1]

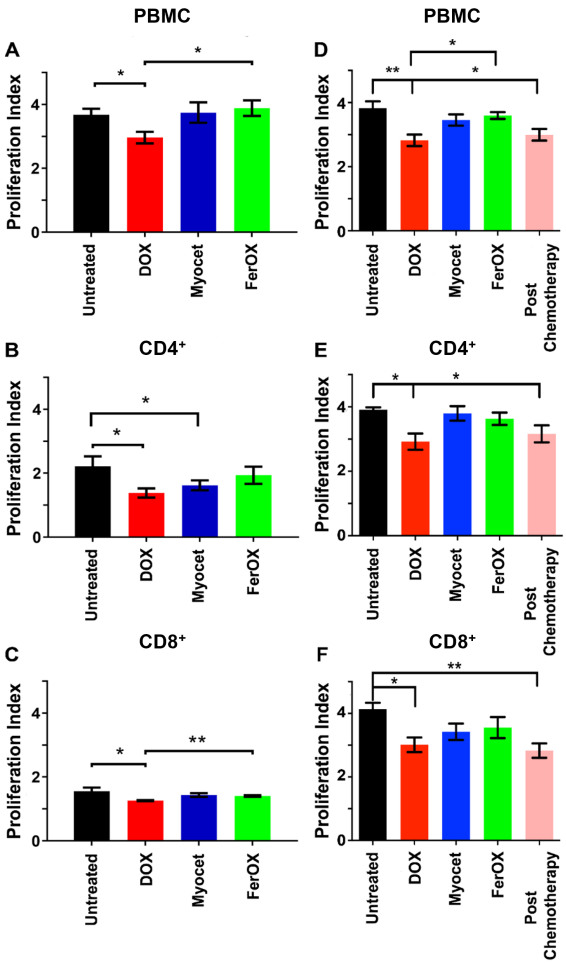
1. *Department of Biomedical and Clinical Sciences, Università degli studi di Milano,* ***2.*** *3. Division of Immunology, Transplantation, and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan,* ***3.*** *Istituti Clinici Scientifici Maugeri IRCCS, Pavia*

Introduction: Despite Doxorubicin (DOX) is associated with relevant side effects, it is still widely used as standard clinical practice in breast cancer (BC) therapy. On one hand, DOX can enhance the antitumor immune response by promoting immunogenic cell death in BC cells. On the other hand, DOX is not cancer-cell-specific, and its off-target cytotoxicity against T-cells may negatively affect the generation of an adaptive immune response. Therefore, new DOX formulations should be developed to preserve antitumor immunity from DOX toxicity. To this aim, we formulated DOX in ferritin nanocages (FerOX) and studied its interaction and major effects on T-cells in comparison with DOX and liposomal DOX (Myocet).

Methods: First, FerOX was produced using a pH dependent loading method. Peripheral Blood Mononuclear Cells (PBMC) were collected from healthy donors and from BC patients before and after neoadjuvant chemotherapy (NAC). To compare free and nanoformulated DOX, cells were incubated with DOX, Myocet and FerOX. DOX uptake and cell viability were studied by confocal microscopy and cytofluorimetry on different T-cells subpopulations ($CD4^+$, $CD8^+$, Central Memory, Naïve, Effector Memory and Terminally Differentiated Effector cells). Furthermore, DOX, FerOX and Myocet were incubated in BC Patient-Derived Organoids (PDO) to assess biological activity in a translational drug screening platform.

Results: Patient-derived PBMC promptly internalized DOX displaying a dramatic proliferative impairment after NAC in comparison to the match-paired PBMC isolated before NAC. In detail, we found that $CD8^+$ T-cells displayed the highest DOX uptake leading to a suboptimal antitumor immune response. To overcome this issue, we compared DOX with Myocet and FerOX. Interestingly, only FerOX was able to reduce DOX uptake in T-cells without affecting their proliferative potential. Moreover, we found that FerOX preserved DOX activity in a panel of PDOs, confirming FerOX strong tumor-targeted efficacy.

Discussion: Overall, this study provides novel understanding on the interaction between HFn-based nanotherapeutic and the immune system. Since the proliferation and activation of T lymphocytes from their resting state are critical to every adaptive immune response, the peculiar behavior of FerOX suggests that it may help in fully preserving T competence and could be an advantageous DOX formulation.



FerOX uptake preserves PBMC proliferation *in vitro* and *ex vivo*. Proliferation index of PBMC, $CD4^+$ and $CD8^+$ T-cells from healthy donors **(A, B, C)** and from BC patients before and post- NAD chemotherapy **(D, E, F)**. T-cells were labelled with CFSE 1µM, stimulated with ConA (5 µg/mL) and incubated with with DOX, Myocet or FerOX 5 µM for 3 hours.

Iconan mazzucchelli 2024.png

Combined action of zinc oxide-based nanoconstructs and external acoustic stimuli against colorectal cancer: from 2D culture to 3D bioprinted models.

Monday, 15th January - 17:07: Nano-oncology - II (Room 507) - Oral - Abstract ID: 81

Ms. Giada Rosso [1], ***Prof. Valentina Cauda*** [1], ***Dr. Giulia Mesiano*** [1], ***Mr. Alessandro Bentivogli*** [2], ***Ms. Giorgia Savino*** [1], ***Dr. Marco Carofiglio*** [3], ***Ms. Marzia Conte*** [1]

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Introduction:

Colorectal cancer (CRC) is one of the most diagnosed and lethal tumours worldwide and current treatments are often affected by the generation of resistances. Nanomedicine represents a promising alternative to treat such life-threatening diseases when the first-line therapies fail. In this work, we propose a combination treatment based on targeted, lipid-coated zinc oxide nanocrystals (L-ZnO), which can be activated by an external acoustic stimulus (ultrasounds or shockwaves). The main idea is to produce site-specific nanoparticles, being decorated with targeting agents, and to activate them on demand through the external stimulus, causing, locally and only when needed, the death of CRC cells.

Methods:

ZnO nanocrystals were synthesized, functionalized, and coated with a lipidic bilayer (L-ZnO), which confers stability and biocompatibility and allows the conjugation with a targeting peptide. L-ZnO and their targeted counterpart, pep-L-ZnO, were administered to HT-29 CRC cells and their combination with acoustic waves was tested. The experiments were carried out firstly on 2D cultures and then translated on two different 3D models: spheroids and 3D bioprinted models. The latter was produced extruding, through a coaxial needle, a tubular structure made of gelatin and alginate, used as support to grow CRC cells.

Results:

After a preliminary characterization of L-ZnO and pep-L-ZnO, attesting the correct lipid coating and targeting, a good hemocompatibility of both nanoconstructs was demonstrated. Then, cytotoxicity, internalization and the combined treatment were evaluated *in vitro*, proving that the targeting consistently and significantly enhances the internalization level of the nanoconstructs, and that the treatment with the nanoconstructs jointly to the external acoustic stimulation leads to a significant depletion of cancer cells in all the models studied, especially in the case of pep-L-ZnO.

Discussion:

This work reports promising results and demonstrates the potentiality of the proposed combination treatment, bringing it a step forward towards the clinical translation against CRC. Possible future directions could be the development of more accurate 3D models and the approach to *in vivo* models and a deepened study of the mechanisms underlying cell death in the combination treatment.

Two fronts of attack: modeling the tumor microenvironment and targeting CEA-expressing colorectal cancer cells using a combinatory nano-strategy

Monday, 15th January - 17:24: Nano-oncology - II (Room 507) - Oral - Abstract ID: 299

Ms. Maria Silveira [1], ***Prof. Maria Oliveira*** [2], ***Prof. Jai Prakash*** [3], ***Prof. Bruno Sarmento*** [4]

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Introduction: The 5-year survival rate for metastatic colorectal cancer (CRC) is limited to 12%, highlighting the urgent need for more effective therapies. In CRC, the immunosuppressive tumor microenvironment (TME) with weak acidity significantly influences treatment outcomes. Carcinoembryonic Antigen (CEA), a glycoprotein overexpressed on the cancer cell surface of most CRC patients, has led to the exploration of engineered antibody fragments, as potent agents. Therefore, this study proposes the development of a combined nano-strategy to both target CEA-expressing CRC cells and challenge the immunosuppressive TME. It involves delivering the conventional chemotherapeutic drug 5-Fluorouracil and IL-12 cytokine (NPs-DMMA-IL12) specifically in the TME.

Methods: Chemical conjugations between anti-CEA scFv and PLGA-PEG as well as, pH responsive monomer, 2,3-dimethylmaleic anhydride (DMMA) and PLGA-NH2 were performed. 5-Fluorouracil and IL-12 were encapsulated into the respective NPs by double-emulsion. The charge conversion of the pH responsive NPs was evaluated by DLS/LDA. To evaluate cell uptake and viability of NPs, a tumor spheroid was developed with macrophages and CRC cell lines expressing CEA high and low levels (CEA^{high} /CEA^{low}). Subsequently, the impact of NPs, both individually and in combination, was evaluated regarding their effects on macrophage polarization using flow cytometry and ELISA.

Results: Encapsulation of 5-Fluorouracil and IL-12 was successfully achieved in well-defined nanoparticles, with a size of approximately 150nm. The pH-responsive shift from cationic to negatively charged NPs-DMMA-IL12 occurred within the pH range of 7.4 to 6.5. In cell uptake studies, NPs demonstrated a three-fold increase in internalization of CEA^{high} cell lines, exhibiting superior anti-cancer properties compared to conventional non-targeted therapies. Additionally, the formulations were deemed safe for macrophages. Finally, the combinatory approach resulted in a significant shift of macrophages towards an anti-tumor phenotype with increased expression of CD86, IFN-γ and TNF-α, and decreased levels of IL-10.

Discussion: In this study, we demonstrated that the developed combinatory strategy enhanced anti-tumor cytotoxic effects. Furthermore, it was confirmed to be safe for macrophages while modulating their anti-tumor phenotype. Nanoparticles carrying IL-12 demonstrated the ability to stimulate IFN-γ and TNF-α production by macrophages. In summary, the combinatory strategy displayed significant potential in modeling the TME and inhibiting tumor progression.

Trojan horse H-Ferritin nanocages improve photodynamic therapy efficiency of ICG in breast cancer cells

Monday, 15th January - 17:41: Nano-oncology - II (Room 507) - Oral - Abstract ID: 196

Dr. Leopoldo Sitia [1], ***Prof. Paola Saccomandi*** [2], ***Dr. Marta Sevieri*** [3], ***Dr. Leonardo Bianchi*** [2], ***Dr. Cristina Sottani*** [4], ***Dr. Raffaele Allevi*** [3], ***Dr. Elena Grignani*** [4], ***Dr. Serena Mazzucchelli*** [3], ***Prof. Fabio Corsi*** [3]

1. *Department of Biomedical and Clinical Sciences, University of Milan,* ***2.*** *Department of Mechanical Engineering, Politecnico di Milano, 20156, Milan,* ***3.*** *Department of Biomedical and Clinical Sciences, Università degli studi di Milano,* ***4.*** *Environmental Research Center, Istituti Clinici Scientifici Maugeri IRCCS, 27100 Pavia*

Introduction: Photodynamic Therapy (PDT) is an emerging cancer treatment characterized by enormous advantages including minimal invasiveness, no drug resistance induction, strong immunostimulation potential and limited side effects. PDT is based on the administration of photosensitizers (PSs) able to produce heat and release cytotoxic substances when irradiated with a focalized laser source. Indocyanine Green (ICG), a clinically approved fluorescent dye, has also been used as PS. However, ICG instability and lack of tumor-tropism strongly limit its PDT efficacy. To improve these limitations, ICG can be formulated into tumor targeting nanoparticles (NPs), such as recombinant Heavy-Ferritin nanocages (HFn). HFn is characterized by a strong interaction with transferrin receptor 1 (TfR1). As TfR1 is overexpressed in most tumors, this leads to a natural tumor tropism potential. In this context, we have already demonstrated that HFn loaded ICG (HFn-ICG) significantly increase its delivery to the target tumors in vitro and in vivo.

Methods: Here, we compared ICG and HFn-ICG properties as PS after irradiation with a diode laser. Then we evaluated their PDT efficacy in two breast cancer (BC) cell lines with different TfR1 expression. Finally, we verified the hypothesis that cells modify their levels of endogenous Heavy Ferritin (H-Fn) as a defense mechanism in response to an external stress, such as PDT treatment. This Trojan-horse like behavior could further favor HFn-ICG tumor uptake and increase its PDT efficacy.

Results: In silico experiments demonstrated that HFn loaded ICG improves its properties as PS. Moreover, we showed that HFn-ICG has a significantly higher PDT efficacy as compared with ICG in BC cells depending on their TfR1 expression levels. Finally, we confirmed that the intracellular levels of H-Fn effectively increase in response to PDT irradiation. This peculiar characteristic, that we unveiled for the first time in literature, could be exploited as a Trojan-horse like mechanism to increase HFn cellular delivery and maximize PDT efficacy.

Discussion: The intrinsic TfR1-mediated tumor targeting ability, the improved PS properties after encapsulation and the peculiar Trojan-horse like mechanism we demonstrated, confirm the promising application of HFn-ICG for PDT. Further, in-vivo studies need to be performed to corroborate this hypothesis.

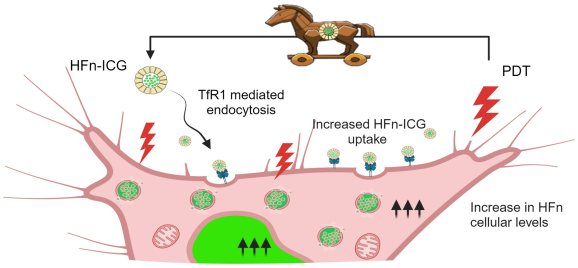

Figure 1: Graphical Abstract reporting the rationale of using HFn-ICG for PDT: HFn-ICG specifically targets tumor cells through a TfR1-dependant uptake mechanism. To protect themselves from oxidative stress generated after irradiation (PDT), cells over-produce and internalize HFn. This protection mechanism is rapidly transformed into a Trojan horse strategy that further increases PDT efficacy.

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Immunoliposomes targeting TROP-2 to enhance the treatment of triple negative breast cancer: co-delivery of metformin and doxorubicin

Monday, 15th January - 17:58: Nano-oncology - II (Room 507) - Oral - Abstract ID: 163

Ms. Daiva Vozgirdaite [1], ***Dr. Katel Hervé-Aubert*** [1], ***Dr. Florence Velge-Roussel*** [1], ***Dr. Nicolas Aubrey*** [2], ***Prof. Igor Chourpa*** [1], ***Prof. Emilie Allard-Vannier*** [1]

1. *EA6295 Nanomedicine and Nanoprobes, University of Tours,* ***2.*** *ISP UMR 1282, INRA, team BioMAP, University of Tours*

Introduction. Despite the advances of nanoparticle drug delivery systems, such as liposomes (LPs), to improve chemotherapy, triple negative breast cancer (TNBC) still poses many challenges due to rapid development of multidrug resistance (MDR), lack of targeted therapy, and metastatic progression. Metabolic modulator metformin (Met) shows great potential as therapeutic adjuvant as it parts of in increasing number of clinical trails in various cancer therapies due to association with reduced cancer risk (De, A., Current Problems in Cancer, 2020). By inhibiting mitochondrial complex I, Met results in improved tumour hypoxia environment and activation of AMP-activated kinase. This reduces tumour growth, MDR to doxorubicin (Dox), and increases immune response (Saini, N., Acta Biochim Biophys Sin, 2018). Moreover, TNBC has been shown to overexpress trophoblast cell-surface antigen 2 (TROP-2), making it a suitable TNBC marker (Aslan, M., NPJ Breast Cancer, 2021). Thus, we aim at improving chemotherapy-based treatment of TNBC by co-delivery hydrophilic Met and hydrophobic Dox inside immunoliposomes (Immuno-LPs), decorated with antibody fragments targeting TROP-2.

Methods. Met-Dox- LPs were formulated using thin-film hydration, followed by conjugation with TROP-2 targeting single chain variable fragments (scFvs) via maleimide – cysteine binding (**Figure 1**). Physical properties and scFv functionality were assessed using DLS, Zetasizer, and indirect ELISA. The effect of nanovectors on MDA-MB-468 cell viability, migration, and internalisation were assessed by MTT assay, flow cytometry, and fluorescence microscopy.

Results. Met-Dox-LPs with size below 200nm and negative surface charge (-20mV) were successfully able to enhance the effect of Dox, by having stronger effect on cell viability with lower concentrations than free Dox or Met (**Figure 2**). Conjugation of scFvs on Met-LPs co-encapsulating Cyanine-5.5 dye did not affect physical properties of nanovectors, nor the functionality of scFvs. The maleimide-cysteine link remained stable when exposed to foetal calf serum (FCS) for 24h. The assessment on MDA-MB-468 by flow cytometry with the Met-Immuno-LPs showed a 4-fold increase in cellular uptake compared to unconjugated liposomes (**Figure 3**).

Conclusion. The formulated liposomes show a promising potential to enhance the chemotherapy-based treatment of TNBC. Next steps in this study is to combine Met-Dox-LPs with TROP-2 conjugation for 2D/3D assessment on TNBC cells.

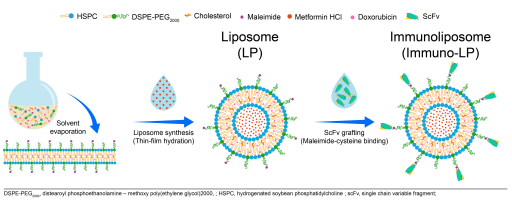


Figure 1. met-dox-immunoliposome synthesis scheme.jpg

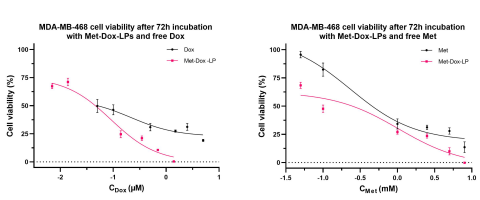


Figure 2. preliminary results mda-mb-468 cell viability after 72h exposure to drug loaded liposomes.jpg

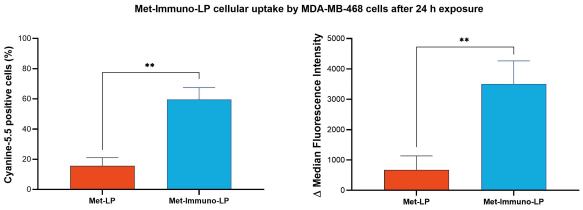


Figure 3. cellular uptake of met-immuno-lps by mda-mb-468 cells after 24 h exposure.jpg

Double porosity nanoassemblies for selective depletion of macrophages and restoration of normoxia in the tumoral tissue.

Monday, 15th January - 18:15: Nano-oncology - II (Room 507) - Oral - Abstract ID: 91

Mr. Jorge Parra [1]***, Mrs. Alicia Arroyo-Nogales*** [2]***, Mrs. Diana Marcos-Fernández*** [1]***, Ms. Sandra Jiménez-Falcao*** [1]***, Ms. Carmen Arribas*** [1]***, Mr. Diego Megías*** [3]***, Prof. Manuel Ramirez-Orellana*** [2]***, Prof. Alejandro Baeza*** [1]

1. *Universidad Politécnica de Madrid,* ***2.*** *Hospital Infantil Universitario Niño Jesus,* ***3.*** *Instituto de Salud Carlos III*

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in children, accounting for up to 12-15% of pediatric cancer mortality. The current treatment is based on the combination of chemotherapy, radiotherapy, surgery and immunotherapy, but the prognosis of the metastatic stages is still poor, with a rate lower than 50%, and there are numerous side effects. Therefore, new therapeutic strategies are needed.

Recently, the tumoral microenvironment (TME) has been studied as a target for cancer treatment. Several types of cells have an important role in the TME, being the tumor-associated macrophages (TAMs) essential for all stages of the tumor development. There is evidence that neuroblastoma cells educate TAMs towards protumoral macrophages.

This project aims to synthetize nanoparticles to induce the death of macrophages, willing to interfere in the TME and to activate the adaptative response of the immune system against the tumor.

RESULTS

Protocell-type nanosystems were developed, consisting of a mesoporous silica core (2.5 nm pore size which allows to load drugs) and mesoporous silica crust (pore size between 8-15nm with the capacity to load macromolecules, in this case, glucose oxidase), covered by a lipid bilayer to prevent the premature release of the cargo loaded in the silica matrix.

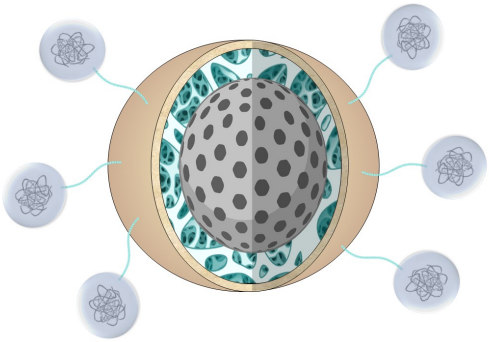
Polymeric nanocapsules of catalase (CAT) were also synthetized and anchored to the surface of the protocell as well as targeting elements (carboxy-D-mannose). to provide the system with specificity against TAMs.

Selective uptake and therapeutic efficacy were evaluated *in vitro.* The nanosystem exhibited a significantly higher cell uptake thanks to the carboxy-D-mannose functionalization, and doxorubicin was the drug which performed a more effective macrophage depletion from those which were tested.

The effect of the enzymatic activity on cell viability was also studied, and although in vitro it did not lead to mortality by itself, in an in vivo model it can help to restore the normoxia in tumor tissue.

CONCLUSION

We developed double porosity-protocells decorated with nanocapsules, which caused a selective macrophage depletion combining the doxorubicin, glucose oxidase and catalase effect. In an in vivo model, we hypothesize according to our results that they can also restore the normoxia in the TME.



Double porosity protocell with nanocapsules jpn.jpg

Wound recovery efficacy of retinol based-micellar formulations in an organotypic skin wound model

Monday, 15th January - 17:07: Nanomedicine for topical delivery (Room 608) - Oral - Abstract ID: 230

Dr. David O. Oluwole [1], ***Dr. Lian X. Liu*** [1]

1. *School of Chemistry and Chemical Engineering, University of Surrey*

The skin plays a crucial role in safeguarding the body against physical, chemical, and environmental threats. When the skin's structural integrity is compromised, it can lead to the formation of acute wounds, which, if not promptly treated, may progress into chronic wounds. Chronic wounds (CWs) affect more than 1% of the global population, and their incidence is on the rise, primarily due to the aging demographic. Current wound management strategies involve methods like debridement, hyperbaric oxygen therapy, antibiotics, and the use of wound dressings such as hydrogels and alginates. However, these approaches lack early intervention and specificity, and contribute to the growing problem of antimicrobial resistance.

In this study, we present a novel approach to wound healing by introducing antibiotics-free retinol micellar formulations developed through a patented procedure by Phytoceuticals Limited. We conducted in vitro investigations to evaluate the wound healing efficacy of these formulations.

Five different formulations with retinol concentrations of 0.3% and 1% were topically applied to an organotypic full-thickness skin wound model, created using a 3 mm punch wound. The treated wound model was incubated in a humidified atmosphere with approximately 5% CO_2 at a temperature of 37 °C for a duration of 6 days. Subsequent histological analysis of the skin wound model was conducted at depths of 60 and 80 µm, as depicted in Figure 1.

Our findings demonstrate that all micellar retinol formulations expedited wound bed contraction, with the 0.3% retinol micellar formulation (RMF) exhibiting the highest efficacy. At depths of 60 and 80 µm, the 0.3% RMF led to inner wound diameter contractions of 58% and 77%, respectively, in contrast to the placebo group, which displayed contractions of 15% and 8%, as illustrated in Figure 2. These results highlight the significant acceleration of wound healing achieved by the retinol micellar formulations and suggest their potential as an early intervention for promoting rapid wound recovery.

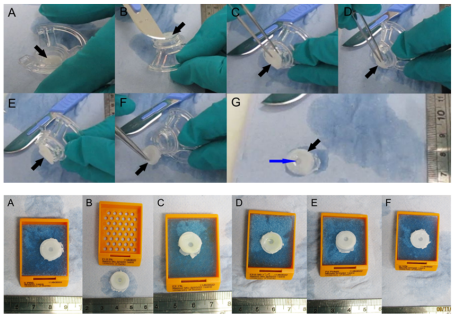


Figure 1. Images depicting careful removal (A through G) of the treated organotypic full-thickness skin wound model from the well. The black arrow indicates the organotypic skin; the blue arrow indicates the punch wound.

Figure 1.png

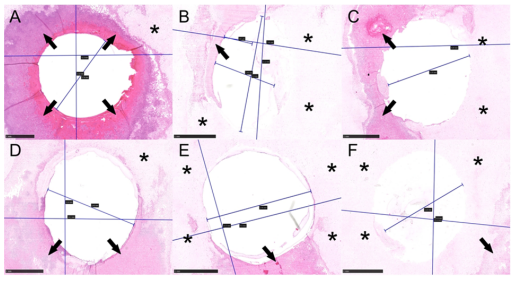


Figure 2. Photomicrographs of the histological sections of the topically treated organotypic full-thickness skin wound model at 80 µm-depth. A, control; B, F1; C, F2; D, F3; E, F4; F, M. Arrows and asterisks indicate epidermis and dermis respectively

Figure 2.png

Optimizing inhaled nanomedicines through the use of metallic nanoparticles

Monday, 15th January - 17:24: Nanomedicine for topical delivery (Room 608) - Oral - Abstract ID: 192

Dr. Susana Carregal Romero [1], ***Ms. Marina Piñol-Cancer*** [2], ***Ms. Laura Fernández-Méndez*** [2], ***Dr. Hugo Groult*** [3], ***Ms. Ainhize Urkola-Arsuaga*** [4], ***Prof. Jesús Ruíz-Cabello*** [1]

1. *1CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), Donostia, Spain 2CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain 3Ikerbasque, Basque Foundation for Science, Bilbao, Spain,* ***2.*** *1CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), Donostia, Spain 2CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain 4Euskal Herriko Unibertsitatea, Donostia, Spain,* ***3.*** *5Biotechnologies et Chimie des Bioressources pour la Santé, Littoral Environment et Sociétés (LIENSs Laboratory), UMR CNRS 7266, La Rochelle, France,* ***4.*** *1CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), Donostia, Spain*

Introduction

Pulmonary administration of nanomedicines, including viral vaccines, presents a promising delivery route due to its direct, noninvasive nature, catering to both systemic delivery and lung targeting. Despite its potential, challenges arise from the same defense mechanisms that safeguard against inhaled pathogens, often resulting in rapid elimination or degradation of nanomedicines. This dilemma is reflected in the limited approval of inhalation drugs, starkly contrasting the rising incidence of lung diseases in an aging population and new infectious threats. Given the direct lung targeting advantage of pulmonary administration, addressing these challenges is essential to meet unmet clinical needs.

Methods

We employ diverse bottom-up synthetic protocols to produce iron oxide nanoparticles, suitable for magnetic resonance imaging (MRI) or bimodal positron emission tomography (PET)/MRI imaging (1). These nanoparticles are then encapsulated or incorporated into drug carriers based on lipids or proteins (2). Subsequently, we investigate nano-bio interactions between these multimodal drug carriers and various lung barriers using techniques such as proteomics and molecular imaging.

Results

Our findings illustrate the utility of magnetic separation in studying the lung surfactant corona formation during pulmonary administration of nanoparticles developed by Raesch et al (3). Additionally, our results demonstrate the potential of molecular imaging for analyzing lung retention time, lung penetration, and cell uptake.

Discussion

We offer examples of multifunctional nanoparticles, proficient in drug encapsulation and labeling with contrast agents, which serve as essential tools for characterizing these interactions across multiple scales. We highlight how different coating agents influence lung retention time, alveolar macrophage clearance, and lung penetration, thereby impacting the potential efficacy of nanomedicines.

References

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2.- Carregal-Romero et al. Biomaterials Advances, 2022, 134, 112551

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Acknowledgments

S.C.R. acknowledges MCIN/AEI/10.13039/501100011033 for the grant PID2019-106139RA-100, the Ramon y Cajal Grant RYC2020-030241-I, and Ramón Areces Foundation for the grant CIVP21S13151. J.R.C.is funded by MCIN/AEI/10.13039/501100011033 (PID2021-123238OB-I00) and by La Caixa Foundation (HealthResearchCall 2020:HR20-00075). We appreciate the research support of the Spanish Foundation against Pulmonary Hypertension and the Basque Government for the R&D Project in Health (grant number 2022333041).

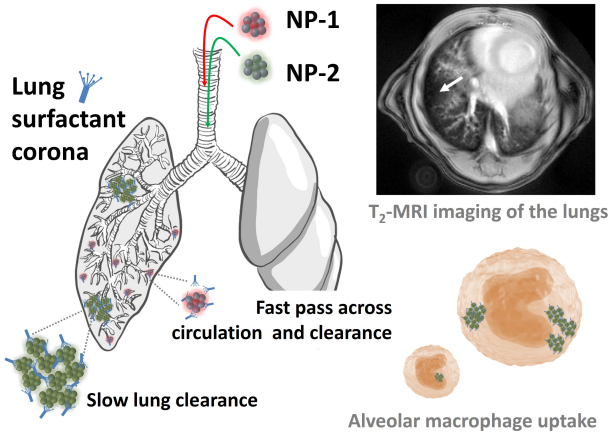

Figure 1. Schematic representation of the pulmonary administration of nanoparticles with different coatings and their fate in the lungs.

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New plant-based nanovesicles based on alkyl polyglucosides surfactants and β-sitosterol as topical drug delivery systems

Monday, 15th January - 17:41: Nanomedicine for topical delivery (Room 608) - Oral - Abstract ID: 249

Ms. Marta Alcaina Hernando [1], ***Dr. Ivana Malvacio*** [2], ***Ms. Ilaria Ferraboschi*** [3], ***Dr. Cristian Huck Iriart*** [4], ***Dr. Annalisa Bianchera*** [5], ***Dr. Santi Sala*** [2], ***Prof. Jan Skov Pedersen*** [6], ***Dr. Lidia Ferrer-Tasies*** [2], ***Prof. Silvia Pescina*** [5], ***Prof. Cristina Sissa*** [3], ***Prof. Nora Ventosa*** [7], ***Dr. Alba Cordoba*** [2]

1. *Nanomol Technologies S.L., Institut de Ciència dels Materials de Barcelona (ICMAB-CSIC), Centro de Investigación Biomédica en Red-Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN),* ***2.*** *Nanomol Technologies S.L.,* ***3.*** *Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, University of Parma,* ***4.*** *Alba Synchrotron Light Source,* ***5.*** *ADDRes Lab, Department of Food and Drug, University of Parma,* ***6.*** *Department of Chemistry and Interdisciplinary Nanoscience Center (iNANO) Aarhus University,* ***7.*** *Institut de Ciència dels Materials de Barcelona (ICMAB-CSIC), Centro de Investigación Biomédica en Red-Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)*

Most of the delivery systems (DSs) used in healthcare are not suitable for vegan consumers or patients, so there is a need for new DSs. We present a new platform of nanovesicles (NVs) formed by self-assembly of β-Sitosterol (Sit) and alkyl polyglucosides, biodegradable and biocompatible components from vegetal origin, that can integrate a large variety of hydrophobic and hydrophilic payloads and deliver them topically.

NVs were formulated by combining Sit and alkyl polyglucosides using DELOS technology, a sustainable production method based on compressed fluids [1]. Physicochemical properties were studied, size, PdI, surface charge (DLS-ELS), morphology by CryoTEM, SAXS, and stability. Skin compatibility was evaluated using reconstructed epidermis models. The capacity to integrate α-Tocopherol (TCP) in the NVs and its activity was evaluated by DPPH assay. Skin penetration in porcine ear explants was determined with Two-photon microscopy.

For the first time, NVs have been produced using Sit and Lauryl Glucoside (LGL) (1:1) as membrane components (size 217±11 nm, PdI 0.22±0.01,) [2]. The addition of negatively charged surfactants, such as Lauryl Glucose Carboxylate (LGC), enabled tuning the surface charge of the NVs. NVs composed by Sit:LGL:LGC (size 182±9 nm, PdI 0.22±0.01) were used to test their loading capacity with several active molecules such as TCP. NVs loaded with TCP were successfully produced presenting similar physicochemical properties (size 131±4 nm, PdI 0.19±0.01) and maintaining the antioxidant capacity of TCP after encapsulation. SAXS analysis demonstrates differences in lamellarity depending on the NVs composition. *In vitro* assays with reconstructed human epidermis showed that the novel delivery platform is compatible with the skin and non-irritant. Finally, skin retention studies demonstrate that the NVs seem to penetrate down to the epidermis.

To conclude, a new drug delivery platform has been developed, using plant-derived ingredients that self-assemble in stable NVs [2]. This novel delivery platform is biocompatible with the skin and can help retain loaded actives in the epidermis, important characteristics for topical delivery applications. Thus, it will cover an unmet need for vegan patients and consumers.

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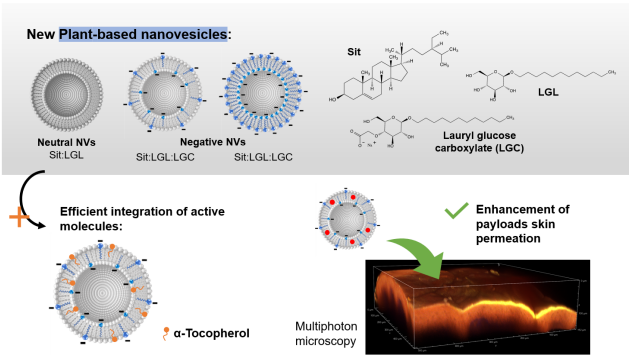


Abstract.png

Microneedle skin patches to improve diagnosis of skin cancer patients in primary care

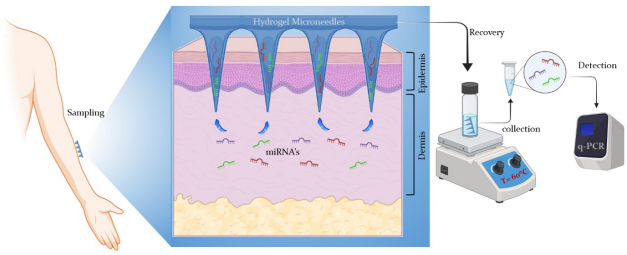
Monday, 15th January - 17:58: Nanomedicine for topical delivery (Room 608) - Oral - Abstract ID: 82

Dr. Ahmad Kenaan [1], ***Dr. Oliver Teenan*** [1], ***Mr. Connor Daniels*** [1], ***Dr. Claire Higgins*** [1], ***Dr. Sylvain Ladame*** [1]

1. *Imperial College London, Department of Bioengineering*

Skin cancer diagnosis is most commonly derived from visual or digital inspection of a skin lesion by a trained professional, ideally including dermoscopy. Identification of suspicious skin lesions in primary care is typically followed by an urgent referral, leading to a skin biopsy and histopathological examination, in suspicious cases. Whilst primary care clinicians are generally very accurate at identifying melanoma skin lesions that require intervention, only 3% of patients sent on the urgent referral pathway end up being diagnosed in secondary care with melanoma. This means that a large volume of patients is subjected to unnecessary invasive procedures (tissue biopsies) resulting in unnecessary morbidity and unnecessary stress. A technology that could help general practitioners (GPs) in primary care more effectively screen out patients who do not require dermatological assessment and biopsy would allow significant savings from the £35M currently spent on unnecessary diagnostic procedures every year in the UK for suspected skin cancers.

Our solution is to (1) identify and validate, within skin interstitial fluid (IF), highly specific and highly sensitive molecular biomarkers for melanoma (and other types of skin cancer); (2) develop new technologies capable of detecting these new biomarkers that could be used by care providers in addition to visual inspection, dermoscopy and digital photographic assessment (with or without artificial intelligence augmentation) to improve the quality of referral. We will be presenting how microneedle patches made of bespoke, highly swellable hydrogels can be engineered to interrogate skin IF in a painless and non-invasive manner. We will also demonstrate their translational potential to diagnose skin cancers, including melanoma, with high sensitivity and specificity based on the detection of cancer-specific microRNA biomarkers. We will be demonstrating how sampling IF proximal to the lesion presents a highly promising strategy to achieve greater levels of microRNA deregulation, thus enabling easier and more accurate diagnosis with unprecedented spatiotemporal resolution.



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DEVELOPMENT OF INNOVATIVE DRUG-ELUTING MULTILAYER ELECTROSPUN NANOSTRUCTURED PATCHES FOR THE ORAL MUCOSA

Monday, 15th January - 18:15: Nanomedicine for topical delivery (Room 608) - Oral - Abstract ID: 26

Prof. Jose M. Lagaron [1]

1. *CSIC*

A large proportion of new drug candidates present poor water solubility, whilst others also present a strong first-pass effect, limiting their use in potential medical therapies. One of the promising disruptive nanotechnologies that can improve the bioavailability of active pharmaceutical ingredients (APIs) is the electrospinning processing technique[1,2]. This technology can be used to generate amorphous dispersions or solutions of an API within a polymer matrix. For the work presented here, novel mucoadhesive patches based on electrospun multilayers are shown as effective drug release platforms. Different poorly soluble drugs were selected, i.e. the antibiotic Ciprofloxacin (CPX) and the non-cardio selective beta-receptor inhibitor Carvedilol (CVD).

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[2]Teno, J.; Pardo-Figuerez, M.; Figueroa-Lopez, K.J.; Prieto, C.; Lagaron, J.M. Development of Multilayer Ciprofloxacin Hydrochloride Electrospun Patches for Buccal Drug Delivery. J. Funct. Biomater. 2022, 13, 170.

Sensitizing P-selectin-expressing brain malignancies to immune checkpoint modulators.

Tuesday, 16th January - 09:00: Plenary Session (Auditorium) - Oral - Abstract ID: 338

Prof. Ronit Satchi-Fainaro [1]

1. *Tel Aviv University*

TBD

(SUPER-) SELECTIVE BIOMATERIALS: A BALANCING ACT OF RIGIDITY AND GEOMETRY AT THE NANOSCALE

Tuesday, 16th January - 09:40: Plenary Session (Auditorium) - Oral - Abstract ID: 332

Prof. Maartje Bastings [1]

1. *EPFL*

Biomaterials have catalyzed a profound transformation of our lives over the last century. Following decades of scientific innovations, biomaterials have become smaller, yet their function increasingly complex. Where macroscopic materials interact with many cells at once, nanomaterials interact only with a fraction of the surface of a single cell. Consequently, where (*specificity*), when (*selectivity*), with how many (*multivalency*) and how strong (*affinity*) these interactions take place, becomes key in determining function. Engineering (super-) selective materials is particularly relevant to advance drug delivery, diagnostics and personalized medicine.
In the Programmable Biomaterials Laboratory (PBL), we are intrigued by the multivalency (strength in numbers) principle and wondered if the selectivity between materials and the bio-interface could be controlled via the engineering of tailored multivalent surfaces. We explored how structural *rigidity vs flexibility* and uniform control over *nano-geometry* interplay toward multivalent selectivity, using DNA as programmable engineering tool. These DNA-based supramolecular macromolecules self-organize into functional nanomaterials through natural base-pairing interactions, providing the advantage that all nanomaterials are exact molecular copies. This allows to explore a new parameter space toward the engineering of selectivity, compared to what is accessible for classic nanomaterials (e.g. polymers, lipids, composites, metals), where properties as size, shape, number of functional sites, and spacing of molecules are inherently an average of the ensemble.
In this talk, I will show that molecular flexibility at interfaces comes with a cost in selectivity and demonstrate that the selectivity of immune-modulating biomaterials can be controlled via mechanical and structural design. Excitingly, we achieved *super-selective* interactions when functional binding units were spatially and geometrically constrained, a new concept we defined as "*Multivalent Pattern Recognition* (MPR)". I will show how geometric patterns play a central role in cellular communication and how materials can truly become cell-type selective when tailoring molecular patterns of interface molecules on DNA-based nanomaterials.

Designing nanomaterials for advanced therapeutics and ultrasensitive biosensing.

Tuesday, 16th January - 10:45: Plenary Session (Auditorium) - Oral - Abstract ID: 340

Prof. Molly Stevens [1]

1. *University of Oxford*

This talk will provide an overview of our recent developments in bioinspired materials for applications in advanced therapeutics and biosensing with focus on establishing translational pipelines to bring our innovations to the clinic [1]. Our group has developed fabrication methods to engineer complex 3D architectures that mimic anisotropic and multiscale tissue structures and generate spatially arranged bioinstructive biochemical cues [2]. I will discuss recent advances in our tunable nanoneedle arrays for multiplexed intracellular biosensing at sub-cellular resolution and modulation of biological processes [3]. We are developing creative solutions for targeted and controlled delivery using microrobots with unique bioinspired characteristics that respond to external stimuli to release a payload [4]. Our therapeutic delivery portfolio includes high molecular weight polymer carriers for enhanced delivery of saRNA therapeutics and photo-responsive nanoreactors inspired in the circadian rhythms [5]. We are exploiting the sensing capabilities of functionalised nanoparticles to engineer nanoprobes for in vivo disease diagnostics that produce a colorimetric response ideal for naked eye read-out and for CRISPR-based preamplification free detection of ncRNAs (CrisprZyme) which we have validated with cardiovascular disease patient samples [6]. I will present advances in Raman spectroscopy for high-throughput label-free characterization of single nanoparticles (SPARTA™) that allow us to integrally analyse a broad range bio-nanomaterials without any modification enabling exciting biosensing applications using extracellular vesicles as disease biomarkers, a growing area of interest in cardiovascular medicine [7]. Finally, I will explore how these versatile technologies can be applied to transformative biomedical innovations and will discuss our efforts in establishing effective translational pipelines to drive our innovations to clinical application while actively engaging in efforts towards the democratisation of healthcare [8].

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Chemically powered nanobots: swimming nanoparticles for biomedicine

Tuesday, 16th January - 11:25: Plenary Session (Auditorium) - Oral - Abstract ID: 343

Prof. Samuel Sánchez [1]

1. *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Catalan Institute for Research and Advanced Studies (ICREA)*

One of the dreams in nanotechnology is to engineer small vehicles and machines, called here nanobots, which can eventually be applied in vivo for medical purposes. Yet, reaching that fascinating goal is not a trivial thing and several challenges need to be addressed. First, researchers need to incorporate efficient but also bio-friendly propulsion mechanisms into the nanobots. Our strategy comprises the use of biocatalysts such enzymes for converting biologically available fuels into a propulsive force. Secondly, nanoparticles' chassis should be generally recognized as safe (GRAS) material, biocompatible and/or biodegradable.

In my talk, I will present how we bioengineer hybrid nanobots combining the best from the two worlds: biology (enzymes) and (nano)technology (nano- micro-particles) providing swimming capabilities, biocompatibility, imaging, multifunctionality and actuation.

Besides the understanding of fundamental aspects (1), and controlling the performance of micro-nanobots (2) I will present some of the proof-of-concept applications of biocompatible nanobots such as the efficient transport of drugs into cancer cells (3) and 3D spheroids (4), sensing capabilities (5), anti-bactericidal applications (6) and the use of molecular imaging techniques like PET-CT (7) or Photoacoustic (8) for the tracking and localization of swarms of nanobots both in vitro and in vivo in confined spaces like mice bladder. Moreover, I will present our recent advances in the treatment of bladder cancer in mice using radionuclide-labelled nanobots (9).

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Journey through the Gut: Nanoparticle dynamics in the healthy and diseased state of the gastrointestinal tract

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 35

Mrs. Yael del Carmen Suarez Lopez [1], ***Mrs. Qianying Chen*** [1], ***Dr. Alexandra Teleki*** [1]

1. *Department of Pharmacy, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

Introduction

The gastrointestinal tract (GIT) is a complex system that poses a challenge to the oral delivery of nanoparticles. Current techniques for investigating the delivery of pharmaceuticals through the GIT have been mostly optimized for studying drug compounds' solubility and stability rather than the dynamic behavior of nanoparticles. Moreover, the impact of GIT pathologies, like inflammatory bowel disease (IBD), on nanoparticle properties after oral administration is poorly understood. To address these issues, this work aims to develop a microfluidic device simulating the GIT in a healthy and IBD state, where simulated fluids can be mixed with nanoparticles to investigate how media composition affects the aggregation and protein corona formation of nanoparticles at the different stages of the GIT.

Methods

Superparamagnetic iron oxide nanoparticles (SPION) were synthesized by flame spray pyrolysis and coated with a silica layer in a single step. They were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). Four commercially available micromixers were tested for their efficiency in creating a uniform mixture. The relative mixing index (RMI) was used to determine the best-performing micromixer.

Media simulating a broad range of inflammatory conditions in IBD were developed. The micromixer with the highest RMI was utilized to study how pH, protein content, bile acid, and lecithin concentrations of healthy and IBD-simulating media affect nanoparticle aggregation, zeta-potential, and protein corona formation. These GI conditions were systematically varied using a design-of-experiments approach. The aggregation and zeta-potential of nanoparticles were characterized by dynamic light scattering and electrophoretic light scattering respectively, while the protein corona was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Results and Discussion

This work presents advances in developing a microfluidic device aiming to investigate nanoparticle dynamics in the GIT. The SPION showed a maghemite crystalline phase, and the silica coating was confirmed by TEM and FTIR (Figure 1). The micromixer with the best mixing efficiency (Figure 2, RMI >96%) was used to study the effects of the media composition on nanoparticle aggregation behavior and protein corona formation in each part (stomach, small intestine, and colon) of the GIT in a healthy or diseased state.

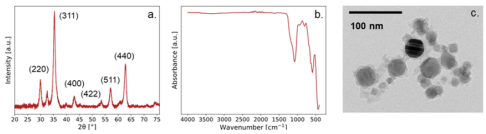

Figure 1. XRD pattern (a), FTIR spectrum (b), and TEM image (c) of flame-made SiO_2-coated γ-Fe_2O_3 nanoparticles.

Figure 1 - xrd ftir and tem of spion.png

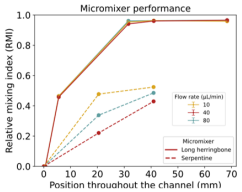

Figure 2. Relative mixing index (RMI) of the long herringbone (solid line) and the serpentine (dashed line) micromixers at different positions throughout the microfluidic channels. The Long herringbone micromixer had an RMI > 96% at the end of the channel at all flow rates tested, while the serpentine micromixer had a mixing efficiency < 60%. Therefore, the long herringbone micromixer was selected for further experiments.

Figure 2 - rmi of best and worst performing micromixers.png

Examining the interaction between thiolate protected metal clusters and lipid bilayers

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 36

Ms. Merve Örer [1], ***Dr. Plinio Maroni*** [1], ***Prof. Thomas Bürgi*** [1]

1. *University of Geneva*

The understanding of the interactions of monolayer protected metal clusters with biological membranes is of fundamental importance for applications of these systems in medicine and for biosensing. At the same time, using metal clusters imposes some restrictions such as toxicity[1] and undesirable immunological responses. In fact, the chemical properties and biological effects of monolayer protected metal clusters can critically depend on the size of the clusters, the protecting ligand layer and chirality. Therefore, different kinds of coatings are used to reduce the negative impacts of metal clusters and increase the beneficial interaction with biological systems. Although thiol groups play an important role for biological systems and have been used as a ligand for the preparation of metal clusters in many areas, the effect of thiolate protected metal clusters on the cell membrane is not well understood.

In our study, lipid bilayers are used as a model of the cell membrane. Different methods have been reported to prepare lipid bilayers[2]. We use several methods to prepare lipid bilayers on solid substrates including Langmuir Blodgett/Schaefer combination and vesicle fusion. We study the interaction between different types of thiolate protected metal clusters and bilayer membranes, by atomic force microscopy (AFM), ellipsometry, quartz crystal microbalance (QCM) and attenuated total reflection infrared (ATR-IR) spectroscopy in a microfluidic and temperature-controlled flow-through cell. We furthermore make use of polarized light to study the orientation of the phospholipid bilayer and their order in presence and absence of metal clusters.

Our research findings show that the use of the Langmuir Blodgett method is beneficial for the preparation of a monolayer of phospholipids, but the use of combinations of methods for the preparation of the bilayer is not appropriate. On the other hand, the vesicle fusion method resulted in high quality phospholipid bilayers. ATR-IR spectroscopy proved to be a potential tool to study the interaction between the monolayer protected metal clusters and the phospholipid bilayers providing detailed molecular-level information.

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Improving Risk Stratification of Preterm Birth via a Screening Blood Test at First Antenatal Appointment

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 123

Mr. Marc Soler [1], ***Dr. Sylvain Ladame*** [1]

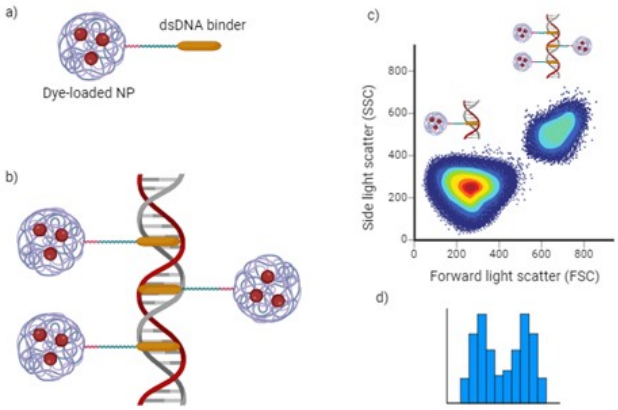
1. *Imperial College London, Department of Bioengineering*

Introduction: Preterm birth (PTB) is a significant global health concern accounting for 1 in 10 births worldwide and is the largest cause of death in children under five. The majority of PTBs occur in women with no identifiable risk factors and in their first pregnancies. Existing predictive tests demonstrate limited predicted values, making them unsuitable for early prediction, effective patient stratification and timely intervention. Few studies have highlighted the relationship between PTB and abnormal foetal fraction (FF) of cell-free DNA (cfDNA) in maternal blood during the first trimester of pregnancy.

Methods: CfDNA sensing was based on the sequence-independent binding of small molecules conjugated to fluorescent nanoparticles (NPs) to double-stranded DNA (dsDNA) via either intercalation between base pairs of covalent crosslinking. The system was designed so that, upon binding to dsDNA, fluorescent DNA-NPs complexes are formed that can be detected with high-throughput by flow cytometry whilst also providing information on cfDNA fragment size.

Results: Progress so far has been made on developing an innovative NP-based nanotechnology for sequence-independent sizing of cfDNA fragments through screening various types of dsDNA binding ligands and fluorescent NPs. Proof-of-concept sensing studies using both flow cytometry and a benchtop cell-counter demonstrated the ability of our NP probe to detect dsDNA fragments as small as 150 base pairs (bp).

Conclusion: CfDNA fragments of the size found in bodily fluids were successfully detected in vitro with high sensitivity using bespoke NP probes. Further work is underway to demonstrate the potential of our nanotechnology for cfDNA size profiling and for the cfDNA detection directly from unprocessed bodily fluids. If successful, this could pave the way to a novel Nucleic Acid Prenatal Test (NIPT) for improving patient stratification in the early stages of pregnancy.



Dsdna sensing scheme.jpg

Versatile Gallium-based Nanoparticles as Antibacterial Agents

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 193

Mr. Shengtao Yu [1], ***Prof. Georgios Sotiriou*** [1]

1. *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm*

Introduction

The demand for novel antimicrobial therapies arises from the increasing tolerance in bacterial infections and the declining antibacterial efficacy of existing antibiotics. Meanwhile, it is valuable to develop novel antimicrobial approaches that are independent from utilizing traditional antibiotics, such as targeting essential nutrients acquisition in bacterial pathogens[1].

Bacteria rely on ferric ions (Fe^{3+}) to maintain their metabolism and enzyme activity. Despite several chemical similarities between gallium ions (Ga^{3+}) and Fe^{3+}, Ga^{3+} cannot be reduced under physiological conditions and participate in redox reactions[2]. Consequently, the introduction of exogenous Ga^{3+} can disrupt bacterial metabolism and replication.

Method

Different sizes and gallium contents of gallium-based nanoparticles (GaNPs) were produced using flame spray pyrolysis (FSP) and comprehensively characterized to determine their physicochemical properties. Flame-made GaNPs were studied for their antibacterial activities through time-kill assays and enumerations of colony-forming units (CFU) to evaluate the effects of size and gallium content on antibacterial efficacy. Furthermore, an antibacterial peptide, LL-37, was loaded onto the GaNPs and assessed the loading efficiency through Pierce bicinchoninic acid (BCA) assays.

Results and discussion

Six distinct sizes of GaNPs were synthesized using FSP by controlling the flame conditions and precursor concentrations, validated by N_2 adsorption and TEM analysis. The specific surface area results exhibited a correlation with the precursor concentration within the flame. The synthesized GaNPs displayed effective inhibition of *Pseudomonas aeruginosa* growth, with MIC > 31 μg/mL. Additionally, the bactericidal potency was influenced by the size and gallium content of GaNPs, owing to release capacity of Ga^{3+}. Beyond their antimicrobial properties, GaNPs can act as drug carriers for delivering antimicrobial peptides. While a synergistic effect may potentially enhance their bactericidal efficacy, further investigation is warranted.

Conclusion

In summary, GaNPs were synthesized using FSP. These GaNPs exhibit dual functionality, serving both as potent antibacterial agents and as versatile drug nanocarriers for delivering antibacterial peptides. Further experiments will provide the necessary framework for their clinical translation.

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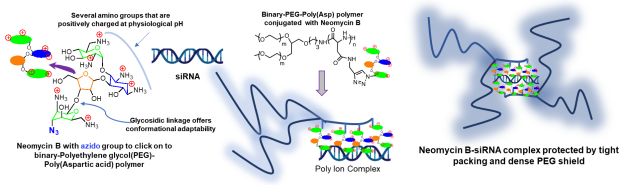
Utilizing the characteristic aminoglycoside-RNA binding for the preparation of a unit poly-ion complex-type siRNA delivery platform

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 197

Dr. West Kristian Paraiso [1], ***Dr. Sabina Quader*** [2], ***Dr. Kotaru Hayashi*** [2], ***Dr. Kazuko Toh*** [2], ***Mr. Shigeto Fukushima*** [2], ***Prof. Kazunori Kataoka*** [2]

1. Universitat Internacional de Catalunya, 2. Innovation Center of Nanomedicine

Even with its vast potential, small-interfering RNA (siRNA) therapeutics are faced with many impediments including instability in biological fluids as well as moderate cellular internalization and endosomal escape activities. Numerous lipid- and polymer-based nanocarriers were developed to address these issues, with the challenge of keeping the balance between stable complexation and endosomal escape. A pragmatic answer might lie within a natural RNA binder, such as neomycin B (Neo), a potent aminoglycoside antibiotic that is well-known for binding to various RNAs motifs. Neo contains six amines which are mostly ionized at physiological pH to facilitate electrostatic interaction with the anionic phosphates of RNAs. The perplexing versatility seen in Neo-RNA binding can be credited to its conformational flexibility arising from glycosidic bonds, an important part of Neo structure. In addition, the amino groups of Neo possess a wide range of pK_as (5.4 to 8.8) which supplement its ability to achieve this intricate balance between complexation and effectual endosomal escape of siRNA in the target cells. In this poster, we report the development of a novel Neo-derived unit poly-ion complex (uPIC), formed by electrostatic interactions between a single siRNA strand and two defined charge-regulated binary polyethylene glycol (PEG)-block-polycation copolymer chains wherein Neo operates as a cationic siRNA captor. This Neo-siRNA uPIC has a small size (around 20 nm) and presents excellent RNA binding and complexation, effective endosomal escape, and sustained blood circulation, suggesting the massive scope of utilizing Neo as a capable component of future PIC-type siRNA delivery platforms which can also be expanded to other nucleic acid-based therapeutics.



Abstract neo.png

On the Amyloid-β transcytosis across the blood-brain barrier

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 199

Mr. Marco Basile [1], ***Dr. Cátia Lopes*** [1], ***Mr. Peter Pfeifer*** [2], ***Mr. Jose Muñoz-López*** [1], ***Dr. Matilde Ghibaudi*** [1], ***Prof. Lorena Ruiz-Perez*** [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia*

Our group has discovered that the blood-brain barrier (BBB) plays a crucial role in regulating the transport of misfolded proteins to and from the Central Nervous System (CNS). We have found that the BBB controls the trafficking of macromolecular cargo by its affinity towards receptors like the low-density lipoprotein receptor-related protein 1 (LRP1) and the low-density lipoprotein receptor-related protein 8 (LRP8). LRP1 primarily transfers amyloid-β (Aβ) across the BBB, while LRP8 requires further studies. We have developed functionalised polymeric nanoparticles that mimic the in vivo process by having multiple ligand-receptor affinities to encourage this process. These investigations are essential in determining how polymeric nanoparticles can enhance the clearance of Aβ from the CNS, which could lead to the development of novel therapies.

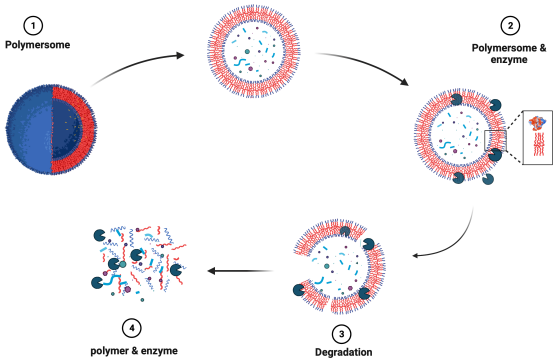
Enzymatic degradation of amphiphilic copolyester for biomedical applications

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 200

Mrs. Maryam Alghamdi [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Department of Chemistry, University College London,* ***2.*** *Institute for Bioengineering of Catalonia (IBEC)*

Amphiphilic block copolymer polymers can self-assemble into either polymersomes or micelles, which have been established for various biomedical applications, especially drug delivery. Using enzyme-responsive biodegradable polymersomes in drug delivery strategies can significantly reduce the side effects experienced during treatment and efficiently achieve drug delivery targets. Therefore, it is crucial to understand the enzymatic degradation mechanism of polymersomes. This study examines the enzymatic degradation mechanism of self-assembled amphiphilic copolyester under physiological conditions (pH=7.4 and T=37°C). Proteinase K and Pseudomonas cepacia Lipase were tested to determine the hydrolysis of amphiphilic copolyester using Dynamic Light Scattering (DLS), Gel Permeation Chromatography (GPC), and Transmission Electron Microscopy (TEM). These techniques were used to study the size, polydispersity, and molecular weight of self-assembled polymer structures during enzymatic degradation.



Degradation pro-3.png

Tuning the size, shape and pore structure of mesoporous silica nanoparticles

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 206

Ms. Marina Llenas Martínez [1], ***Ms. Marta Florensa Marquez*** [1], ***Mr. Ruben Garcia Fontarosa*** [1], ***Dr. Stefania Sandoval Rojano*** [1], ***Dr. Gerard Tobías Rossell*** [1]

1. *Institut de Ciència dels Materials de Barcelona (ICMAB-CSIC)*

Mesoporous silica nanoparticles (MSN) have been widely used in recent decades due to their unique properties, such as controllable size, shape and porosity, ordered pore structure, very high specific surface area and good chemical stability, making them attractive for a variety of applications like catalysis, separation processes or sensing. In addition, MSN also present important biocompatibility, high loading capacity and ease of functionalization making them gain a tremendous attention as drug delivery systems. As novel platforms for biomedical application, the control of their physicochemical properties is extremely important as they play a key role in the biodistribution and tissue accumulation of these nanoparticles. In the present work, MSN with different characteristics have been prepared by fine control of the synthesis parameters; namely, the precursors and their ratios, the amount of surfactant, the reaction time, the temperature or the agitation conditions, among others. The obtained nanoparticles have been characterized by complementary techniques, including electronic microscopy, spectroscopy, thermal analyses and adsorption studies. Particles with different size, morphology and pore size and structure have been obtained thus shedding light on how the selected synthetic approach and employed conditions modify the MSN properties, highly relevant for the targeted biomedical application [1].

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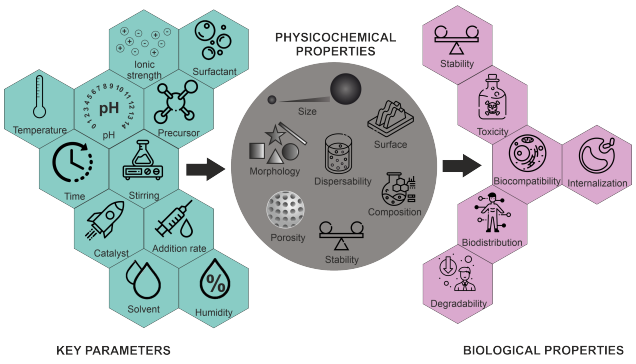

Key parameters.png

Design of phenotypic anti-inflammatory nanomedicines

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 212

Ms. Claudia Codano [1], ***Mr. Peter Pfeifer*** [2], ***Prof. lorena Ruiz*** [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia*

In recent decades, biodegradable synthetic polymers have been largely studied for many biomedical applications. Among these, poly(propylene fumarate) (PPF) has been investigated for its wide versatility of usage, from bone tissue engineering to drug delivery and regenerative medicine. PPF is a linear polyester characterised by biocompatibility and biodegradability. Upon hydrolysis of its ester linkages, the polymeric backbone releases propylene glycol and fumarate as degradation products in the cell milieu.

The challenge presented herein is to prove if such fumarate metabolites could mimic the anti-inflammatory properties of dimethyl fumarate (DMF), a drug already approved for the treatment of autoimmune disorders such as multiple sclerosis and psoriasis.

We designed a poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)-PPF copolymer that combines the anti-inflammatory features of fumarate derivatives with the superselective targeting properties of PMPC towards macrophages and dendritic cells, the most important actors in managing inflammation[1]. The self-assembling behaviour of PMPC-PPF copolymer into a supramolecular multivalent scaffold transposes the anti-inflammatory properties of DMF to the nanoscale, making the nanoparticle the nanodrug itself and physically driving its activity through phenotypic targeting.

PPF was synthesised by step-growth polymerisation of its diester intermediate, bis(hydroxypropyl) fumarate, to accomplish this goal in a 2-step synthesis. PPF was either functionalised with 4-cyanopentanoic acid dithiobenzoate (CPADB) for reversible addition-fragmentation chain-transfer (RAFT) polymerisation or with 2-Bromoisobutyl-bromide for atom transfer radical polymerisation (ATRP) of PMPC monomer, monitoring the polymerisation to achieve the desired length of PMPC chains. Each polymer product was analysed by gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR) to ensure its quality and conversion grade. After the copolymer characterisation, PMPC-PPF micelles were obtained by solvent-switch and characterised by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) to evaluate, respectively, their hydrodynamic diameter and morphology, aiming for a homogeneous population.

PMPC-PPF micelles were then tested on differentiated monocytes to evaluate their cytotoxicity and anti-inflammatory properties. Preliminary studies revealed a reduction in the expression of main pro-inflammatory cytokines both in pro-inflammatory and undifferentiated phenotypes, paving the way for further analysis to confirm the potentiality of this PPF-based copolymer.

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Designing Enzymatically-Powered PLGA Nanobots and Exploring their Swarming Behavior

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 253

Mr. Carles Prado [1], ***Dr. Juan Fraire*** [2], ***Dr. Maria Crespo*** [1], ***Ms. kristin fichna*** [1], ***Prof. Samuel Sánchez*** [3]

1. *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST),* ***2.*** *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST),* ***3.*** *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Catalan Institute for Research and Advanced Studies (ICREA)*

Nanobots have been widely investigated as the next generation of vehicles for drug delivery. Active motion, and especially their collective behavior (swarming), have shown an enormous advantage in terms of movement in complex medias, overcoming biological barriers, drug delivery and tumor penetration. Not only that, but also in vivo therapeutics outcomes have been observed. Nevertheless, there is a general concern about the composition and simplicity of the different designs used, which may hinder their clinical applications. There is still the need to develop a simple nanobot based on organic materials which would be more appealing for industry and clinicians. Here, we present and compare the synthesis and characterization of two new urease-nanobots designs. Both are based on an organic biocompatible and biodegradable chassis of poly(lactic-co-glycolic acid), PLGA (FDA approved material and already used in clinics). For their functionalization with urease, amine groups must be incorporated in the chassis surface. As PLGA is negatively charged, two different strategies have been developed. In one case, the core is combined with Chitosan (during synthesis), a natural positive polymer used as a dietary supplement. On the other hand, polyethylenimine (PEI) is used for providing a positive layer around the PLGA core (after synthesis). Moreover, the versatility of the chassis synthesis allowed us to encapsulate with high-efficiency hydrophobic (oil in water emulsion, OW) and hydrophilic (water in oil in water emulsion, WOW) standard drugs.

Studying its collective behavior, it was demonstrated how in the presence of the fuel (urea), nanobots experiment a sudden expansion that allows them to explore and reach further areas, if compared with passive controls. In parallel, we have seen how an oil surface acts as a barrier for nanobots. However, in the presence of the fuel, their catalytic reaction provokes the mixing of the oil/aqueous interface, displacing upwards the remaining oil phase. That allows the nanobots to cross this barrier. This phenomenon could be explored for skin applications, where there is a protective oil limiting nanoparticles crossing.

High-Z nanoparticles for enhanced radiation therapy

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 256

Mr. Ruben Garcia Fontarosa [1], ***Ms. Marina Llenas Martínez*** [1], ***Dr. Esperanza Medina-Gutiérrez*** [1], ***Dr. Stefania Sandoval Rojano*** [1], ***Mr. Manuel Altabas González*** [2], ***Dr. Xavier Maldonado*** [2], ***Dr. Jordi Giralt*** [2], ***Dr. Gerard Tobías Rossell*** [1]

1. *Institut de Ciència dels Materials de Barcelona (ICMAB-CSIC),* ***2.*** *Vall d'Hebron Institut d'Oncologia (VHIO)*

Cancer is one of the main causes of death worldwide [1]. Once cancer has been diagnosed, radiation therapy is a commonly employed treatment, along with chemotherapy and surgery. However, reduction of the side effects caused by radiation is one of the main challenges that still lies ahead.

Gold nanoparticles (AuNPs) are attracting wide interest for their use as radioenhancers in external radiation therapy. Their high atomic number (Z) increases the radiation damage to cancer cells, thus allowing the reduction of the administered dose [2]. AuNPs can have specific size and shape, low toxicity, good biocompatibility, longer half-life than drugs in the systemic circulation and favourable accumulation in solid tumours due to the enhanced permeability and retention effect (EPR), making them attractive candidates for clinical use.

The enhancement of the radiotherapeutic dose is highly dependent on the particle size. Small AuNPs are more effective than large AuNPs, but small AuNPs have demonstrated cytotoxicity and are usually rapidly cleared from the blood compared with large AuNPs [3]. Therefore, a rigorous control of this parameter becomes a key factor to obtain efficient nanostructures potentially useful for cancer therapy.

The present work entails the synthesis of AuNPs with different physico-chemical properties and the subsequent evaluation *in vitro*. Their cytotoxicity, internalisation and radiotherapeutic effect are determined in a cancer cell line, thus evaluating their potential interest in the oncological field as radioenhancers.

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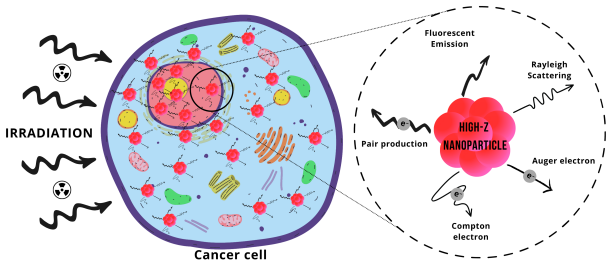

Irradiation of cancer cells with high-z nps.png

RNase enzyme functionalized Vaults for the treatment of Pancreatic cancer

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 258

Mr. Pablo Caruana [1], ***Mr. Roger Fernandez Palomeras*** [2], ***Dr. Jessica Castro*** [3], ***Ms. Maria Edwards*** [1], ***Dr. Josep Balart*** [1], ***Mr. Francisco Rodriguez Lozano*** [1], ***Dr. Antoni Benito*** [3], ***Dr. Jose Luis Corchero*** [4], ***Dr. Maria Virtudes Céspedes*** [1]

1. *Biomedical Research Institute Sant Pau (IIB Sant Pau),* ***2.*** *Institut de Biotecnologia i de Biomedicina and CIBER-BBN,* ***3.*** *Laboratori d'Enginyeria de Proteïnes, Departament de Biologia, Universitat de Girona,* ***4.*** *Institut de Biotecnologia i de Biomedicina and CIBER-BBN, Universitat Autònoma de Barcelona*

Pancreatic cancer is a highly lethal and difficult-to-cure disease. Despite treatments including surgery, chemotherapy, radiotherapy and targeted therapies, the five-year survival rate remains critically low.Applying nanoparticles in drug delivery is a promising approach to enhance the efficacy of treatments and minimize side effects. One of these avenues involves leveraging naturally occurring self-assembling protein nanoparticles named vaults. Vaults hold significant potential due to their high drug-loading capacity, stability, and lack of immunogenicity.

Here, we introduced a novel approach and protocols to produce recombinant vaults in human cells through transient gene expression of a His-tagged variant of the major vault protein (MVP-H6). We developed an innovative affinity-based purification technique tailored for these recombinant vaults. This approach simultaneously addresses the one-step biofabrication and encapsulation of a cargo recombinant protein within these vaults through their co-expression in human cells providing a streamlined and efficient means to biofabricate and purify engineered vaults loaded with virtually any INT-tagged cargo protein. Altogether, it significantly reduces production time, offering a faster and simpler strategy to engineer and produce more effective drug delivery systems.

Internalization assays in Panc-1 pancreatic cells, using flow cytometry, showed an increased internalization (5-fold) after 2h of exposure at 10nM nanoparticles conjugated with a tumor penetrating peptide (TPP) ligand in comparison to unconjugated nanoparticles. In addition, nanoparticles functionalized with TPP and loaded with the cytotoxic RNase enzyme TNR-INT (TPP-TNR-INT) demonstrated a cytotoxic effect (57%) in Panc-1 cells at 5 nM for 24h. Conversely, the non-loaded nanoparticles have no effect on pancreatic cells under the same conditions. In in vivo experiments, the biodistribution analysis after intravenous administration of 400 ug TPP-TNR-INT in Panc-1 xenografted mice demonstrated tumor uptake at 5h and a well-tolerated performance without inducing pathological effects.

Conclusions: -1. Protocols proposed here allow the easy and straightforward biofabrication and purification of engineered vaults loaded with virtually any INT-tagged cargo protein. 2. RNase enzyme functionalized vaults displayed cytotoxic activity against pancreatic tumor cells and they are well-tolerated after iv administration, regarding tumor tissue uptake in Panc-1 pancreatic xenograft model.

Funding: PI20/00770 grant to JLC, and PI20/00623 to MVC (ISCIII); IDEAS18038BENI (AECC) and PID2021-126941OB-I00 (MCIU) to AB.

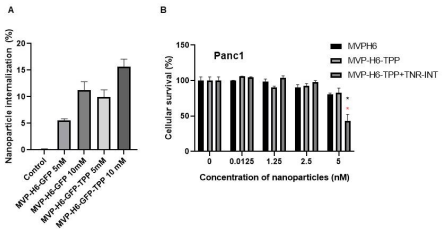

Fig 1. Nanoparticle internalization and cytotoxicity in panc-1 cell line. **A)** Percentage of cells that internalized the nanoparticle at given doses. **B)** Percentage of viability cells relative to control 48h after administration of nanoparticles at given doses.

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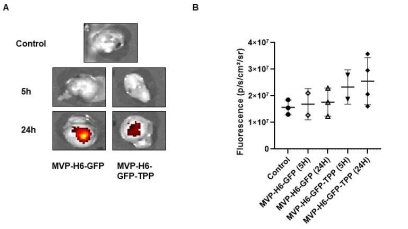

Fig 2. Nanoparticle biodistribution in panc-1 subcutaneous mouse model. Nanoparticles were loaded with GFP to measure FLI. **A)** Image of the tumors 5 and 24h after intravenous injection of nanoparticles (400 µg) using In Vivo Imaging Software. **B)** Graphical representation of the fluorescence of panc-1 subcutaneous tumors 5 and 24h after intravenous injection of nanoparticles (400 µg)

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Development of a proteoliposome-based therapy for Chronic Granulomatous Disease

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 281

Ms. Perrine Rochas [1], ***Ms. Isabelle Petit-Hartlein*** [1], ***Mr. Sylvain Beaumel*** [2], ***Ms. Bénédicte Vigne*** [2], ***Dr. Michel Thépaut*** [1], ***Prof. Franck Fieschi*** [3], ***Dr. Marie José Stasia*** [1]

1. *Université Grenoble Alpes, CNRS, CEA, UMR5075, Institut de Biologie Structurale, Grenoble, France,* ***2.*** *Centre Hospitalier Universitaire Grenoble Alpes, Pôle Biologie, CDiReC, Grenoble, France,* ***3.*** *Institut Universitaire de France (IUF), Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation, Paris, France*

Introduction:

Chronic Granulomatous Disease (CGD) is a rare innate immunodeficiency syndrome mainly diagnosed in children facing recurrent severe infections. This disease is caused by defective activity of phagocytes' NADPH oxidase complex that normally produces microbicidal superoxide anions (ROS) during infection. This enzyme is composed of a redox element formed by two membrane proteins NOX2 and $p22^{phox}$, and by cytosolic subunits $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$. The main form of CGD is caused by genetic mutations in the CYBB gene encoding NOX2 (X-CGD)[1]. Absence of NOX2/$p22^{phox}$ expression in X-CGD phagocytes leads to life-threatening infections, often in the lungs.

Prophylaxis using antibiotic drugs is the main treatment for CGD patients. Curative therapies such as allogeneic bone marrow transplantation or gene therapy can be performed but are not adapted to emergency situations. Acute lung infections often become refractory to conventional antibiotic medication. Thus, the only alternative is transfusion of donor granulocytes. However, this remains difficult to organize in emergency situations and compromises the success of future bone marrow transplantation because of increased graft-versus-host disease risks.

Our project is to make a proof of concept of the efficacy of a local protein-based emergency therapy using proteoliposomes in experimental X-CGD cell models. The future will be to evaluate this approach in an acute lung infection X-CGD mouse model, the essential step to consider an application in humans.

Methods:

NOX2/$p22^{phox}$ membrane subunits are overexpressed in HEK cells using lentiviral transduction[2], purified by affinity chromatography and incorporated into proteoliposomes (NOX2/$p22^{phox}$ PLs). Membrane incorporation of NOX2/$p22^{phox}$ PLs and restoration of NADPH oxidase activity are analyzed by flow cytometry and confocal microscopy using X-CGD cell models[3]. Cytotoxicity of PLs treatment on the cells is checked using the MTT test.

Results:

We succeeded to produce 850µg purified NOX2/$p22^{phox}$ per 10^{10} HEK cells. When incorporated into liposomes, the NOX2/$p22^{phox}$ heterodimer can produce ROS *in vitro*. NOX2/$p22^{phox}$ PLs are capable of delivering NOX2/$p22^{phox}$ to the plasma membrane when incubated with HEK cells. Restoration of NADPH oxidase activity of X-CGD cell models by NOX2/$p22^{phox}$ PLs is under investigation.

References:

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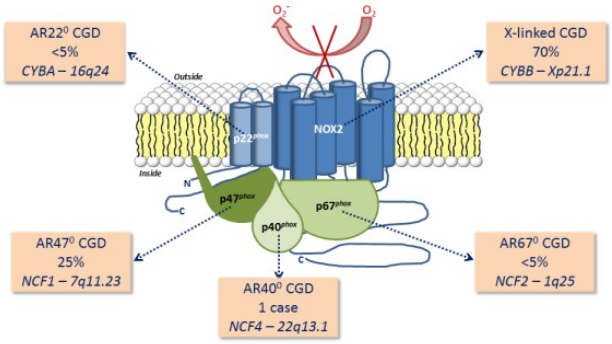
[2]Elegheert *et al.*, 2018, doi:10.1038/s41596-018-0075-9

[3]Brault *et al.*, 2017, doi:10.2147/IJN.S128611



Classification of cgd forms related to mutations of the different membrane and cytosolic subunits of the phagocyte nadph oxidase complex.jpg

PSar-VitE compounds as non-immunogenic alternative to PEG

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 283

Mr. Rafael Miravet-Martí [1], ***Ms. Laura Bano-Benito*** [1], ***Ms. Lucía Barahona*** [1], ***Dr. Teresa Pellicer*** [1], ***Dr. Daniel Morelló-Bolumar*** [1], ***Dr. Irene Dolz*** [1], ***Dr. Josep Garcia Garcia*** [1], ***Dr. Aroa Duro-Castaño*** [1], ***Dr. Vicent J. Nebot*** [1]

1. *Curapath S.L.*

TPGS is a non-ionic amphiphilic polymer composed by polyethylene glycol (PEG) and VitE derivative which was approved by the FDA as a save adjuvant for pharmaceutical purposes. Its characteristic physicochemical properties allow TPGS use in a wide variety of applications including its use as emulsifier, solubilizer for poor soluble drugs, and absorption and permeation enhancer [1].

Despite the widely use of TPGS, it has some disadvantages related to its hydrophilic part, PEG. PEG use is extended in drug delivery and nanotechnology therapies due to their stealth properties and biocompatibility resulting in prolonged circulation times in the body [2]. However, after a repeated inoculation PEG can cause an immunogenic response resulting in an increased blood clearance and reduced efficacy for the TPGS based therapies [3]. Furthermore, PEG is not biodegradable and its residues can be accumulated in the body.

To overcome PEG drawbacks described above, at Curapath we have developed a bioinspired alternative to TPGS, PSar-VitE, in which PEG is replaced by polysarcosine (PSar). PSar is a neutral and hydrophilic polypeptoid with similar physicochemical and stealth-like properties of those of PEG [4]. Moreover, PSar has been recently postulated as a non-immunogenic alternative to PEG [5].

In this work, PSar-VitE compounds have been tested as: (i) solubilizer of hydrophobic drugs, (ii) skin permeation enhancer and (iii) shielding lipid in LNP formulations and compared with those benchmark lipid-PEG compounds normally used for these purposes.

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Phenotypic targeting of the LRPs receptors

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 284

Dr. Matilde Ghibaudi [1], ***Dr. Virginia M Gouveia*** [2], ***Dr. Ned Hoyle*** [2], ***Dr. Denis Cecchin*** [2], ***Dr. Cátia Lopes*** [1], ***Ms. Claudia Codano*** [1], ***Mr. Marco Basile*** [1], ***Ms. Giulia Maria Porro*** [1], ***Dr. Valentino Barbieri*** [3], ***Mr. Alessandro Ronzoni*** [3], ***Mr. Xie Zhendong*** [3], ***Prof. Giuseppe Battaglia*** [4]

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The family of low-density lipoprotein receptor-related proteins (LRPs) is a crucial class of proteins that play a role in controlling trafficking and signalling in numerous physiological processes. They are particularly important in regulating the transport of macromolecules across the blood-brain barrier (BBB). The BBB is the interface between the brain and the systemic circulation, consisting of specialized endothelial cells, pericytes, and several brain cells. As the BBB is a critical element in the management of brain homeostasis, it is an important target for the development of numerous therapies.

To target cells based on their phenotypic expression of receptors, we have developed a new approach that uses the promiscuous ligand agiopep-2. This ligand is able to interact with several members of the LRPs family, including BBB markers such as LRP1, LRP2, and LRP8. We formulated a synthetic vesicle, known as a polymersome (PO), with several copies of the ligand and studied its biodistribution as a function of its phenotypic interaction with the LRP receptors.

We selected six PO formulations with different degrees of AP functionalisation that can cross the BBB, simulated in vitro by brain endothelial cells. Based on these results, we investigated the biodistribution of the POs in healthy C57BL/6J mice of seven weeks old, in vivo. After one single intraperitoneal injection of POs, we studied their biodistribution in fourteen organs, calculating the percentage of the injected dose per gram of tissue collected at 30 minutes, 2 hours, 4 hours, and 24 hours. This study is critical to further select the POs to be employed as therapeutic carriers in central nervous system disease. We used the data to fit the overall distribution using physiologically-based pharmacokinetics models to create predictive algorithms for modulating dosage.

Targeting cells through their unique metabolic phenotype

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 296

Mr. Víctor Mejías Pérez [1]***, Dr. Joana Fort*** [2]***, Dr. Manuel Palacin*** [3]***, Prof. Giuseppe Battaglia*** [4]***, Dr. Iris Batalha*** [5]

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Tuberculosis (TB) remains a substantial global health burden, with an estimated 10 million new cases and around 1.5 million deaths annually. The overuse or misuse of antibiotics has led to the evolution of drug-resistant strains of *Mycobacterium tuberculosis*, which are more difficult to treat due to their resistance to multiple first-line and even second-line drugs. Nanomedicine holds promise in addressing some of these challenges, particularly related to the targeted and sustained delivery of drugs and the effective crossing of biological barriers. We have previously reported that polymeric nanobiotics containing isoniazid and clofazimine presented lack of toxicity, dose-dependent response, and improved therapeutic efficacy when compared to free drugs *in vivo.* However, the challenge of delivering antibiotics specifically to infected cells persists. MHC class I-like related (MR1), an evolutionarily conserved protein, plays a pivotal role in presenting metabolites to specialized T cells, notably the Mucosal-associated invariant T (MAIT) cells. Upon metabolite binding, MR1 associates with ß2 microglobulin (ß2m) and translocates to the cell surface. this presentation triggers the activation and response of MAIT cells, leading to the release of cytokine and the initiation of an inmune response to combat the infection.
Methods: MR1-ß2m heterodimer have been expressed in *E. Coli* in the form of inclusion bodies, refolded in the presence of metabolites of interest, and purified by sequential anion exchange and size exclusion Fast-Protein Liquid Chromatography (FPLC). Using phage display technology, we then screened for peptides that bind specific MR1-metabolite complexes.
Results: SDS-PAGE and native mass spectrometry confirmed the presence of the metabolite in the MR1-ß2m heterodimer after purification. Phage display experiments revealed enriched peptides with a strong affinity for the target.
Conclusions and Future Prospects: The ability of MAIT cells to recognize a wide range of microbial metabolites presented by MR1 underscores its significance in immune surveillance and its potential for therapeutic development. Our next steps are to functionalise nanobiotics with our lead peptides for the targeted delivery of anti tubercular drugs to infected cells

3D Printing of Capsular shell for The Delivery of Cyclosporin-loaded Solid Self-nanoemulsifying Drug Delivery Systems

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 311

Dr. Mohammed Algahtani [1], ***Dr. Javed Ahmed*** [1], ***Mr. Abdul Aleem Mohammed*** [1]

1. *Department of Pharmaceutics, College of Pharmacy, Najran University, Kingdom of Saudi Arabia*

Cyclosporine (CsA) is a potent immunosuppressant agent that has been used since 1980 for the treatment of several autoimmune diseases and is widely used to increase the survival rate of patients and grafts post-organ transplant surgeries. CsA is a poorly soluble drug and has a narrow therapeutic window with an inter-subject variation that is associated with graft rejection, nephrotoxicity and other severe adverse effects. Here, we investigate a novel method that combines solubility improvement of CsA using solid self-nano emulsifying drug delivery systems (SNEDDS) formulation and dispenses the CsA doses in a personalized manner using 3D printing technology. The combination of caproyl 90 and octanoic acid was chosen as the oil phase and the combination of cremophore El and PEG 400 was chosen as the Smix phase. The optimized liquid SNEDDS was solidified using PEG 6000 (Table 1). A capsular shell designed and printed using an FDM printer with an oval base that ascends to form a dome with an opening at the top. This opening is used to fill the molten CsA-loaded SNEDDS formulation using a pipette or syringe (Figure 1). The CsA-loaded SNEDDS formulation was characterized by FTIR, DSC and SEM/EDX. The *in-vitro* release of CsA showed complete release within sixty minutes in a zero-order release manner. Neither, the shell opening size (Figure 2) nor the amount of the loaded formulation impacted the drug release profile (Figure 3). This innovative method will allow healthcare practitioners to dispense tailored doses in simple steps without the need for deep experience in 3D printing technology.

S. No.	Ingredients of liquid SNEDDS	%composition
1.	Caproyl 90	15.0
2.	Octanoic Acid	15.0
3.	Cremophore EL	20.0
4.	PEG-400	10.0
5.	PEG 6000	40.0

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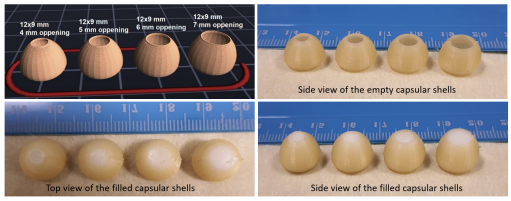

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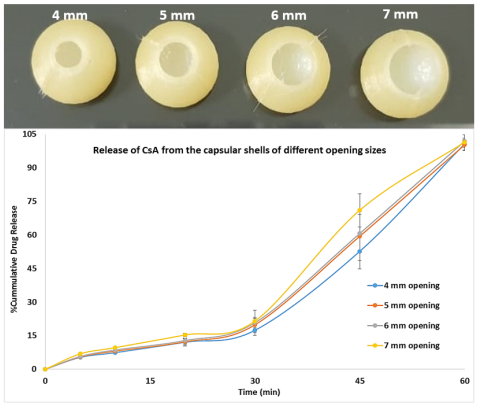

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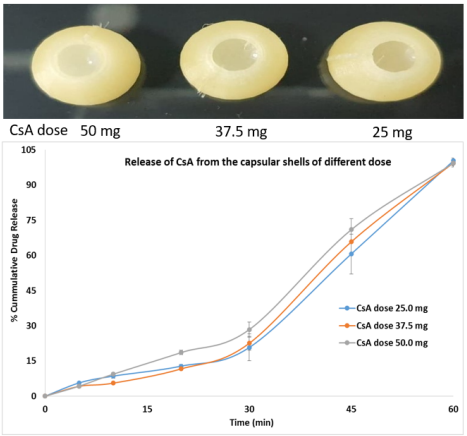

Figure 3 iconan.png

Evaluation of the toxicity of graphene oxide and its pegylated conjugate in human endothelial cells.

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 317

Dr. Arisbel Cerpa naranjo [1], ***Ms. Niza Albo*** [1], ***Dr. Mariana P. Arce*** [1], ***Dr. Javier Perez-Piñeiro*** [1], ***Dr. María Fuencisla Gilsanz*** [1], ***Dr. Darío Gallach*** [1], ***Dr. Marisol Fernández-Alonso*** [2], ***Ms. Marta Sanz-Gómez*** [2], ***Dr. Elvira Bragado-García*** [2]

1. *Universidad Europea,* ***2.*** *Universidad Complutense*

Graphene oxide (GO) is a material that has enabled significant advances and due to its unique properties, it can be used in a wide range of applications, including the healthcare field [1-5]. New methods of synthesis of graphene oxide and the possibility of functionalizing it with polyethylene glycol (PEG) are enabling its widespread use in medicine [1-3]. This work presents the synthesis of pegylated graphene oxide (GO-PEG) from GO, as well as its structural and morphological characterization of both starting materials and conjugates. Infrared Spectroscopy (IR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) techniques have been used for the structural and morphological characterization [4]. The cytotoxicity of GO and GO-PEG in human endothelial cells (EA.hy926 cell line) with different biological media (DMEM, DMEM+BSA, DMEM+FBS) was also investigated using the MTT assay. In addition, GO internalization in human endothelial cells was studied by TEM. The results obtained show that cell viability decreases as the concentration of GO and GO-PEG increases, and their cytotoxicity depends on the biological medium and the type of nanoparticle [5]. With the internalization images, it is concluded that cell survival depends mainly on the size, shape, and dose of the nanoparticle and pegylation favors internalization.

Recent Publications

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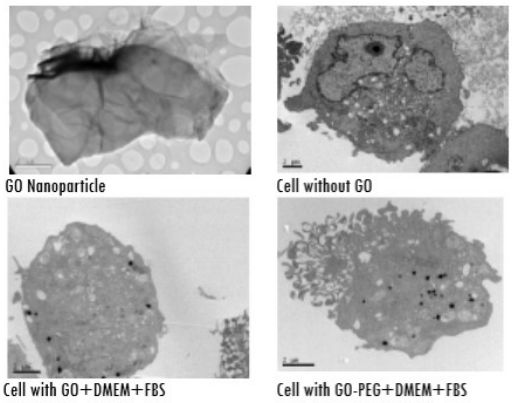


Figure 1. Internalization of GO and GO-PEG in human endothelial cells (EA.hy926 cell line) in presence of different biological media.

Figure 1.jpg

Portable biosensor for easy pathogen detection in aquaculture using Electrochemical Impedance Spectroscopy

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 323

Mr. Piotr Poręba [1], ***Ms. Paulina Janicka*** [1], ***Mrs. Michalina Alicka*** [1], ***Mr. Tomasz Filip*** [1], ***Dr. Katarzyna Pala*** [1], ***Mr. Marcin Prządka*** [1]

1. *Food4Future Technologies*

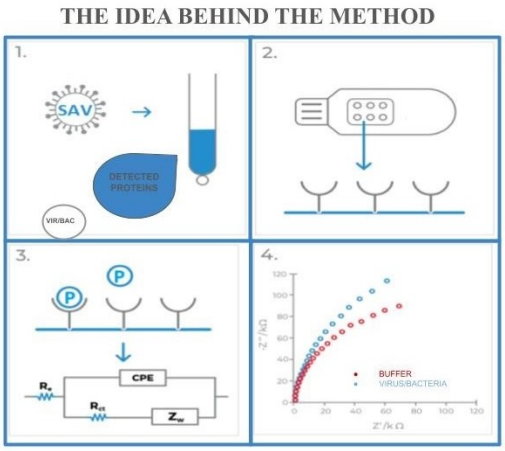
Aquaculture's role in meeting the global population's growing food demand is becoming increasingly critical. The expansion of seafood consumption and the prevalence of Recirculating Aquaculture Systems (RAS) underscore the need for rapid and precise pathogen detection techniques. Pathogens in aquaculture are not only economically damaging but also pose risks of zoonotic disease transmission.

Food4Future Technologies has developed a pioneering Rapid Pathogen Detection Test prototype. This initiative focused on targeting specific bacterial/viral pathogens in salmon and shrimp. Our portable device, utilizing Electrochemical Impedance Spectroscopy (EIS) is compatible with a biosensor featuring a specially synthesized bioreceptor immobilized as a layer on a gold biosensor surface. This bioreceptor is tailored to identify particular proteins specific to viral or bacterial pathogens. The reaction is based on a change of impedance upon adding a sample containing a protein able to selectively and specifically bind to the bioreceptor. This causes a change in electrical properties at the biosensor surface - changes resistance to the flow of an alternating current (impedance). The detection process is efficient and versatile, suitable for both laboratory and on-site aquaculture farm applications, and completes in just 20 minutes. An integrated decision criterion, based on a machine learning algorithm analyzes the data in real-time during this process.

In recent years, our team has focused on enhancing pathogen detection in aquaculture. We've developed biosensor-based detection kits capable of identifying a variety of pathogens in shrimp ei. White Spot Syndrome Virus, Taura Syndrome Virus, Yellow Head Virus, and Vibrio parahaemolyticus and in salmon such as: Piscine orthoreovirus, Infectious Salmon Anaemia Virus, Infectious Pancreatic Necrosis Virus, Piscine Myocarditis Virus, Viral Haemorrhagic Septicaemia and Aeromonas salmonicida.

This development was supplemented with comprehensive animal studies conducted across Norway, Denmark, the USA and Vietnam. The data gathered from these studies were crucial in refining our approach. Our data scientists worked to analyze the obtained results, leading to the creation of an algorithm adept at distinguishing between samples infected with these pathogens and healthy ones.

The utilization of EIS in non-laboratory settings and the creation of a pathogen-specific, ready-to-use biosensor represent significant advancements in the field of aquaculture pathogen detection.



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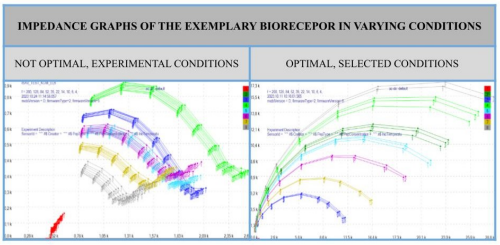

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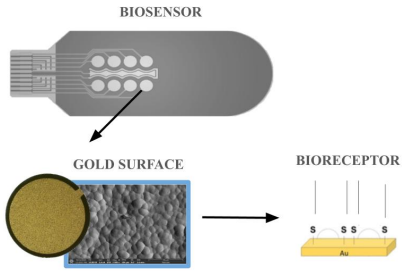


Abstract 3.jpg

Abstract 4.jpg

Plant-On-A-Chip: Studying Plant cell-Nanomaterial Interactions using Microfluidics

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 325

Mr. Jordi Floriach-Clark [1], ***Mr. Martijn van Galen*** [1], ***Prof. Joris Sprakel*** [1], ***Prof. Ben Scheres*** [1], ***Prof. Viola Willemsen*** [1]

1. *Wageningen University*

Nanotechnologies have transformed healthcare by new imaging and sensing tools and the development of targeted (drug) delivery. This success was build upon fundamental knowledge of the interaction between the animal cell or organism with the nanomaterial and its physicochemical and biological properties. After decades, the “nano-revolution” has not arrived yet to the agricultural field, despite food safety being under challenge by a growing population and climate change.
What lacks is a strong understanding of plant cell-nanomaterial interactions, a different scenario with new challenges we can now look at with experienced eyes.
By knowing how to overcome plant cell barriers (e.g. cell wall and membrane), (targeted) delivery of nucleic acid, protein and small metabolites may become possible, overcoming old bottlenecks such as plant transformation, regeneration and architecture research.

For the successful organisation in the three dimensions of plant architecture, plant cells need to sense space and divide asymmetrically. Part of this sensing is based in multiple mechanical and chemical cues. The exact nature of these cues and their sensing is hard to study in physiologically relevant conditions and at a cellular level. Microfluidic chips provide a precise and reproducible strategy to create chemical gradients or local treatments and monitor their effects, and they have been hardly used for plant research, mainly due to the size and complexity of plant organs. We use a simple plant model that shows great compatibility with microfluidic devices to test the effects of local biochemical treatments at the cellular level. With such a system, plant hormonesand other biochemical cues (e.g. peptides) can be tested in vivo, and their effects visualised over days under the microscope.
Furthermore, the same platform assists in our study of plant cell-nanoparticle interactions, a matter of increasing interest for the development of phyto-nanotechnology. We adopted a systematic approach for testing multiple nanoparticle types (differing in e.g. size, charge, decorations, composition) on single cells. The outcome of this study may provide key knowledge to understand how different properties can affect the travel across the cell wall and membrane in highly reproducible conditions.

Design and characterization of novel cinnamyl-modified copolymer micelles for cannabidiol delivery

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 235

Prof. Denitsa Momekova [1], ***Dr. Natalia Toncheva-Moncheva*** [2], ***Dr. Erik Dimitrov*** [2], ***Dr. Georgi Grancharov*** [2], ***Prof. Petar Petrov*** [2], ***Prof. Stanislav Rangelov*** [3]

1. *Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav Street, 1000 Sofia, Bulgaria,* ***2.*** *InstituteInstitute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria,* ***3.*** *Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Polymer micelles are promising nanosized carriers for delivery of drugs with low aqueous solubility and unfavorable pharmacokinetics. Notwithstanding their considerable potential as drug delivery vehicles, some important characteristics still need to be addressed to attain better colloidal stability, prolonged circulation and enhanced bioavailability of their cargo. Cannabidiol (CBD) is a phytocannabinoid with pleiotropic therapeutic effect, but poor aqueous solubility and low bioavailability hampering its full clinical translation.

The aim of the study is the design and characterization of novel block copolymer micelles for enhanced solubility, higher encapsulation efficiency and controlled CBD release.

The micelles based on novel amphiphilic block copolymers comprising two outer hydrophilic polyglycidol (PG) blocks and a middle hydrophobic block of poly(e-caprolactone) bearing pendant cinnamyl moieties (P(CyCL-co-CL)) were prepared by solvent evaporation method and loaded with CBD following two different protocols—loading during micelle formation (protocol A) and loading into preformed micelles (protocol B). Their key characteristics such as size, size distribution, zeta potential, molar mass, critical micelle concentration, morphology, and encapsulation efficiency were determined by using dynamic and static multiangle and electrophoretic light scattering, transmission electron microscopy, and atomic force microscopy. The antiproliferative activity of micellar CBD was assessed in comparative way vs. free drug in a panel of human tumor cell lines.

The elaborated micelles are characterized with small size (100-120 nm) and spherical morphology. The micelles prepared by protocol A have higher encapsulation efficiency than those by protocol B and further the nanoformulations based on the novel modified copolymer showed invariably greater encapsulation efficiency (90-95 %) and slower CBD release than the non-modified micelles which is apparently due to the introduction of cinnamyl-bearing units. The comparative evaluation of the antiproliferative effect of micellar CBD vs. the free drug against the acute myeloid leukemia-derived HL-60 cell line and Sezary Syndrome HUT-78 demonstrated that the newly developed systems have pronounced antitumor activity.

These results evident the effective design of the novel cynnamil-modified copolymer and the potential of its micelles as enhanced delivery vehicles of CBD.

Acknowledgements: The support by the European Union-Next Generation EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, Project BG-RRP-2.004-0004-C01

Copolymer	R_h^{90} (nm)			ζ potential (mV)			EE (%)	
	Blank	Loaded Prot. A	Loaded Prot. B	Blank	Loaded Prot. A	Loaded Prot. B	Loaded Prot. A	Loaded Prot. B
PG_{45}-*b*-PCL_{35}-*b*-PG_{45}	52.0± 1.7	56.0 ± 2.7	50.0 ± 3.8	2.94± 1.97	-2.61 ± 2.10	2.90 ± 2.40	91.0	82.0
PG_{50}-*b*-PPO_4-*b*-[P(CyCL)$_4$-*co*-(CL)$_{40}$]-*b*-PPO_4-*b*-PG_{50}	60.0 ± 1.6	51.0± 1.8	57.0± 1.4	-5.90± 1.23	-6.61 ± 3.10	4.19 ± 2.50	95.0	92.0

Figure 1..png

Design and evaluation of cannabidiol-loaded sterically stabilized niosomes

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 236

Dr. Viliana Gugleva [1], ***Dr. Rositsa Mihaylova*** [2], ***Dr. Natalia Toncheva-Moncheva*** [3], ***Prof. Stanislav Rangelov*** [3], ***Dr. Aleksander Forys*** [4], ***Prof. Barbara Trzebicka*** [4], ***Prof. Denitsa Momekova*** [5]

1. *Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University of Varna "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria,* ***2.*** *Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria,* ***3.*** *Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria,* ***4.*** *Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland,* ***5.*** *Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Street, 1000 Sofia, Bulgaria*

Introduction

Long circulating niosomes are non-ionic surfactant based vesicles, which structure is further modified with suitable polymers to prevent their elimination by the mononuclear phagocyte system. Cannabidiol (CBD) is a phytocanabinoid derived from *Cannabis sativa* characterized by variety of pharmacological effects, among which a pronounced antineoplastic activity. The current study is focused on the development, characterization and pharmacological evaluation of long-circulating cannabidiol-loaded niosomes.

Methods

Cannabidiol-loaded niosomes composed of Span 60, Tween 60 and cholesterol modified with amphiphilic linear or star-shaped polyglycidol-poly(ε-caprolactone)-based copolymers (0.5, 1, 2.5 mol %) were prepared by thin film hydration method followed by subsequent probe sonication. The vesicles were characterized by dynamic light scattering, cryo-transmission electron microscopy, spectrofluorimetry, cannabidiol entrapment efficiency and *in vitro* release. The cytotoxicity, pro-apoptotic and anti-inflammatory of elaborated niosomes were tested against human malignant cell lines (MJ, T-24, HUT-78) vs. free cannabidiol.

Results

The prepared niosomes have small sizes (150-240 nm) suitable for systemic delivery, spherical-shaped morphology, high cannabidiol entrapment efficiency (89-94%) and sustained drug release profile. The niosomal cannabidiol exhibits antineoplastic activity corresponding to free agent and superior pro-apoptotic and anti-inflammatory effects.

Discussion

The copolymer modified niosomes exhibit larger size compared to the conventional ones, which may be related to the formation of a protective shield by the hydrophilic polyglycidol chains and less curved bilayer membranes following the inclusion of rigid poly(ε-caprolactone) moieties. The formulation based on Tween 60/Span60 and cholesterol modified with 2.5 mol % star-shaped 3-arm copolymer provides controlled and lower CBD release in physiological relevant media (cf. with the unmodified vesicles) which confirms the stabilizing effect of modifying copolymer. Based on the obtained results it can be concluded that the elaborated niosomes may serve as feasible platform for systemic delivery of cannabidiol.

Acknowledgements: This work was supported by grand from the Bulgarian National Science Fund (КП-06-43/3-2020).

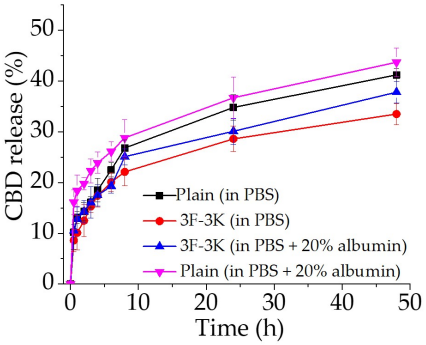


Figure release.jpg

IC_{50} (μM ± SD) in Terms of CBD Concentration			
Cell line Sample	**T-24**	**MJ**	**HUT-78**
CBD (as solution)	12.2 ± 2.1	58.6 ± 5.9	30.1 ± 5.9
Tw60:Sp60:Ch:3F-3K	32.1 ± 6.3	74.5 ± 4.4	63.9 ± 1.8
Tw60:Sp60:Ch:4F-3K	37.3 ± 5.2	74.2 ± 3.8	64.6 ± 3.7
Tw60:Sp60:Ch (plain)	58.9 ± 6.5	69.1 ± 6.2	86.2 ± 3.1

Table 1.png

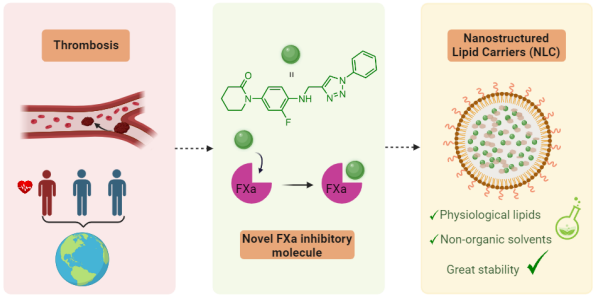
Design and optimization of an innovative lipid nanosystem for encapsulation of a novel FXa inhibitory molecule

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 147

Ms. Karla López [1], ***Dr. Andrea Ravasio*** [2], ***Dr. José Vicente González*** [3], ***Dr. Flavia Zacconi*** [4]

1. *Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago 7820436, Chile,* ***2.*** *Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago 7820436, Chile.,* ***3.*** *Escuela de Química y Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago 7820436, Chile,* ***4.*** *Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago 7820436 Chile*

Thrombosis, a significant contributor to cardiovascular diseases fatalities (33% of annual deaths worldwide), poses a formidable challenge in the development of safe anticoagulants. The direct inhibition of Factor Xa (FXa) in the human coagulation system has emerged as a pivotal pharmacological strategy to prevent thrombi formation, giving rise to direct oral anticoagulants (DOACs). However, the inherent risks of DOACs, such as severe uncontrolled bleeding, require innovative solutions. In response, our research group synthesized a novel FXa inhibitor using green chemistry techniques, incorporating an eco-friendly approach, and demonstrating its *in vitro* efficacy. Despite its potential, the molecule's elevated lipophilia could hinder its clinical application. To overcome this limitation, we propose the use of Nanostructured Lipid Carriers (NLCs), an innovative type of lipid nanoparticles. NLCs, are colloidal carriers formulated without organic solvents, and represent a new generation of Solid Lipid Nanoparticles (SLN) with enhanced stability and drug incorporation efficacy. The nanosystems unique solidified lipid nucleus, with specific crystalline patterns, includes liquid lipid compartments that prevent lipid phase recrystallization. This structure facilitates the encapsulation of active lipid ingredients, safeguarding them against degradation and enabling targeted delivery. Our research aimed to design and optimize NLCs using a low-energy conventional microemulsion technique to effectively encapsulate the new FXa inhibitory molecule. The resulting nanocarriers exhibited favorable physicochemical properties, including sizes of 138±15 nm, polydispersity index (PI) of 0.168, and zeta potential (ZP) of -19±2 mV. The prototypes demonstrated excellent storage stability for over 20 weeks, physiological stability for more than 48 hours, and gastrointestinal stability for up to 4 hours. Once the inhibitory molecule was encapsulated, the nanosystems maintained a consistent size of 135±6 nm, PI of 0.236, and a ZP of -24±6 mV. The encapsulation efficiency greater than 50% was confirmed using ^{19}F NMR detection. In summary, this study presents an innovative and eco-friendly NLC formulation, marking the first-time encapsulation of a novel FXa inhibitory molecule. The stable lipid nanostructures offer promising prospects for overcoming the challenges associated with thrombosis treatment.



Graphical abstract klll.png

Simultaneous Dual-Colour Imaging and Polarity Assessment in Organelles Using a Single Fluorescent Probe

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 263

Mr. Lei Hu [1], ***Ms. Dandan Chen*** [2], ***Dr. Hui Wang*** [3], ***Prof. Xiaohe Tian*** [2], ***Prof. lorena Ruiz*** [4], ***Prof. Giuseppe Battaglia*** [5]

1. *1. Institute for Bioengineering of Catalonia (IBEC). 2. Department of Organic Chemistry, University of Barcelona, Barcelona, Spain Barcelona (Spain).,* ***2.*** *West China Hospital of Sichuan University,* ***3.*** *Wannan Medical College,* ***4.*** *1. Institute for Bioengineering of Catalonia (IBEC). 4. West China Hospital of Sichuan University, Chengdu (China).5. Department of Applied Physics, University of Barcelona, Barcelona, Spain Barcelona (Spain).,* ***5.*** *1. Institute for Bioengineering of Catalonia Barcelona, (Spain). 2. Department of Organic Chemistry, University of Barcelona, Barcelona, Spain Barcelona (Spain).*

Cellular polarity is pivotal for protein transport and biological signal transduction, with its dysregulation implicated in various diseases. Understanding the polarity within different organelles is crucial for pathophysiological research and early disease diagnosis. However, simultaneous, dual-colour imaging and quantifying polarity in distinct organelles have yet to be hindered by the lack of single fluorescent probes (SF probes) tailored for this purpose. This study introduces a polarity-sensitive fluorophore, **LE-TPA**, designed for concurrent dual-colour imaging and quantifying polarity in lipid droplets (LDs) and the endoplasmic reticulum (ER). **LE-TPA** exhibits a unique fluorescence response to water content in 1,4-dioxane/water mixtures, with an enhancement of up to 10% water and a reduction after that, alongside a significant red shift from 577 nm to 622 nm. This behaviour allows for the distinction between LDs and ER, as demonstrated by green and red fluorescence, respectively, corresponding to the subtle differences in their water content. We established a linear correlation between emission wavelength and solvent polarity, which enabled us to determine the polarities within LDs and ER quantitatively. Notably, **LE-TPA** also facilitated the visualisation of LD formation within the ER. This study lays the groundwork for further research into the dynamics of LDs within the ER and the polarity differences between organelles in healthy and cancerous cells, offering a novel tool for pathophysiological studies and the early detection of cancer.

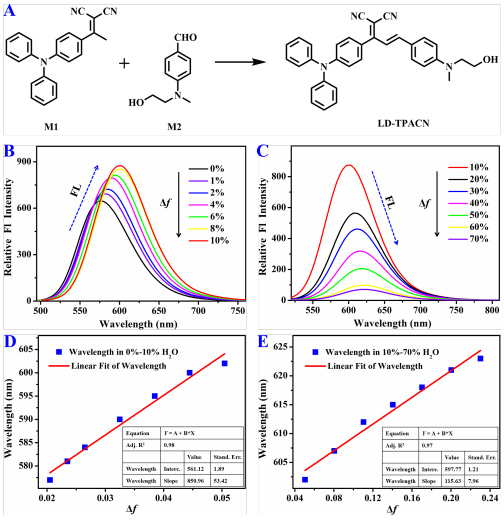


Fig.1.png

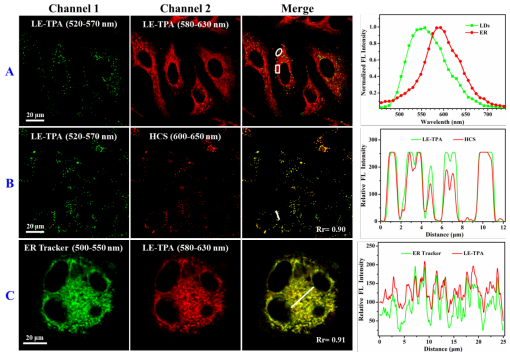


Fig. 2.png

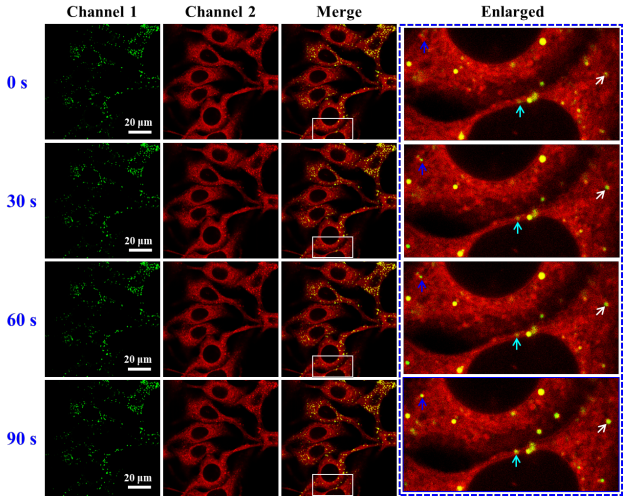


Fig.3.png

Covalent organic framework nanosheets for diabetes: unveiling the molecular mechanism of glucose-induced insulin release

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 273

Ms. Seoyoung Lee [1], ***Dr. Farah Benyettou*** [1], ***Prof. Ali Trabolsi*** [1], ***Prof. Serdal Kirmizialtin*** [1]

1. *New York University Abu Dhabi*

Diabetes is directly linked to approximately 1.5 million deaths every year and insulin injection is the primary method of treatment for diabetes. The development of oral delivery of insulin is important as a noninvasive treatment approach as it addresses the side effects of routine subcutaneous injections. In our previous study, we developed gastro-resistant insulin-loaded covalent organic framework (COF) nanosheets that exhibited hyperglycemia-induced insulin release in vitro and in vivo for the oral delivery of insulin. However, the molecular mechanism of glucose-induced release of insulin, which is important to design better delivery systems, remains untapped. To understand the mechanism of hyperglycemia-induced insulin release, we conducted molecular dynamics (MD) simulations of the COF nanosheets with an insulin molecule in the absence and presence of glucose (Fig. 1). Free energy simulations revealed that insulin binds less strongly to COF in the presence of glucose (Fig. 2), confirming the experimental observation. To understand the physical principles of glucose-responsive binding, we computed contact probabilities and pair correlation functions between the host (COF) and the guest (insulin, glucose) molecules. Our analysis suggests that insulin binds to the nitro groups on the surface of the COF. The presence of glucose molecules resulted in non-specific interactions around the surface of the COF and insulin, which shields the protein-COF interaction. The glucose covering the protein surface decreases the stability of the COF-insulin complex, inducing insulin release. The findings of this computational study are in good agreement with the experimental results and provide a physical explanation of the glucose-dependent insulin release mechanism. An accurate computational description of insulin-COF interactions can help in the effective screening of COFs in drug loading for insulin.

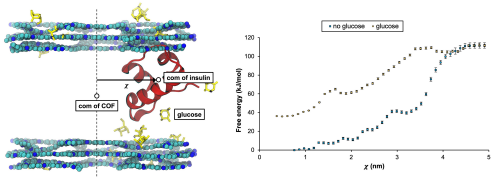


Fig. 2 definition of left and free energy profile of glucose and no glucose systems right .jpg

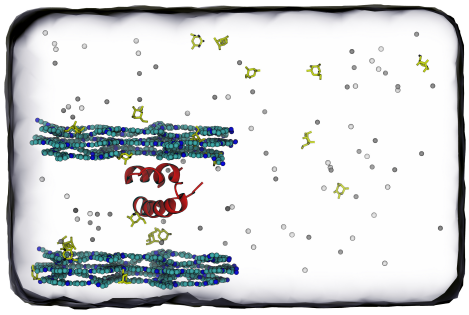


Fig. 1 simulation box of insulin red loaded cof nanosheets blue in a high glucose yellow environment in water box outline .jpg

Real time in line size monitoring with the commercially available DLS system: Accuracy and Repeatability assessment

Monday, 15th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 344

Tuesday, 16th January - 13:30: Poster Session (Poster Area) - Poster - Abstract ID: 344

Dr. Hanna Anop [1]

1. *Cordouan Technologies*

Poster of the exhibitor **CORDOUAN Technologies**

Nano-Ghosts: Cell-Free nano-vesicles for off-The-shelf cancer targeting and Immunotherapy

Tuesday, 16th January - 14:30: Desing of novel nanomedicines - I (Auditorium) - Oral - Abstract ID: 20

Prof. Marcelle Machluf [1]

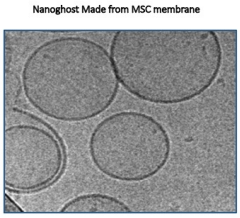
1. *Technion*

Introduction: Mesenchymal stem cells (MSCs) have gained the most attention in this regard, owing to their ability to traverse physiological barriers, and target different sites of inflammation including primary and metastatic tumors, while exhibiting relative allogeneic safety. However, once transplanted, MSCs undergo changes that alter their targeting capabilities tropism and increase their immunogenicity, only permitting them to exert a short hit-and-run effect. We hypothesised that overcoming these challenges can be realised by combining the safety and tumor-targeting capabilities of MSCs in an inanimate platform that can withstand limiting host influences. The foundations for this combination are laid by a novel class of nano-vesicles (200 nm), termed immuno-nano-ghosts (iNGs), equipped with the membrane proteins of from MSCs and exogenous ones inspired genetic engineering such as PD-1 and TRAIL.

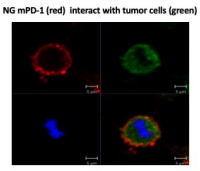
Methods: We have developed a scalable and cGMP compliant technology for the production of iNGs from the whole MSC membranes, and their loading with diverse bioactive compounds as well as engineering them to express ligands such as PD-1 and TRAIL. The NG were loaded with different therapeutics and studied in vitro and in vivo on intracranial glioma cancer model as well as melanoma and pancreatic tumors.

Results: Our data highlight the NGs as a targeted delivery platform that can be effectively loaded and used to selectively deliver diverse therapeutics including biological drugs, small molecules, and nucleic acids. Never the less' surprising data demonstrate that the NG by themselves can modulate inflammation via cell-cell interaction. Their abundance of natural targeting mechanisms allows the NGs (injected i.v.) to bypass the BBB and penetrate the entire tumor bulk and rapidly deploy their payload directly into the target cancer cells leading to unprecedented tumor growth inhibition and increased animals' survival in intracranial glioma model. The NGs also interact with the cancer stem cells modulating the response of the tumor to chemotherapy. NGs can retain MSCs' tropism towards inflamed tissues and function as immunomodulators using the PD-1/PDL1 mechanism.

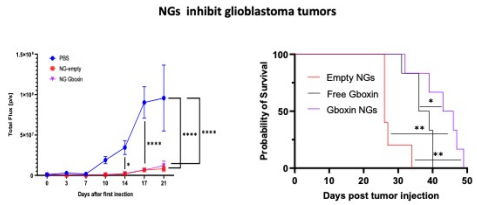
Conclusions: Our results, so far, clearly demonstrate the translational potential of NGs, both as targeted delivery platform as well as a novel immunomodulatory biologic, for oncological and immunological applications.



Presentation 2.jpg



Presentation3.jpg



Presentation1.jpg

Low molecular weight hydrogelators as nanocarriers for drug delivery

Tuesday, 16th January - 14:47: Desing of novel nanomedicines - I (Auditorium) - Oral - Abstract ID: 7

Ms. Eleonora Calicchia [1], ***Prof. Giuseppe Portale*** [1], ***Prof. Anna Salvati*** [1]

1. *University of Groningen*

Low molecular weight hydrogelators (LMWHGs) are an interesting class of materials that have gained significant interest in the biomedicine field for their potential use as drug delivery systems [1]. They consist of small molecules capable of forming a gel-like structure in water due to supramolecular interactions, leading to the formation of an extensive network known as physical gel [2]. LMWHGs have been mainly investigated for biomedical application in the form of bulk gels, while their potential use as drug carriers for intracellular drug delivery has remained less explored. To this end, the gelation needs to be confined in order to obtain nano-sized carriers (50-200 nm) capable of entering cells.

Here, we have explored two different methods to confine the gelation of LMWHGs, in either a water-in-oil nanoemulsion or inside liposomes. For this we have used a LMWHG based on lysine (Fmoc-Lys(Fmoc)-OH) [3] known to gelate water upon pH modulation.

After optimization of the conditions required to form stable nanocarriers, we used Dynamic Light Scattering (DLS), Small Angle X-Ray Scattering (SAXS), Transmission Electron Microscopy (TEM), and Cryo-Electron Microscopy (Cryo-EM), to assess the nanocarriers properties.

In order to render both systems suitable for biological applications, we optimized different methods based on centrifugation to transfer the nanodroplets formed in the nanoemulsion from the oil phase to water and to remove the non-encapsulated hydrogelator outside the liposomes.

The results showed that the nanoemulsion method allowed us to obtain droplets containing the LMWG which could be transferred in water by resuspension with a Tween80 solution.

Similarly, liposomes containing the LMWG were also obtained. Further studies are ongoing to optimize the generated droplets, test their capacity to encapsulate poorly soluble drugs, as well as to characterize their interactions with cells (uptake, intracellular behavior and potential toxicity).

[1] E. R. Draper, D. J. Adams; *Chem*, **2017**, *3*, 390-410

[2] E. R. Draper, D. J. Adams; *Langmuir*, **2019**, *35*, 6506-6521

[3] S. M. M. Reddy et al.; *Soft Matter*, **2015**, *11*, 8126-8140

Unlocking the potential of Janus micelles: a multivalent platform for precision nanomedicines with dual phenotypical targetings

Tuesday, 16th January - 15:04: Desing of novel nanomedicines - I (Auditorium) - Oral - Abstract ID: 220

Mr. Jose Muñoz-López [1], ***Prof. lorena Ruiz*** [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia*

Targeted therapies rely on the precise selectivity of ligands towards their target receptors. In synthetical systems, selectivity can be expertly harnessed through the implementation of multivalent systems, such as nanoparticles, when these are thoughtfully functionalized with multiple low-affinity ligands. This approach seizes the substantial influence of combinatorial entropy on binding affinity, which leads to on-off association profiles. Herein, Janus micelles, distinguished by their unique biphasic geometry and dissimilar corona properties, emerge as a multivalent scaffold to target two different phenotypes when each domain is tailored with distinct ligands, performing in the same fashion as antibodies. In this work, we present a novel ABC amphiphilic triblock copolymer system comprising poly(ethylene glycol)-polylactide-poly(N-vinylpyrrolidone), denoted as PEG-PLA-PVP, with the ability to form Janus micelles, which are generated by solution-mediated self-assembly of the PEG and PVP hydrophilic, and PLA hydrophobic blocks. Triblock synthesis lies in two steps: first, poly(ethylene glycol)-polylactide-2-bromo-2-methylpropanoate (PEG-PLA-Br) diblock macroinitiator is synthesized by the ring-opening polymerization of DL-lactide with commercial poly(ethylene glycol) and quenched with 2-bromo-isobutiryl-bromide. Following, triblock copolymer is synthesized by the atom transfer radical polymerization (ATRP) of *N*-vinylpyrrolidone monomer (NVP) initiated by the PEG-PLA-Br diblock produced in the former synthetic step. The characterization of the produced diblock and triblock copolymers were carried out by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). The morphologies adopted by PEG-PLA-Br and PEG-PLA-PVP in solution were investigated by both transmission electron microscopy (TEM) and cryogenic electron microscopy (cryo-TEM), confirming the formation of micelles for both block copolymers. TEM images showed differences in the negative stained micelles generated in the diblock and triblock copolymers systems, indicating an asymmetric distribution of both hydrophilic blocks in the self-assembling of the triblock system. Presently, we are working on a library of functionalized PEG-PLA and PVP-PLA micelles to fine-tune the optimal polymer brush density and polymer tether. Our ultimate goal is to extend this research to tertiary mixture systems, combining these two functionalized diblocks with the triblock micellar scaffold. Thus, we aspire to create a versatile micellar platform to unfold the creation of nanodrugs with similar precision and efficacy observed in the field of antibody-based therapies.

Design of a microfluidic platform to study the stability of therapeutic protein formulations (TPFs)

Tuesday, 16th January - 15:21: Desing of novel nanomedicines - I (Auditorium) - Oral - Abstract ID: 191

Dr. Osvaldo Bortone [1], ***Dr. Samuele Fiorenza*** [2], ***Dr. Maurizio Baldassarre*** [3], ***Dr. Nunzia Falco*** [4], ***Prof. Paolo Antonio Netti*** [1], ***Prof. Enza Torino*** [1]

1. *Department of Chemical, Materials and Production Engineering, University of Naples Federico II, 80125 Naples, Italy,* ***2.*** *Department of Information Technology and Electrical Engineering, University of Naples Federico II, 80125 Naples, Italy,* ***3.*** *Global Drug Product Development, Merck Serono S.p.A, Switzerland,* ***4.*** *Global Drug Product Development, Merck Serono S.p.A, Italy*

Therapeutic protein formulations (TPFs) (*e.g.*, mAbs, nAbs) are exceptionally promising for the care of challenging diseases thanks to their unparalleled pharmacological properties. However, their spread in the clinics is limited by their instability (*e.g.,* aggregation), causing TPFs to be immunogenic. Their stability is threatened by several factors, including heat or mechanical stress. TPF stability is arduous to be studied due to their multicomponent composition (active protein ingredient plus stabilizing co-solutes), determining an intricate network of intermolecular interactions and challenging rheological properties (*e.g.*, high viscosity). Current batchwise approaches probing the TPF stability are poorly controllable, low-throughput and, in some cases, uncapable of replicating the real destabilizing sources encountered in the TPF lifecycle (*e.g.*, manufacturing stress or combination of physical stresses). To improve the clinical outcomes, stability of therapeutic proteins must be ensured through ameliorated formulations. Parallelly, there is a need for cost-effective technologies that can better mimic real stressors, providing a broader scientific knowledge of the protein behavior - destabilizing sources intercorrelation. Thanks to its high versatility and minimum volume requirement, microfluidics can help address these needs.

An automated modular microfluidic platform is designed and developed to study the stability of TPFs by subjecting them to physical stresses: thermal, high shear (typical of manufacturing processes) and combined stimulations. Different nAb-based formulations are exposed to overmentioned stresses and then analyzed through conventional (SE-HPLC) and orthogonal *quasi-real-time* techniques (DLS, FTIR).

The microfluidic platform can replicate standard *in-batch* thermal stress, returning the same SE-HPLC outcomes. Moreover, the exposure of TPFs to microfluidic shear stress can induce both colloidal and structural modifications that are not caught by conventional analyses and are not triggered by standard batchwise mechanical stress protocols (agitation). Finally, the microfluidic platform might highlight unrevealed, non-obvious instability behaviors by exposing the TPFs to combined thermal-mechanical stresses.

The platform can generate data returning advanced stability information. The latter, integrated to that obtained from current approaches, can help clarify the intercorrelation between formulation instability and destabilizing sources. This could enable the setting of improved mitigation strategies (improved selection of stabilizing co-solutes), thus impacting on the clinical outcomes, including therapy efficacy and patients' satisfaction.

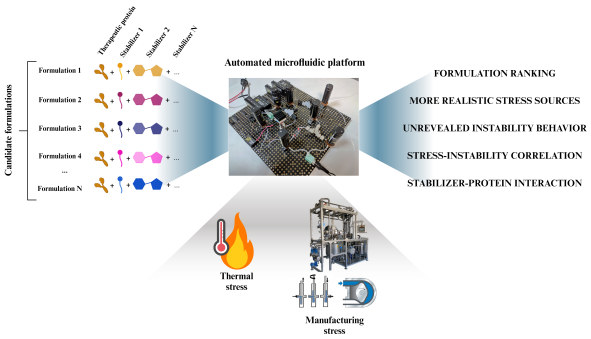

Therapeutic protein
Stabilizer 1
Stabilizer 2
Stabilizer N
Candidate formulations
Formulation 1
Formulation 2
Formulation 3
Formulation 4
...
Formulation N
Automated microfluidic platform
FORMULATION RANKING
MORE REALISTIC STRESS SOURCES
UNREVEALED INSTABILITY BEHAVIOR
STRESS-INSTABILITY CORRELATION
STABILIZER-PROTEIN INTERACTION
Thermal stress
Manufacturing stress

Figure.png

Nanosized delivery systems for therapeutic proteins and drugs via multifunctional (co)polymer engineering

Tuesday, 16th January - 15:38: Desing of novel nanomedicines - I (Auditorium) - Oral - Abstract ID: 40

Prof. Francesco Cellesi [1]

1. *Politecnico di Milano*

Recent advancements in polymer chemistry and nanomedicine have given rise to a diverse range of nanocarriers engineered for targeted delivery of therapeutic molecules [1]. Utilizing cutting-edge macromolecular engineering techniques, these nanocarriers can be precisely tailored to exhibit specific physicochemical characteristics, including controlled size, self-assembly, amphiphilicity, and multifunctionality[2]. Our research has focused on the development of polymers with complex, well-defined structures and functional groups to generate bioactive and traceable nanomaterials.

Biodegradable polyester-based copolymers, such as polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA), copolymerized with hydrophilic poly(ethylene glycol) (PEG) or poly(glycerol methacrylate) (PGMA) [2-3], as well as conjugatable poly(glycidyl methacrylate), were synthesized using controlled living polymerization and customized bioconjugation methods.

These macromolecules are engineered with branched and multi-armed topologies, enhancing their ability to cross biological barriers, encapsulate drugs and elicit specific cellular responses[3]. Furthermore, by incorporating fluorinated repeating units, we have created nanoscopic ^{19}F magnetic resonance imaging agents that were also capable of in situ drug release.

To address the challenges posed by poor pharmacokinetics, short circulation half-life, stability, and immunogenicity of therapeutic proteins, we developed a synthetic approach based on site-specific protein modification, followed by grafting-from polymerization technique[4]. Proteins were conjugated with hydrophilic PGMA and PEG macromolecular brushes with predetermined molecular weight and topology. The preservation of activity under proteolytic and high-temperature conditions was influenced by the structure of the repeating unit and the macromolecular architecture. These results highlighted the potential of PGMA as a promising alternative to PEG, effectively extending the half-life of biotherapeutics. This polymerization method may inspire the design of a novel class of protein–polymer conjugates, combining optimal macromolecular composition and topology for enhanced therapeutic applications.

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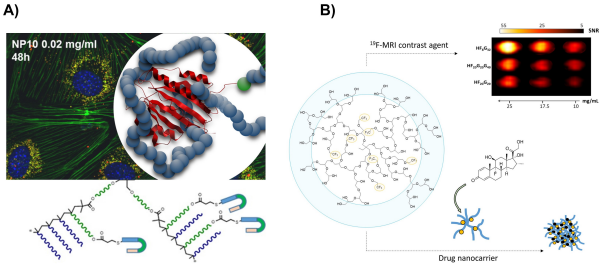


Figure.jpg

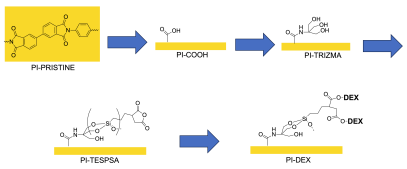
Surface functionalization of polyimide-based intraneural devices with covalently bonded dexamethasone to develop innovative drug delivery systems.

Tuesday, 16th January - 15:55: Desing of novel nanomedicines - I (Auditorium) - Oral - Abstract ID: 225

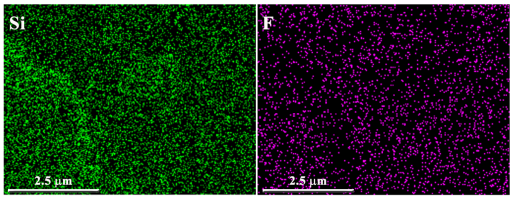
Dr. Giulia Turrin [1], ***Dr. Chiara Bisquoli*** [1], ***Dr. Fernanda Narvaez*** [2], ***Dr. Davide Barboni*** [3], ***Dr. Martina Catani*** [3], ***Prof. Stefano Caramori*** [3], ***Prof. Claudio Trapella*** [3], ***Prof. Maria Asplund*** [4], ***Dr. Stefano Carli*** [1]

1. *Department of Environmental and Prevention Sciences, University of Ferrara,* ***2.*** *University of Freiburg,* ***3.*** *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara,* ***4.*** *Chalmers University of Technology*

Foreign Body Respone (FBR) is an inevitable process that occurs whenever intraneural devices are implanted. It is due to an excessive inflammatory response that extends over a period of several weeks or months after the surgical implantation. This leads to the growth of fibrotic tissue around the inserted device, isolating it from the target physically and electrically. This work is aimed to provide a strategy to covalently link dexamethasone (DEX, a potent anti-inflammatory drug), to the surface of polyimide (UPILEX-S®), the inert material the device is made of. Hence, the main and innovative idea of this study is to consider the polyamide, which represents the largest area of the overall device, as a drug delivery platform rather than including the drug over the electroactive surface area (as done so far). The synthetic pathway consists of modifications of the polyimide surface, to permit the attachment of DEX. Briefly, the surface imide functions of the polymer are hydrolyzed in alkali media: this generates carboxylic functions, that can be exploited for the subsequent coupling with a specific amine enriched in hydroxylic functions at one end (Trizma® base). These hydroxyl groups are now an ideal platform for the next silanization process, which is achieved by using the commercially available triethoxysilylpropyl succinic anhydride silane (TESPSA). Here, the succinic anhydride acts as an anchor site for the insertion of one-to-two molecules of DEX, through the formation of an ester bond. The presence of the covalently incorporated drug was confirmed by ATR-FTIR and SEM/EDX analyses. In addition, HPLC was used to evaluate the kinetic of release as well as the overall drug loading. The drug release is expected to occur by the hydrolysis of the ester bond between DEX and the underlaying Si-functionalized polyimide. It was found that the drug release extends over a time window of about four weeks, which is expected to mitigate the initial FBRs and possibly reducing the chronic adverse reactions. This can pave the way to construct future neural/nerve devices that can face the well-known chronic challenge.



Chemical route to obtain pi-dex.png



Atomic distribution map of fluorine and silicon.png

Nanozyme catalysed CRISPR assay for preamplification-free detection of non-coding RNAs and low-resource multi-omic diagnostics

Tuesday, 16th January - 14:30: Nanomedicine for Gene Therapy and Genomics (Room 507) - Oral - Abstract ID: 119

Mr. Schan Perera [1], ***Dr. Pargat Singh*** [1], ***Dr. Leah Frenette*** [1], ***Dr. Marta Broto*** [1], ***Prof. Molly Stevens*** [2]

1. *Imperial College London, Department of Materials,* ***2.*** *Imperial College London, Department of Bioengineering*

Introduction

CRISPR (clustered regularly interspaced short palindromic repeats) systems have emerged as promising tools for molecular diagnostics owing to their programmable target recognition. The ability to engineer new guide RNAs against different nucleic acid targets makes this platform particularly promising, not only for widespread deployment against infectious diseases but also as a monitoring tool for non-communicable illnesses such as cardiovascular diseases (CVDs). Currently, target pre-amplification using complex molecular techniques is essential to reach clinical levels of sensitivity, limiting the accessibility of this technique while also hindering the development of multiplexed CRISPR devices.

Methods

Previously, we have developed a nanozyme based CRISPR assay (CrisprZyme) for the pre-amplification free detection of non-coding RNAs (Figure 1). Platinum-gold core shell nanostructures (nanozymes) were synthesised and conjugated to 5'-FAM-UUUUUC-biotin-3' CRISPR/Cas13 reporter molecules. Following a standard CRISPR reaction, these reagents flow up a lateral flow strip, where the test line is chemically amplified via a substrate oxidation reaction catalysed by the porous nanozymes. This 'post-amplification' step significantly improves the sensitivity of the CRISPR assay. In bypassing the need for complex pre-amplification and associated primers and polymerases, we open the door to additional downstream multiplexing, in particular the simultaneous detection of proteomic samples from the sample matrix.

Results

We have shown that Crispr-Zyme can be deployed in a lateral flow-based readout – providing a colourimetric readout from a CRISPR reaction in 30 minutes, achieving limits of detection as low at 12.5 pM. As a proof of concept, we have also shown that nanozyme workflow is compatible with proteomic detection using orthogonally conjugated nanozymes to detect spiked natriuretic peptide antigens directly from the CRISPR reaction matrix with minimal cross reactivity (Figure 2).

Discussion

In combining the specificity of CRISPR/Cas13 and the robust signal amplification capacity of our nanozymes we hope to bridge the gap and deliver a platform for accessible multi-omic diagnostics. This proof of concept has been designed with cardiovascular diseases in mind, detecting long non-coding LIPCAR and NTproBNP peptides, it is likely that target selection will play a considerable part in the design of future assays as cross reactivity may be system and matrix dependent.

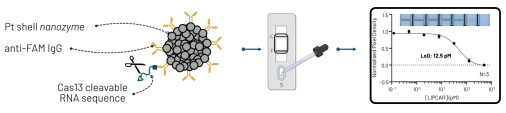


Figure 1.png

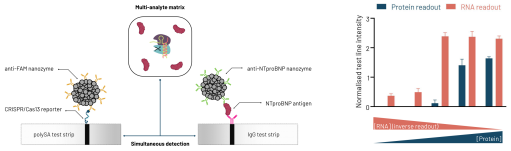


Figure 2.png

Bioconjugation of the Adeno-Associated Virus (AAV) capsid: improving the tissue-specific gene delivery

Tuesday, 16th January - 14:47: Nanomedicine for Gene Therapy and Genomics (Room 507) - Oral - Abstract ID: 145

Ms. Maia Marchand [1], ***Dr. Pierre-Alban Lalys*** [2], ***Mr. Mohammed Bouzelha*** [1], ***Dr. Juliette Varin*** [1], ***Dr. Audrey Bourdon*** [1], ***Dr. Dimitri Alvarez-Dorta*** [3], ***Ms. Karine Pavageau*** [1], ***Mr. Anthony Mellet*** [1], ***Mr. Mickaël Guilbaud*** [1], ***Dr. Sébastien G. Gouin*** [2], ***Dr. Thibaut Larcher*** [4], ***Dr. Oumeya Adjali*** [1], ***Dr. Caroline Le Guiner*** [1], ***Dr. Thérèse Cronin*** [1], ***Prof. David Deniaud*** [2], ***Dr. Mathieu Mével*** [1]

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Introduction

Adeno-associated viruses (AAV) are currently extensively investigated as vectors for gene therapy. Nevertheless, one of the remaining challenges concerning these vectors is their important tropism: this results in a restrained gene delivery to the cells of interest.

Methods

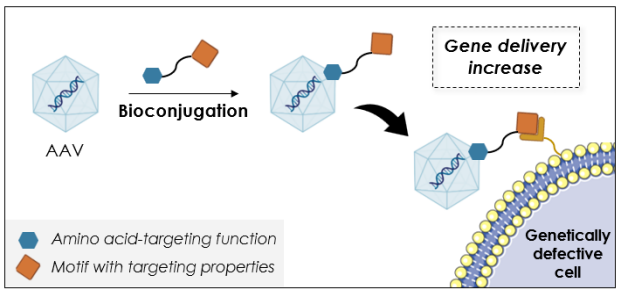
In the purpose of improving AAVs efficacy, the strategy developed by our group is the direct chemical bioconjugation of their proteins-composed capsid to introduce molecules with targeting properties. Bifunctional anchors were synthetized to bear the motif of interest, linked to an amino-acid conjugation function. Phenyl isothiocyanate or aryl diazonium salt derivatives were chosen to selectively modify lysine (K) or tyrosine (Y) residues respectively. With this strategy, functionalization of AAV capsid with carbohydrates such as *N*-acetyl galactosamine and mannose was explored, as their receptors are particularly overexpressed in liver and retina, tissues extensively investigated in gene therapy clinical studies. The methodology was also extended to coat the capsid with click chemistry anchors, to evaluate the possibility of post-functionalization with proteins (*e.g.* nanobodies) having high interaction potency with specific cell receptors. The obtained viral vectors were characterized by dot blot, western blot, mass spectrometry, and their transduction efficiency was evaluated, *in vitro* and *in vivo*.

Results

Covalent conjugation of carbohydrates and click anchors was confirmed, as well as post-functionalization with a relevant nanobody. Of note, during the transduction evaluation study, carbohydrates-modified vectors were significantly more efficient *in vivo* after systemic or subretinal injection in rodents. In more details, an interesting increase in transduction area and in the number of transduced cells could be observed, in both liver and retina.

Discussion

Those first results suggest that chemical functionalization of viral vectors has the potential to significantly improve current gene therapies. The key strength of the strategy demonstrated here is the possibility to coat viral capsids, in one step or two steps, with a broad range of functionalities, from relevant small molecules to single-domain antibody for potential highly specific targeted gene delivery. This contributes to the rational design of new treatments adapted to each genetic disease, paving the way to develop next-generation nanotherapeutics.



Bioconjugation of the aav capsid to improve tissue-specific gene delivery.png

coupled Hydrodynamic flow focusing for optimal design of microRNAs-materials complexation to improve their bio-nano interactions

Tuesday, 16th January - 15:04: Nanomedicine for Gene Therapy and Genomics (Room 507) - Oral - Abstract ID: 228

Dr. Felicia Roffo [1], ***Dr. Francesca Maria Orlandella*** [2], ***Ms. Neila Luciano*** [3], ***Prof. Giuliana Salvatore*** [2], ***Prof. Paolo Antonio Netti*** [4], ***Prof. Enza Torino*** [4]

1. *Interdisciplinary Research Center on Biomaterials, CRIB, University of Naples Federico II, P.le Tecchio 80, 80125 Naples, Italy,* ***2.*** *Dipartimento di Scienze Motorie e del Benessere, Università degli studi di Napoli Partenope, Ammiraglio Ferdinando Acton 38, 80133 Naples, Italy.,* ***3.*** *Dipartimento di Scienze Biomediche Avanzate, Università degli studi di Napoli Federico II, Pansini 5, 80125 Naples, Italy,* ***4.*** *Department of Chemical, Materials and Production Engineering, University of Naples Federico II, 80125 Naples, Italy*

The microRNAs have emerged as cancer therapeutics due to their involvement in a set of genes related to tumor initiation and development. Nevertheless, their employment is restricted by their low stability in blood circulation, limited transmembrane transport and degradation in endosomal/lysosomal vesicles. With the aim improving the bio-nano interactions of microRNAs and consequently their delivery potency, we exploited the coupled Hydrodynamic Flow Focusing (cHFF) for rational design lipid-polymer hybrid NPs (LiPoNs). Indeed, the LiPoNs are made up of a chitosan core to stabilize the microRNAs loading and the lipid bilayer to enhance the cellular uptake of the whole complex.

The one step microfluidic cHFF was performed by sheeting a microRNA-polymer solution between two lipid sides, and additionally a contrast agent, Gd-DTPA, is added to the middle solution to provide a theranostic properties to the LiPoNs. The physiochemical properties of the vectors were characterized by NanoSight, Zetasizer Nano and TEM. The changes of relaxations times were evaluated by Minispec Bench Top Relaxometer. The transfection efficacy of the LiPoNs was assessed on MDA-MB-231 cells in comparison with common transfection agent and microRNAs alone. Moreover, the expression levels of the target genes of the microRNAs were quantified from the RNA extracted by the cells with the real-time PCR. The migration ability of the cells upon the treatment with different microRNAs formulations was performed.

microRNAs and Gd-DTPA co-loaded LiPoNs reported average size around 130 nm and a slightly negative charge. The microRNAs delivery mediated by LiPoNs effectively regulate the gene of interests, that are manly related to the acquisition of invasion ability of cancers cells. Therefore, a reduction of cell mobility of MDA-MB-231 cells is reported upon the treatment with microRNAs loaded LiPoNs.

The fine processing of coupled HFF enables to control the entrapment of the microRNAs, the vector structure and consequently its features, making the LiPoNs promising theranostic tools for cancer treatment, where the lipid-polymer interface improves the stability and delivery of miRNAs. Therefore, the combination of materials provides new properties to carriers, that are not only the simple addition of the advantages of individual materials but arise from their integration.

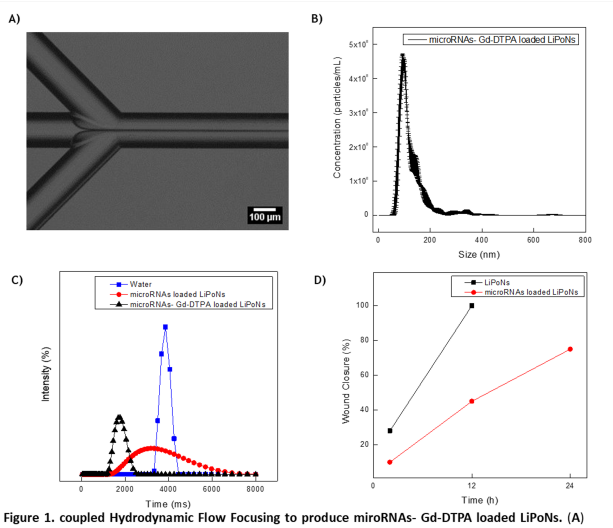


Figure 1. coupled Hydrodynamic Flow Focusing to produce miroRNAs- Gd-DTPA loaded LiPoNs. (**A**) Transmission Optical Microscopy Image of coupled Hydrodynamic Flow Focusing (cHFF) pattern; (**B**) Size and concentration distribution of microRNA-Gd-DTPA loaded LiPoNs; (**C**) Comparison of longitudinal relaxation time distributions of water, microRNA loaded LiPoNs and microRNAs- Gd-DTPA loaded LiPoNs; (**D**) Effects of the microRNAs delivered by LiPoNs on migration ability of MDA-MB-231 cells.

Figure 1.png

Amphiphilic Poly-(β-amino ester)s as a siRNA nanocarrier system for the treatment of pulmonary diseases

Tuesday, 16th January - 15:21: Nanomedicine for Gene Therapy and Genomics (Room 507) - Oral - Abstract ID: 189

Mr. Joschka Müller [1], ***Mr. Adrian Kromer*** [1], ***Ms. Katharina Steinegger*** [1], ***Prof. Olivia M. Merkel*** [1]

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Introduction:

RNA interference (RNAi) has emerged as a promising strategy for combatting pulmonary diseases. This process involves the incorporation of small-interfering RNA (siRNA) into the RNA-induced silencing complex (RISC). The RISC, facilitated by the Argonaute 2 (AGO2) protein, orchestrates mRNA degradation. To address this limitation, we investigated an amphiphilic polymer-based nucleic acid carrier system that forms micelleplexes upon contact with negatively charged siRNA, enabling gene knockdown through RNAi.

Methods:

Micelleplexes were extensively characterized using Dynamic Light Scattering (DLS), Laser Doppler Anemometry (LDA), Transmission Electron Microscopy (TEM) and validated by Molecular Dynamic Simulations. siRNA encapsulation efficiency was assessed with a SYBR Gold assay. *In vitro* eGFP knockdown was evaluated by H1299-eGFP cells using micelleplexes with varying proportions of hydrophobic side chains, and the data were analyzed by flow cytometry. To mimic a more complex *ex vivo* setting, micelleplexes encapsulating siRNA against GAPDH gene were nebulized using different vibrating mesh nebulizers (VMN) and applied to human Precision-Cut Lung Slices (PCLS) to achieve a GAPDH knockdown.

Results:

The micelleplexes, as shown in figure 1, demonstrated advantageous charasteristics with a hydrodynamic diameter between 50 and 150 nm and a low Polydispersity Index (PDI). Furthermore, the micelleplexes exhibited a slightly positive zeta potential at relevant N/P ratios while achieving complete encapsulation of siRNA. In the case of eGFP knockdown (figure 2), an increase in hydrophobic side chains resulted in a stronger knockdown. Notably, in primary human lung cells (PCLS), a knockdown of more than 60% was achieved.

Discussion:

The physicochemical data underscore good reproducibility and the potential for *in vivo* applicability. Importantly, the eGFP knockdown showed an enhancement with higher proportions of hydrophobic content, suggesting improved endosomal escape. Even following nebulization, the micelleplexes retained their effectiveness, as confirmed in the PCLS experiment. In conclusion, our nanocarrier system exhibits great promise for pulmonary RNAi delivery, offering a potential solution for treating pulmonary diseases with encouraging results.

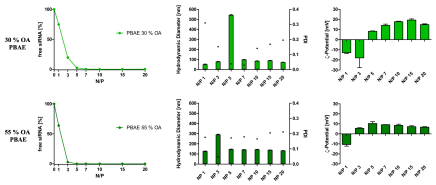


Figure 1 physicochemical characterisation.png

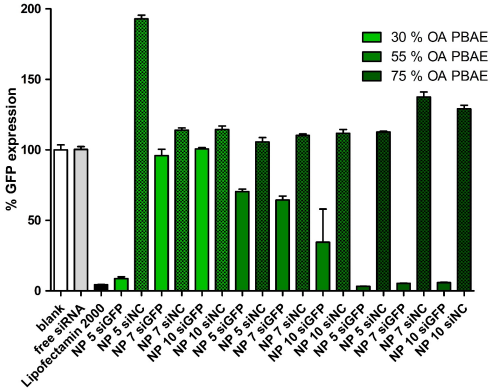


Figure 2 h1299-egfp knockdown.jpeg

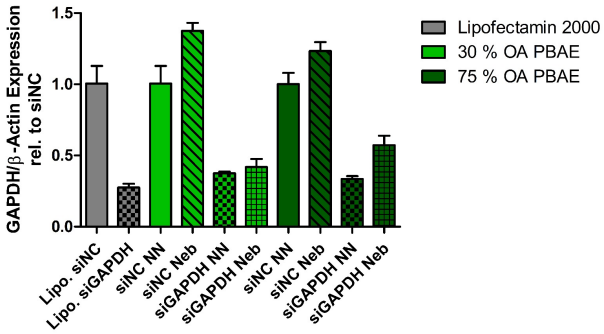


Figure 3 hpcls gapdh knockdown.jpeg

Excision tissue regeneration potential of Lysinibacillus fusiformis-formulated silver nanoparticles cream against multidrug-resistant Pseudomonas aeruginosa-infected wounds in Wistar rats

Tuesday, 16th January - 15:38: Nanomedicine for Gene Therapy and Genomics (Room 507) - Oral - Abstract ID: 15

Dr. Richard Omole [1], ***Dr. Nkem Torimiro*** [2], ***Dr. Oluwole Adeyemi*** [3], ***Prof. Saravanan Muthupandian*** [4], ***Dr. Karthik Ganesh*** [5]

1. *Microbiology Unit, Department of Applied Sciences, Osun State College of Technology, Esa-Oke,* ***2.*** *Department of Microbiology, Obafemi Awolowo University,* ***3.*** *Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University,* ***4.*** *AMR and Nanotherapeutics Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS),* ***5.*** *Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS)*

- **Introduction**

Due to the rising global trends of MDR bacterial infections, there is a need to assess the *in vivo* tissue regeneration potentials of novel therapeutic agents against multidrug-resistant (MDR) bacteria-infected wounds.

- **Methods**

Lysinibacillus fusiformis isolated previously from soil in Ile-Ife, Nigeria was used to synthesize silver nanoparticles (AgNPs). Two grams of wet biomass of the bacteria was inoculated into 1 mM $AgNO_3$ solution (300 mL) and incubated at 30 °C for 72 h. The AgNPs were observed for the colour change, confirmed by UV-vis spectroscopy, and characterized via SEM, DLS, EDX, TEM, FTIR and XRD analyses. The AgNPs cream was formulated following the *British Pharmacopoeia* protocol. Excision wounds of an approximate area of 250 mm^2 were created on Wistar rats and infected with MDR *Pseudomonas aeruginosa.* The chronic wounds were treated daily with the nano-formulated cream until complete tissue regeneration occurred. The cream base vehicle and the untreated groups served as the controls. The data obtained were expressed as mean ± standard error of the mean and were statistically analyzed.

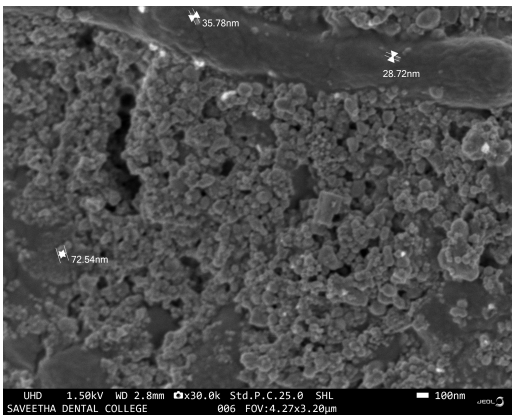
- **Results**

Lysinibacillus fusiformis produced an AgNPs solution with a deep brown colour and UV-vis absorption peak between 381 – 404 nm. The AgNPs are spherical and not agglomerated, with a size distribution of 98.88±1.46 nm, and 73.89% of zerovalent silver. However, TEM revealed the AgNPs had an average diameter of 18.42±1.47 nm. FTIR revealed amine groups, while XRD showed Bragg's peaks of crystalline silver. In the Wistar rat models, the AgNPs-formulated cream showed complete tissue regeneration against MDR Pseudomonas aeruginosa-infected wounds within 14 days.

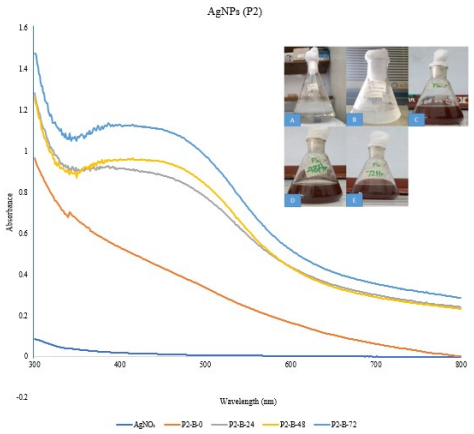
- **Discussion/Conclusion**

The study concluded that the AgNPs nano-formulated cream has antibacterial activities that could increase wound tensile strength, hasten wound contraction, and allow epithelialization and collagen synthesis to occur rapidly in multidrug-resistant wound infections, which could serve as a leader in human clinical trials.

Keywords: ***Lysinibacillus fusiformis,*** **silver nanoparticles, multi-drug resistant bacteria, chronic wounds, creams, tissue regeneration, Wistar rats**



Omole et al. 2023 agnps sem image.jpeg



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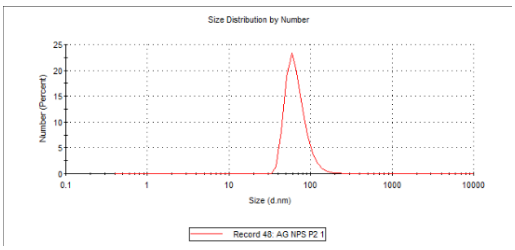

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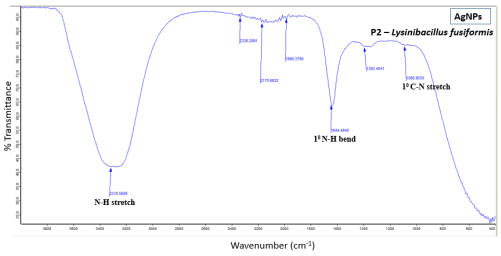

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Engineering stealth mRNA nano-vaccines for antitumor prophylaxis and therapeutics

Tuesday, 16th January - 15:55: Nanomedicine for Gene Therapy and Genomics (Room 507) - Oral - Abstract ID: 10

Dr. Coral Garcia-Fernandez [1], Dr. Tommaso Virgilio [2], Dr. Irene Latino [2], Prof. Marta Guerra-Rebollo [1], Dr. Santiago G. Fernandez [2], Prof. Salvador Borrós [1], Prof. Cristina Fornaguera [1]

1. *Grup d'Enginyeria de Materials (Gemat), Institut Químic de Sarrià (IQS), Universitat Ramon Llull (URL).,* ***2.*** *Institute for Research in Biomedicine, Faculty of Biomedical Sciences, Universita della Svitzzera italiana (USI)*

INTRODUCTION: mRNA-based vaccines symbolize a new paradigm shift in personalized medicine for the prophylaxis and treatment of infectious and non-infectious diseases. Nanomedicine has notably impacted in their development, but nanosystems available are still far from being optimized. They are not targeted and thus, distributed ubiquitously to the whole organisms and they are usually prematurely cleaned up by circulation due to immune system recognition and elimination. Thus, the design of nanoplatforms for safe and efficient mRNA delivery is pivotal to implementing mRNA-based therapies.

METHODS: We present our novel ZION polymeric nanoparticles, based on zwitterionic grafted cationic polymers, for mRNA vaccination purposes. They are a modification of our poly(beta aminoester) polymeric platform, that includes, in addition to the cationic end-terminal oligopeptides for the mRNA electrostatic encapsulation; zwitterionic grafting in the side chains, as well as a targeting peptide (Figure 1). The zwitterionic component of the carrier is meant to act as antifouling and stabilizer component in the formulation. The outstanding stealth capacity of the zwitterionic polymers was evidenced by their combination with peptide targeting, allowing efficient targeted delivery of the active ingredient only to target antigen presenting cells (APCs), overcoming side inflammatory responses due to off-site transfection.

RESULTS AND DISCUSSION: Our platform has demonstrated preclinical safety and efficient immunization. ZION nanoparticles produced remarkable levels of specific antibodies. In addition, immune cellular response in terms of CD8+ T cell proliferation evidenced the promising applicability of these carriers for cancer vaccination. This was further confirmed by *in vivo* tumor vaccination. To sum up, in this communication we present a novel family of stealth polymers that successfully deliver mRNA in a tunable way, showing unprecedented results in both prophylactic and therapeutic tumor vaccination approaches *in vivo (Figure 2)*.

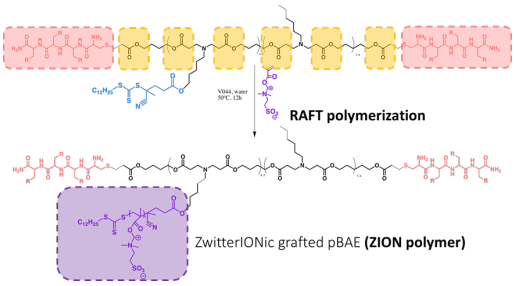

Figure1.png

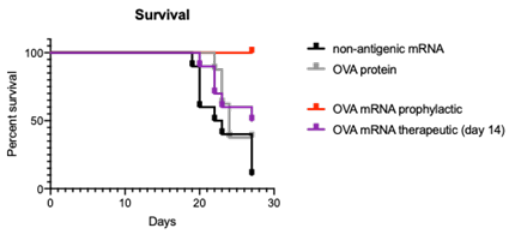

Figure2.png

Chitosan luminescent rare-earth doped nanoparticles as cancer cell tracking for imaging tumors

Tuesday, 16th January - 14:30: Imaging, diagnostics and theragnostic (Room 608) - Oral - Abstract ID: 322

Dr. Patricia Juarez Camacho [1], ***Ms. Rodrigo Osorio-Arciniega*** [1], ***Ms. Eduardo Lucero-Meza*** [1], ***Dr. Bonifacio Can-Uc*** [2], ***Dr. Pierrick GJ Fournier*** [1], ***Dr. Israel Rocha*** [1], ***Dr. Gustavo Hirata*** [2]

1. *Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California,* ***2.*** *Universidad Nacional Autonoma de Mexico*

Background: Cancer can be hard to diagnose due to the high false negatives or positives derived from technical and human mistakes. Contrast agents are used for both cancer diagnosis and tumor visualization facilitating tumor resection procedure. Nevertheless, they accumulate in specific tissues, have low-penetration, and rapid-clearance from the circulation. New contrast agents are needed to improve tumor detection.

Here, we synthesized two different luminescent nanoparticles (LNPs) conjugated with chitosan (Ch) and functionalized with folic acid (FA) for breast cancer detection. We compared *in vitro* its physicochemical characteristics and effects on cancer cell proliferation. Also, evaluated its impact on the immune system *in vivo,* and measured cluster cells using Light Sheet Microscopy (LSM).

Results: LNPs synthesized by sol-gel method and functionalized with FA using EDC□NHS coupling revealed high-intensity yellow emission at 540 nm wavelength with a quantum yield of ~ 36%. *In vitro* viability assay demonstrated biocompatibility and high improvement of cellular uptake in MDA-MB-231, and T-47D cancer cells. LSM analysis of cell-LNP interactions to visualize tagged MDA-MB-231 clusters with LNP/Ch-FA showed positive interactions due to higher stability and adherence.

Biodistribution and effects on the immune system by LNP/Ch-FA nanoparticles inoculated in mice were evaluated by flow cytometer analysis. LNP/Ch-FA were well tolerated *in vivo.* No adverse effects were observed macroscopically. Peripheral blood analysis from animals injected with LNP/Ch-FA after 2 or 6 days of the treatment showed that a single injection of the different NPs did not have any effects on the amount of circulating neutrophils or monocytes in peripheral blood when assessed 2 or 6 days after the injection. With T cells, the core NP induced a significant decrease in circulating T cells (-31%, p=0.045) that seemed specific to the CD4+ T cells (-33%, p=0.0479). However, conjugation of the NP to chitosan prevented this effect.

Conclusions: Our work presented significant evidence that allow a better understanding of LNPs-Ch-FA for possible upcoming clinical applications that could work singly or in synergy with the present detection techniques to boost accuracy and precision for cancer detection.

Low dimensional carbon-materials doped with metals as safe nanoparticles for medical imaging and temperature sensing.

Tuesday, 16th January - 14:47: Imaging, diagnostics and theragnostic (Room 608) - Oral - Abstract ID: 280

Dr. Lucia Cardo [1], ***Mr. Michele Cesco*** [1], ***Dr. Cecilia Wetzl*** [1], ***Prof. Jesús Ruíz-Cabello*** [2], ***Prof. Maurizio Prato*** [3]

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Introduction

Low dimensional (<10 nm) carbon-based materials are quasi-spherical particles, made of carbon, nitrogen and oxygen, owning excellent luminescence properties, water solubility, high biocompatibility and tuneable surfaces available for post-synthetic bio-conjugations. These nanomaterials, promising also in nanomedicine, are often known as carbon dots (CDs).[1] Our group has focused on the preparation and biological evaluation of several CDs doped with different metal ions and heteroatoms, aiming to achieve safe-by-design materials to be employed as probes for imaging and/or therapy.[2,3] Here, we describe some of our most recent CDs doped with gadolinium, manganese and fluorine (Gd, Mn, and F-CDs), as dual probes for Fluorescence Imaging (FI) and MRI, and with neodymium (Nd-CDs), as novel luminescence-based nanothermometers.

Methods

All metal-CD systems were prepared by microwave-assisted hydrothermal treatment of small organic precursors in the presence of the metal salt ('one-pot' bottom-up approach, Figure 1A). This allows fast/inexpensive preparations and controlling the tunability of the properties of the material. Importantly in the field of CDs, we established several purification strategies of the material (by combining chromatography and gel electrophoresis), to achieve homogeneous and artifact-free formulations. The behaviour of CDs for MRI has been evaluated both *in vitro* (cells) and *in vivo/ex vivo* (mice), whilst temperature-dependent emission studies of Nd-CDs were performed to assess their capability as temperature biosensors.

Results

All doped CDs obtained are highly stable with amorphous morphologies and size ranging between 5-10 nm. They are cell permeable, accumulating into the cytosol, as observed in X-ray fluorescence spectroscopy (XRF) at synchrotron facilities (Figure 1B). F, Gd and Mn-CDs show relaxivity values comparable or higher than commercially available contrast agents and, most importantly, they are detectable by MRI in *in-vivo* biodistribution experiments (e.g., Gd-CDs, Figure 1C and D).[3] Besides, our Nd-CD sensor displays emission profiles (λ_{ex} 808 nm, biological window II) linearly depending on temperature variations (5-60 °C, Figure 1E) with good reversibility/stability and signal detection through *ex-vivo* tissues up to 5 mm depth.

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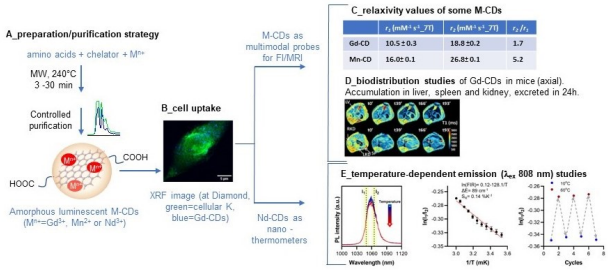
[2] D. Mattinzoli, et al. *Materials Today Bio*. **2022**, 16, 100286.

[3] L. Cardo et al. *Small* **2023**, 19, 2206442.



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Dual Functional Nanocone Clusters as Cavitation Nuclei for Nanoparticle-Mediated Histotripsy

Tuesday, 16th January - 15:04: Imaging, diagnostics and theragnostic (Room 608) - Oral - Abstract ID: 234

Mr. Mohammad Abou Chakra [1], Mr. Waleed Mustafa [1], Ms. Sarah Hall [2], Ms. Cemran TOYDEMIR [1], Prof. Ozge SENSOY [1], Dr. Eli Vlaisavljevich [2], Dr. Yasemin Yuksel Durmaz [1]

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Histotripsy is a non-invasive and non-thermal ultrasound ablation technique, which mechanically ablates the tissues using very short, focused, high-pressured ultrasound pulses to generate dense cavitating bubble cloud [1]. Histotripsy requires very large negative pressures (≥28MPa) to generate cavitation in a target tissue. Recently, we have developed nanoparticle-mediated histotripsy (NMH) to significantly reduce the cavitation pressure of histotripsy. Perfluorocarbon (PFC) filled nanodroplets (NDs) were used as cavitation nuclei for histotripsy, as they are able to significantly lower the cavitation threshold[2]. Nanocone clusters (NCC) were the next generation agent of NMH which addresses the limitations of previously designed NDs. NCC can be obtained by simply mixing FDA-approved cyclodextrins (CD) and suitable PFC, which result in smaller size aggregates, detectable PFC amount, and more stable long-term storage[3]. Moreover, NCC can be functionalized to allow bioconjugation and combination of different treatments[4]. Even if histotripsy allows effective tumor ablation, remaining trace of cancer cell can be treated with drug loaded NCC since they can be selectively accumulated into tumor tissue through their special surface modification.

Experimentally and computationally studies were done to understand the organization of inclusion complexes of CD and PFC around free PFC droplets. βCD as a building block was monofunctionalized with the groups like azide, alkyne, and amine to obtain functional NCC for most used bioconjugation such as targeting, PEGylation and fluorescence labeling (Figure 1). Moreover, incorporation of Doxorubicin (Dox) as anticancer agent into NCC was systematically studied (Figure 2). Obtained bioconjugated and drug loaded NCC were tested as cavitation nuclei using a 500 kHz histotripsy transducer to apply single cycle histotripsy pulses with a pulse repetition frequency of 0.5 Hz (Figure 3).

Functional NCC was used for bioconjugation to obtain labelled or targeted particle to show the potential for future combinational treatment. Dox has been incorporated into NCC eventhough it competes with PFH. Both bioconjugated and drug loaded NCC were able to lower the cavitation threshold indicating the potential as dual functional particles for tumor ablation and fighting against remaining cancer cells.

[1]Xu,Z., *Int. J. Hyperth.* **2021**,*38* (1),561.[2]Vlaisavljevich,E,*Theranostics*, 2013, 851.[3]Kaymaz,B.; *Molecular Pharmaceutics*,**2022**, *19* (8), 2907. [4]Toydemir,C.; *Biomacromolecules*,**2022**, *23* (12), 5297.

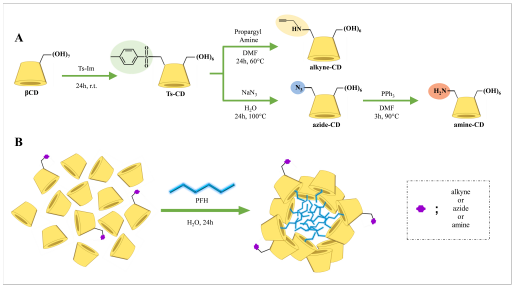

Figure 1. preparation of functional ncc.png

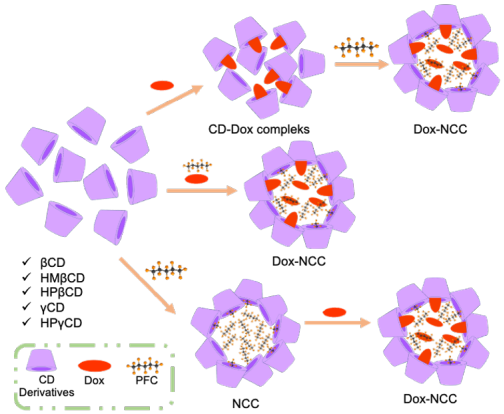

Figure 2. strategies to load dox into ncc.png

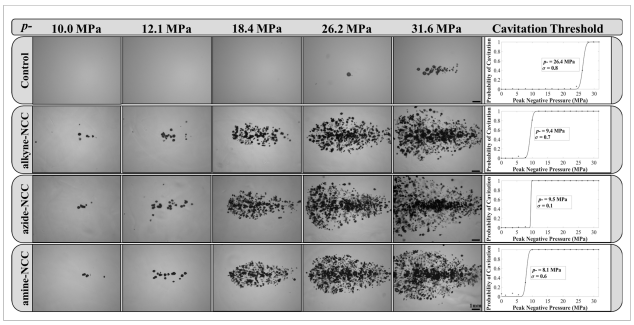

Figure 3. functional ncc lowers the cavitation threshold of histotripsy.png

Application of λ-Carrageenan oligosaccharides as innovative sugar-based biofunctional coating for theranostic ferrite nanoparticles

Tuesday, 16th January - 15:21: Imaging, diagnostics and theragnostic (Room 608) - Oral - Abstract ID: 157

Dr. Manon Porta Zapata [1], ***Dr. Susana Carregal Romero*** [2], ***Mr. Clément Daviaud*** [1], ***Dr. Lydia Martinez-Parra*** [3], ***Dr. David Castejón*** [4], ***Ms. Ainhize Urkola-Arsuaga*** [5], ***Ms. Chanez Manseur*** [1], ***Prof. Jean-Marie Piot*** [1], ***Prof. Ingrid Fruitier-Arnaudin*** [1], ***Prof. Jesús Ruíz-Cabello*** [2], ***Dr. Hugo Groult*** [1]

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Polysaccharides (PS) are well-known as valuable coatings for designing multifunctional **nanoparticles (NP)** intended for advanced biomedical applications.[1] However, these applications typically **focus on specific PS varieties, neglecting numerous other families** with untapped potential, **including λ-carrageenan (λ-CAR)**. The λ-CAR is **a galactan-based PS that displays promising antitumoral effects**. Still, its ***in vivo*** **use is strongly restrained due to high viscosity and adverse proinflammatory** and anticoagulant properties.[2] **The depolymerization** of the λ-CAR **into oligosaccharides (λ-COS)** can overcome these issues, offering new candidates with improved innocuity and biological specificity.[3] **Surprisingly, such λ-COS have not yet been included as coatings for any NP**. In this study, we proposed ferrite λ-COS coated NP to assess ground-breaking pharmacokinetic (PK) properties and antitumor performances.

Native λ-CAR was depolymerized using an H_2O_2 method to produce a 4 kDa λ-COS derivative. **Confirmation of the removal of the inflammatory and anticoagulant activities was obtained** through dosage of typical markers in macrophage cell culture and coagulometry tests. Conversely, **the preservation of a specific anticancer activity-namely the inhibition of heparanase (HPSE)**, a key enzyme involved in tumour progression[4] **was validated** using FRET-based assays. **λ-COS coated ferrite NP were next prepared through a single-step microwave-assisted method**, adapted from a previous published protocol.[5] **A comprehensive physicochemical description of the NP was conducted**, including electronic microscopy (TEM, SEM), advanced spectroscopy (EDS, XPS, Raman, FTIR), magnetic measurements and colloidal stability analysis (DLS, ELS). It showed **stable NP** (Fe/Mn ratio of 1.5) of 44 nm, with ζ-potential of -27 mV, and relaxivity values **suitable for T1/T2 versatile use as MRI contrast agents**. **The antitumor performance of the λ-COS was effectively imparted when used as NP coating**, as demonstrated on breast cancer cells-based assays. Finally, their PK properties after intravenous administration. was studied in healthy mice by MRI monitoring, supported by *ex vivo* dosage of Mn in the organs. The results revealed the **coveted combination of a prolonged vascular lifetime (>3 hours) and relatively fast hepatobiliary clearance**. These findings and perspective of therapeutic assays on a rodent-based cancer model will be discussed in the context of the current challenges in the field of nanotherapeutics.

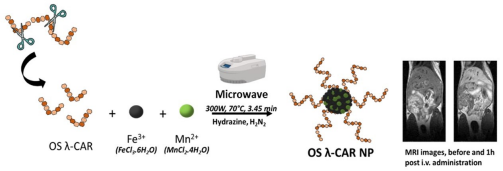

Image abstract iconan.jpg

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(2) Guo, Z.; Wei, Y.; Zhang, Y.; Xu, Y.; Zheng, L.; Zhu, B.; Yao, Z. Carrageenan Oligosaccharides: A Comprehensive Review of Preparation, Isolation, Purification, Structure, Biological Activities and Applications. *Algal Research* **2022**, *61*, 102593. https://doi.org/10.1016/j.algal.2021.102593.

(3) Groult, H.; Cousin, R.; Chot-Plassot, C.; Maura, M.; Bridiau, N.; Piot, J.-M.; Maugard, T.; Fruitier-Arnaudin, I. λ-Carrageenan Oligosaccharides of Distinct Anti-Heparanase and Anticoagulant Activities Inhibit MDA-MB-231 Breast Cancer Cell Migration. *Marine Drugs* **2019**, *17* (3), 140. https://doi.org/10.3390/md17030140.

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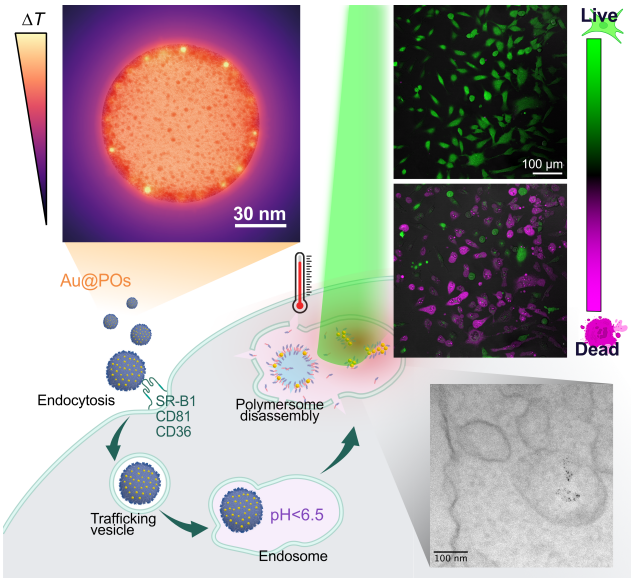
Designing hybrid polymersomes for intracellular thermoplasmonics

Tuesday, 16th January - 15:38: Imaging, diagnostics and theragnostic (Room 608) - Oral - Abstract ID: 276

Dr. Valentino Barbieri [1], ***Mr. Javier González Colsa*** [2], ***Dr. Diana Matias*** [3], ***Dr. Aroa Duro Castaño*** [4], ***Ms. Anshu Thapa*** [4], ***Dr. Pablo Albella*** [2], ***Prof. lorena Ruiz*** [5], ***Prof. Giorgio Volpe*** [6], ***Prof. Giuseppe Battaglia*** [7]

1. *Department of Chemistry, University College London (UK). Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC, Spain),* ***2.*** *Group of Optics, Department of Applied Physics, University of Cantabria (Spain),* ***3.*** *Department of Chemistry, University College London (UK). Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC, Spain). Instituto de Medicina Molecular João Lobo Antunes (iMM), Lisbon, (Portugal).,* ***4.*** *Department of Chemistry, University College London (UK),* ***5.*** *Institute for Bioengineering of Catalonia (IBEC, Spain). Department of Applied Physics, University of Barcelona, Barcelona (Spain),* ***6.*** *Department of Chemistry, University College London,* ***7.*** *Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC, Spain). Department of Chemistry, University College London (UK). Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, (Spain).*

For decades gold nanoparticles (AuNPs) have been proposed as photothermal therapeutic agents thanks to their thermoplasmonic properties. However, their colloidal instability in crowded biological environments highly reduces their cellular uptake and bioavailability. Polymersomes – vesicles self-assembled from amphiphilic block copolymers – have shown great potential as nanocarriers for the precise intracellular delivery of therapeutic agents and diagnostic probes. Hence, the integration of AuNPs within polymersome membranes represents a stepping stone to enable the use of both these nanomaterials in medical scenarios that benefit from photothermal stimulation. Yet, it is unclear whether such hybrid polymersomes could be produced with efficient thermoplasmonic properties without altering the typical morphology, stability and cell-targeting functionality of polymersomes in biological environments. Here we show that hybrid polymersomes (Au@POs) produced by the *in situ* synthesis of 2-nm AuNPs in the membranes of poly[(2-methacryloyl)ethyl phosphorylcholine]-*block*-poly[2-(diisopropylamino)ethyl methacrylate] (PMPC–*b*-PDPA) polymersomes, can be synthesized with excellent morphological control and superior stability. The efficient collective absorption of AuNPs within Au@POs can generate a strong thermoplasmonic response resulting in temperature increments well above 10 K upon laser illumination of dilute suspensions. To rationalize our findings, we develop a multiscale theoretical framework able to predict the global temperature evolution in Au@PO ensembles starting from structural and compositional information. Our fully analytical theoretical model holds potential towards the rational design of novel photothermal agents in accurate medical scenarios. We then proceed to characterize the Au@PO uptake and intracellular accumulation of Au-polymer assemblies. The interaction between the PMPC blocks in the polymersome brush and the SR-B1, CD36 and CD81 receptors expressed on the surface of cancer cells, induces a 40-fold increase in uptake compared to that of benchmark PEGylated AuNPs. We demonstrate that after Au@PO uptake, hybrid Au-polymer assemblies accumulate in trafficking organelles, where they can be stimulated by a low-power scanning laser to induce the rapid photothermal death of cancer cells *in vitro*. We envision that our nanotechnological platform could be translated to other ligand-receptor combinations by engineering the Au@PO surface to design effective photothermal agents capable of selectively providing intracellular heating to specific cell phenotypes and tissues.



Graphical abstract.png

Porphyrin-Based Single-Chain Polymer Nanoparticles for Photodynamic Therapy

Tuesday, 16th January - 15:55: Imaging, diagnostics and theragnostic (Room 608) - Oral - Abstract ID: 22

Prof. Binnur Aydogan Temel [1], ***Ms. Zehra Demir*** [2], ***Ms. Esma Samuk*** [2], ***Prof. Gokhan Temel*** [3], ***Dr. Dilek Ozturk Civelek*** [4], ***Prof. Demet Karaca Balta*** [5]

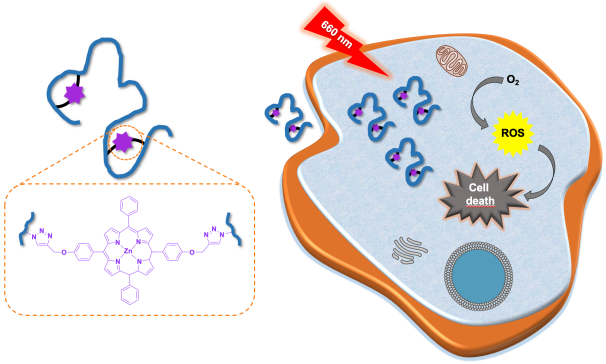
1. *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, Turkey,* ***2.*** *Department of Biotechnology, Institute of Health Sciences, Bezmialem Vakif University, Istanbul, Turkey,* ***3.*** *Department of Polymer Material Engineering, Faculty of Engineering, Yalova University, Yalova, Turkey,* ***4.*** *Department of Pharmacology, Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, Turkey,* ***5.*** *Chemistry Department, Yildiz Technical University, Istanbul, Turkey*

Recently, photodynamic therapy (PDT) which involves accumulation of photosensitizers (PSs) in cancer cells, activation of PSs with specific wavelength of light and formation of reactive oxygen species (ROS) via energy transfer, has become one of the most attractive approaches in cancer treatment. Upon irradiation, PSs in the excited state react with the oxygen in the tissue, causing the formation of cytotoxic species such as singlet oxygen and toxicity in cancer cells. Therefore, the photosensitizer plays an important role in PDT and determines its efficacy. Porphyrin and its derivatives have received great attention as second generation photosensitizer due to production of effective singlet oxygen and low side effects in the body. However, porphyrin PSs are still not widely applied in clinical PDT, as most of them are aromatic molecules with limited selectivity, poor water solubility, and limited bio-dispersion. Various approaches have been developed to overcome these limitations and to improve the properties of porphyrin photosensitizers such as introducing hydrophilic segments onto porphyrin structure and conjugation porphyrins to lipids or polymers. One of these approaches could be the use of single□chain polymer nanoparticles (SCNP) which are formed by collapsing or folding a single polymer chains through intrachain crosslinking in dilute solution. Due to the individual folding or collapse of linear polymer chains, single-chain polymer nanoparticles are much smaller than conventional polymer nanoparticles (3-30 nm in size).

In this study, we aimed to synthesize SCNPs by using porphyrin structures directly as crosslinkers and investigate their potential in photodynamic therapy. Various hydrophilic or amphiphilic copolymers were synthesized via reversible addition-fragmentation chain-transfer (RAFT) polymerization. Porphyrin containing SCNPs were formed by click reaction. The resulting polymers and SCNPs were characterized using GPC, FT-IR, ^{1}H NMR, UV-Vis, fluorescence and DSC analyses. The sizes of single-chain polymer nanoparticles were characterized by DLS and TEM. The stability of nanoparticles and their ability to produce singlet oxygen were investigated and in vitro cell uptake, singlet-oxygen production and phototoxicity were determined by cell studies.

Keywords: Single-chain polymer nanoparticles, photodynamic therapy, porphyrin, singlet oxygen.

Acknowledgements: This work was financially supported by the Scientific and Technological Research Council of Turkey (Project No: 122Z054).



Schematic representation of porphyrin-based single-chain polymer nanoparticles for pdt..png

NanoSwimmers: Precision Navigation & Amyloid Protein Repair

Tuesday, 16th January - 16:50: Nanorobotics and Biomimetics (Auditorium) - Oral - Abstract ID: 86

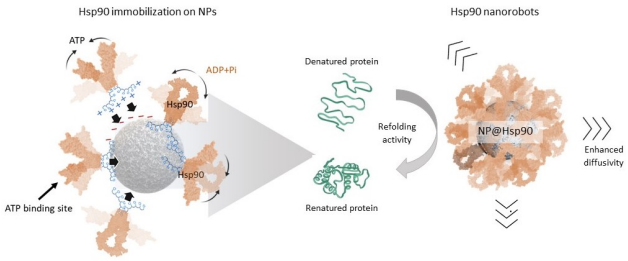
Prof. Mónica L. Fanarraga [1]

1. *Instituto de Investigación Valdecilla-IDIVAL; Universidad de Cantabria, Avda. Herrera Oria s/n, Santander, 39011, Spain*

This study presents a novel self-propelled versatile nanosystem for nanoscale biocompatible tissue exploration, powered by ATP, which is the universal cellular energy. These hybrid nanoparticles act as nanorobots with enhanced mobility, but also with the remarkable ability to perform critical tasks, including environmental sensing and real-time protein repair. These properties are due to their unique biocoating comprising the heat shock protein Hsp90. This is a molecular chaperone with a natural affinity for capturing, repairing, and refolding many client proteins, including amyloid. Nanoswimmers function effectively in a variety of biological media, depending on the concentration of ATP.

In view of the increasing prevalence of neurodegenerative diseases associated with the accumulation of amyloid deposits, we investigate their potential to prevent the assembly of amyloid oligomers, which is crucial in neurodegenerative diseases. Our results highlight the efficacy of these nanorobots as in vitro inhibitors of amyloid formation.

In essence, this research highlights how the fusion of biotechnology and material science is ushering in a transformative era in nanomedicine, with unprecedented prospects in healthcare and beyond. This innovation opens up a wide range of applications, from precision drug delivery to real-time protein repair and the prevention of amyloid-related diseases.



Nanorobot toc.jpg

Self-powered nanoparticles for smart cancer therapy: Design, functionalisation, and in vitro studies

Tuesday, 16th January - 17:07: Nanorobotics and Biomimetics (Auditorium) - Oral - Abstract ID: 277

Dr. Arathyram Ramachandra kurup Sasikala [1]

1. *University of Bradford*

Introduction:

In recent years, there has been growing interest in developing nanomedicine treatment for cancer due to its multifunctional properties and improved performance. The use of multifunctional nanoparticles in conjunction with light, magnetic field, electric field, and ultrasound has resulted in several non-invasive cancer treatment approaches with promising results. Piezoelectric nanoparticles have recently been used as a theranostics platform due to their biocompatibility, nonlinear optical properties, high X-ray attenuation coefficient, near-infrared absorbance, and long circulation half-life. Here, we report the development of an ultrasound-responsive smart nanocarrier system (USNC) using piezoelectric barium titanate nanoparticles (BTNPs) as a potential cancer theranostics platform.

Methods:

The hydrophilic and nanosized BTNPs were prepared by a muti-step approach with some modifications. An anticancer drug doxorubicin (DOX) was covalently conjugated to the carboxyl group of BTNPs in a pH-dependent manner. The USNCs were characterised using TEM, DLS, XRD, FTIR and Raman. Piezoelectric properties of the USNCs were measured using the ultrasound-driven voltage generation from the nanoparticles. In vitro studies such as the USNCs viability, intracellular localisation and anticancer effects were studied in human neuroblastoma (SHSY-5Y) and breast cancer (MCF7) cell lines in 2D and 3D cell cultures.

Results and Discussion:

The characterisation studies confirmed that the nanoparticles are of size less than 100 nm with excellent piezoelectric properties and enhance colloidal stability after functionalisation. The DOX release profile confirmed ultrasound and pH-sensitive release from the nanocarriers. The label-free Second Harmonic generation (SHG) imaging capability of the USNCs enabled us to track their uptake by cells in the 2D and 3D cell cultures. The excellent piezoelectric property of the USNCs enabled the effective penetration of the tumour spheroids with the help of ultrasound. Thus, the theranostics effects of these nanoparticles demonstrated that the pulsed US improved the penetration of these nanocarriers and the delivery of DOX in both 2D monolayers and 3D tumour spheroids cultures resulting in an enhanced anticancer effect.

Conclusion

The uniquely modified BTNPs (USNCs) can be used as an excellent anticancer theranostics platform with smart properties.

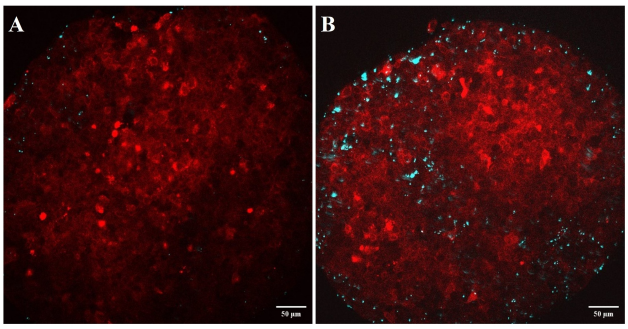


Figure1: Two photon microscopy image showing the ultrasound induced translocation of the USNCs in 3D breast cancer spheroid models (at 140 µm depth). A) US untreated group, B)US stimulated group. Here cyan colour indicates the SHG signals from the USNCs. For imaging of the breast cancer spheroids, MCF cells were pre-labelled with Cell Tracker Deep Red.

Figure 1.jpg

Chemotaxis of asymmetric liposomes

Tuesday, 16th January - 17:24: Nanorobotics and Biomimetics (Auditorium) - Oral - Abstract ID: 233

Ms. Barbara Borges Fernandes [1], ***Dr. Subhadip Ghosh*** [1], ***Dr. Ian Williams*** [2], ***Dr. Joe Forth*** [3], ***Prof. lorena Ruiz*** [1], ***Prof. Giuseppe Battaglia*** [4]

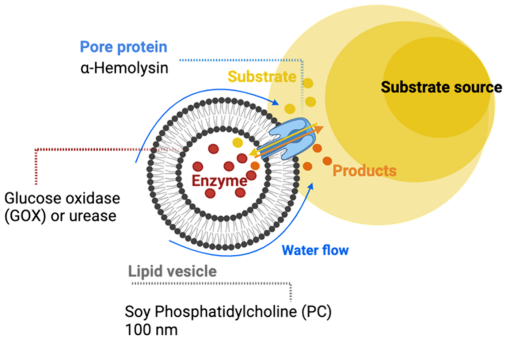
1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Department of Physics, University of Surrey,* ***3.*** *Department of Chemistry, University of Liverpool,* ***4.*** *Institute for Bioengineering of Catalonia*

Active systems have gained significant interest across various scientific disciplines. Active motion is an important phenomenon in nature, particularly when related to concentration gradients, a behavior known as chemotaxis. Examples include the directed movement exhibited by bacteria and neutrophils, orchestrated through intricate signaling pathways. In synthetic systems, active and chemotactic behavior has been demonstrated in Janus particles and colloidal structures such as polymersomes and liposomes.

This study investigates a novel active system composed of liposomes with encapsulated enzymes. Asymmetry is introduced by incorporating the pore protein alpha-hemolysin, facilitating the diffusion of substrate and products across the vesicle membrane. The resulting asymmetric distribution of species creates a slip velocity on the liposome's surface, resulting in self-propulsion.

The nature of the substrate proves to be a critical factor influencing the velocity and direction of the drift of liposomes in a microfluidic channel. Phenomena such as diffusioosmosis and diffusioosmophoresis emerge as inherent components of the motion we measured by tracking polystyrene beads in a gradient of urea and glucose. These phenomena are also present in the movement of porated liposomes. In addition to them, a chemotaxis component is also observed for the liposomes that have pores. The drift direction and velocity result from all these events and depend on the enzyme/ substrate pair.

This research sheds light on some fundamental principles governing liposome chemotaxis with encapsulated enzymes. This system can offer insights into the chemotactic behavior of some natural vesicles and can also be applied in diverse fields, including drug delivery. The interplay between substrate properties, surface interactions, enzyme reactions, and asymmetry opens new horizons for further exploration of chemotaxis in biochemical systems.



Graphic representation of a chemotactic asymmetric liposome.png

Swarms of enzyme powered nanomotors with photothermal and magnetic properties for immunogenic cell death

Tuesday, 16th January - 17:41: Nanorobotics and Biomimetics (Auditorium) - Oral - Abstract ID: 137

Dr. Juan Fraire [1], ***Ms. Lena Kesio*** [1], ***Dr. Anna Bakenecker*** [1], ***Dr. Maria Guix*** [2], ***Prof. Samuel Sánchez*** [3]

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Immunogenic cell death (ICD) is a process where damage-associated molecular patterns (DAMPs), such as ATP and calreticulin (CLRT), are released or exposed at the cell's surface. ICD has emerged as a promising strategy for enhancing the efficacy of cancer immunotherapy. Recent studies have demonstrated that ICD can be induced by means of light-triggered effects without the need for chemotherapeutics with potential side effects.[1] Light-responsive nanomaterials could improve ICD induction further if they could collectively displace and penetrate more efficiently into tumors. Advanced nanomaterials able to convert chemical energy into motion or nanomotors (NMs) are being actively explored due to their ability to overcome different biological barriers and to collectively displace in the form of swarms.[2] At the upfront are urease-powered NMs due to their biocompatibility, biodegradability, and the possibility of using urea as fuel at physiological concentrations to power their motion.[3] When iron oxide as chassis of these motors it is possbile to combine motion capabilities with photothermal and magnetic properties.[4]

In this work we investigated the magnetic navigation capabilities of swarms of urease-powered iron oxide nanomotors (IONMs), guided by external magnetic fields, for enhanced and selective displacement and accumulation in desired regions of 3D-printed phantom models. In addition to their navigational abilities, we evaluated the photothermal properties of the iron oxide nanoparticles for induction of vapor nanobubbles (VNBs) formation upon irradiation with a pulsed laser to induce selective cell killing. As light-triggered cell death by means of VNBs holds the potential for generating DAMPs crucial to activating anti-tumor immune responses, we proceed to characterize the release of ATP and CLRT exposure in treated samples.

The combination of magnetic navigation and photothermal properties of IONMs proved to have clear potential for the selective displacement and accumulation of NMs and to induce the release and exposure of ICD hallmarks upon irradiation.

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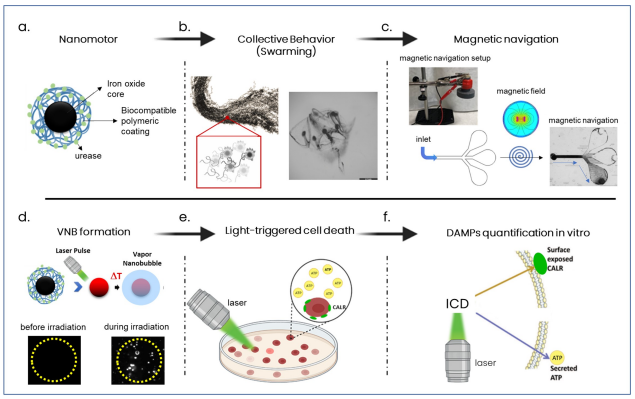
(3) Arqué, X.; et al. *Chem. Sci.* **2022**, *13* (32), 9128–9146.

(4) Fraire, J. C.; et al. *ACS Nano* **2023**, *17* (8), 7180–7193.



Swarms of enzyme powered nanomotors with photothermal and magnetic properties for immunogenic cell death

a) Synthesis and characterization of nanomotors,
b) Motion analysis and collective behavior (swarming).
c) Magnetic navigation and control of the collective propulsion.
d) Vapor nanobubbles formation upon pulsed laser irradiation..
e) Light-triggered cell death.
f) Release of DAMPs associated to ICD upon irradiation of the nanomotors.

Figure 1.jpg

Cellsomes as novel biomimetic nanocarriers for the treatment of cardiovascular diseases

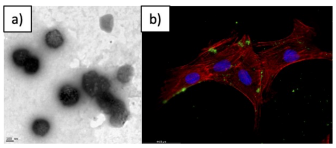
Tuesday, 16th January - 17:58: Nanorobotics and Biomimetics (Auditorium) - Oral - Abstract ID: 327

Prof. Pablo Taboada [1], ***Ms. Mariangel Luna*** [1], ***Ms. Alba Costa*** [1], ***Mr. Osvaldo Beltrán*** [1], ***Mr. Alejandro Varela*** [1], ***Mr. Oscar Abelenda*** [1], ***Mr. Noé Escareño*** [1], ***Dr. Silvia Barbosa*** [1]

1. *University of Santiago de Compostela*

One of main aims of nanotechnology is to develop newdrug delivery platforms that effectively reduce systemic toxicity of currently used drugs while retaining their pharmacological activity. To this purpose, organic and inorganic materials have been manipulated at the micro- and nanoscale to synthesize drug nanocarriers. In the development of such materials, bioinspired approaches have emerged as an alternative to counteract the extraordinary ability of our body to recognize, label, sequester, and clear foreign objects. The translation of the structural complexity, and features of cell membranes to nanocarriers offers exciting opportunities to fabricate next-generation biomimetic nanomaterials with enhanced pharmacokinetic, biointerfacing and tissue-specific homing capabilities while preserving the key functionalities of the parent cells.

In this work, we developed biomimetic cell membrane-based nanovesicles and cell membrane-based particle nanocoatings (cellsomes) from murine macrophages and platelets (see Figure), which play a key role in atherosclerosis genesis and development, the main underlaying factor in cardiovascular diseases. Thanks to the biomimicry and homing ability of such cellsomes, the statin drug pravastatin and cytokine IL-10 encapsulated inside these cellsomes as bioactive cargoes were targeted to the inflamed endothelium and their therapeutic activity locally exploited in order to achieve the reduction of cholesterol accumulation and reactive oxygen species levels as well as the increase in athero-protective anti-inflammatory cytokines and cholesterol efflux levels, as demonstrated in vitro and in vivo.



a) TEM image of cellsomes; b) internalization of cellsomes in endothelial cells.

Figure.png

DNA origami directed virus capsid polymorphism

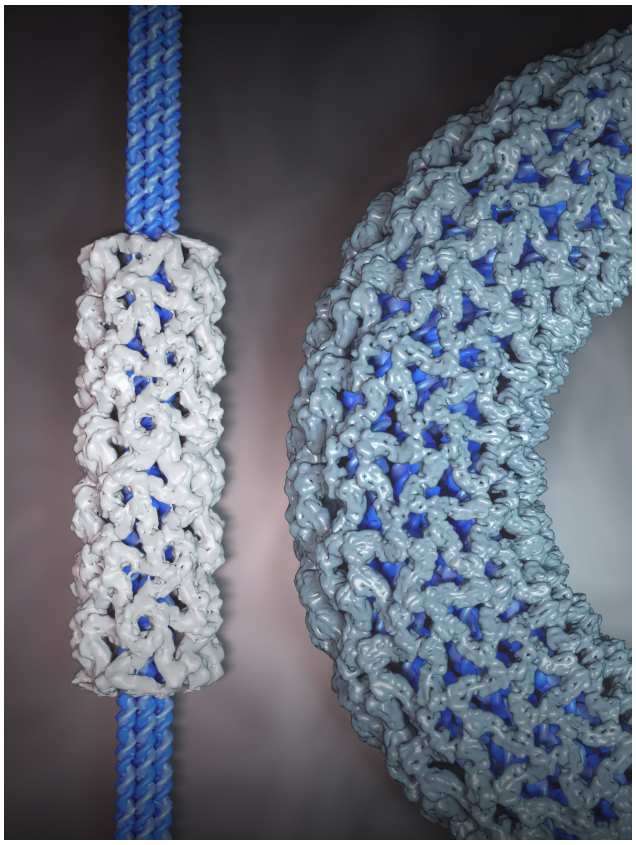
Tuesday, 16th January - 18:15: Nanorobotics and Biomimetics (Auditorium) - Oral - Abstract ID: 172

Prof. Mauri Kostiainen [1]

1. *Aalto University*

Most known viruses protect their genome by encapsulating it inside a protein capsid. Viral capsids can adopt various geometries, most iconically characterized by icosahedral or helical symmetries. The assembly process of native capsids is highly cooperative and governed by the protein geometry, protein-protein as well as protein-nucleic acid interactions. Importantly, the high control over the size and shape of virus capsids would have advantages in the development of new vaccines and delivery systems. However, tools to direct the assembly process in a programmable manner are exceedingly elusive or strictly limited to specific structures. Here, we introduce a modular approach by demonstrating DNA origami directed polymorphism of single protein subunit capsids. We achieve control over the capsid shape, size, and topology by employing user-defined DNA origami nanostructures as binding and assembly platforms for the capsid proteins. Binding assays and single-particle cryo-electron microscopy reconstruction show that the DNA origami nanoshapes are efficiently encapsulated within the capsid. Further, we observe that helical arrangement of hexameric capsomers is the preferred mode of packing, while a negative curvature of the origami structure is not well tolerated. The capsid proteins assemble on DNA origami in single- or double-layer configurations depending on the applied stoichiometry. In addition, the obtained viral capsid coatings are able to efficiently shield the encapsulated DNA origami from nuclease degradation. Our approach is, moreover, not limited to a single type of virus capsomers and can also be applied to RNA–DNA origami structures. We have for example demonstrated folded mRNA structures and identified key folding strategies to enable protein translation, without a separate origami unfolding step. Therefore, these findings may in addition find direct implementations in next-generation cargo protection and targeting strategies.

Seitz, I.; Saarinen, S.; Kumpula, E.-P.; McNeale, D.; Anaya-Plaza, E.; Lampinen, V.; Hytönen, V. P.; Sainsbury, F.; Cornelissen, J. J. L. M.; Linko, V.; Huiskonen, J. T.; Kostiainen, M. A. DNA Origami Directed Virus Capsid Polymorphism, *Nature Nanotechnology*, **18**, 1205-1212 (2023). (https://doi.org/10.1038/s41565-023-01443-x)



Scheme 1.jpg

Targeting macrophage polarization states for precision immunotherapy

Tuesday, 16th January - 16:50: Nano-immunology - I (Room 507) - Oral - Abstract ID: 140

Ms. Lara Victoria Aiassa [1], ***Dr. Pablo Scodeller*** [2], ***Mr. Peter Pfeifer*** [3], ***Prof. Loris Rizzello*** [4], ***Prof. Giuseppe Battaglia*** [5]

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Macrophages are crucial immune system components, safeguarding our tissues from external threats such as injuries, toxins, and infections [1]. When faced with an insult, resident macrophages initiate the inflammatory process, transitioning from a resting state (M0) to an activated state and changing their effector function into a pro-inflammatory (or M1) and anti-inflammatory (or M2) phenotype [2]. This dynamic activation of macrophages plays a pivotal role in disease progression and can lead to unresolved inflammation if impaired. To address this, macrophage-targeting nanomedicines have emerged as a revolutionary approach for treating a wide range of human diseases, including infections, chronic inflammatory disorders, neurodegenerative diseases, and cancer. Traditionally, targeted strategies have relied on high-affinity ligands like antibodies. However, these interactions can lead to indiscriminate targeting of any cell expressing the corresponding receptor, resulting in a loss of selectivity. One strategy to overcome such a challenge involves employing low-affinity ligands within a multivalent scaffold, thereby achieving super-selectivity [3]. This approach relies on the collective effect of individual affinities, ensuring that associations only occur when receptors are expressed at specific densities, effectively targeting cells expressing the desired receptor while minimizing non-specific interactions. We propose using engineered polymer-based self-assembled nanoparticles (polymersomes) where multiple ligands are expressed alongside polymers that prevent non-specific interactions and act as steric modulators (Fig.1) [4]. *In-vitro* experiments show that nanoparticle binding to the cell surface is non-linear, dependent on the number of ligands (NL) present (Fig. 2). This behavior allows for identifying an optimal ligand density, creating on-off association profiles that enable precise targeting of specific macrophage phenotypes. Through this approach, we can achieve phenotypic targeting of macrophages while enhancing selectivity and therapeutic efficacy.

1] T.A. Wynn, A. Chawla, and J.W. Pollard, **Nature**, 496, 7446, 445–455 (2013).
[2] P.J. Murray et al., **Immunity**, 41(1), 14–20 (2014).
[3] Martinez-Veracoechea, F. J. & Frenkel, **Proc. Natl Acad. Sci. USA**,108,10963–10968 (2011).
[4] S. Acosta-Gutiérrez et al., **ACS Cent. Sci.**, 8, 7, 891–904 (2022).

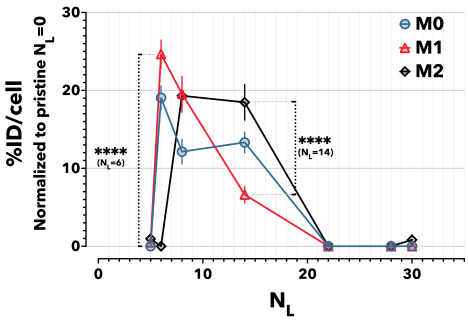

Fig2.on-off association profile np binding as a function of the nl.jpg

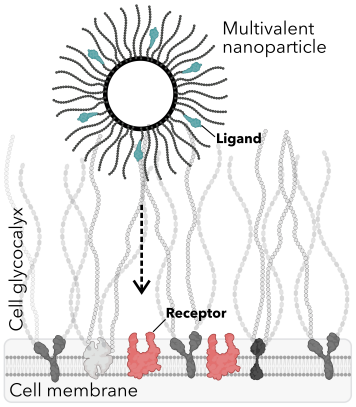

Fig1.cell-np interaction simplified.jpg

Development of gold nanoparticle-bacterial biohybrids for biomedical applications

Tuesday, 16th January - 17:07: Nano-immunology - I (Room 507) - Oral - Abstract ID: 28

Dr. Lorena Vázquez-Iglesias [1], ***Ms. Lara González Cabaleiro*** [1], ***Dr. Gustavo Bodelón*** [2], ***Prof. Jorge Pérez-Juste*** [1], ***Dr. Isabel Pastoriza-Santos*** [3]

1. CINBIO, Universidade de Vigo, Campus Universitario As Lagoas Marcosende, Vigo 36310, Spain; Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO, Vigo 36310, Spain, 2. CINBIO, Universidade de Vigo, Campus CINBIO, Universidade de Vigo, 36310 Vigo. Galicia Sur Health Research Institute (IIS Galicia Sur), Vigo.Departamento de BC, 3. CINBIO, Universidade de Vigo, Campus Universitario As Lagoas Marcosende, Vigo 36310, Spain

Introduction

Bacterial cells have been extensively investigated as an alternative approach for cancer therapy [1]. The integration of **LIVING BACTERIAL CELLS** with **INORGANIC COMPONENTS** to create functional **LIVING HYBRIDS** has revolutionized the utilization of materials and unlocked novel functionalities stemming from the fusion of biology and materials science[2]. Meanwhile, gold nanoparticles have great potential as a nanomedicine for drug delivery, photothermal, magnetothermal, and photodynamic therapy, as well as imaging agents by surface-enhanced Raman scattering (SERS)[3]. Thus, the synergistic combination of bacterial cells and the nanomaterials to yield the so-called biohybrid microrobots[4] can offer multiple applications to combat cancer. In this work, we aim to establish robust protein-protein interactions that will facilitate the assembly of gold nanoparticles onto the bacterial surface, offering a versatile and efficient platform for potential applications. We report the development of an efficient Bacterial-AuNP hybrids mediated by Spytag/SpyCatcher covalent binding[5] and the application of this live biohybrid microrobots composed of gold nanoparticles and genetically engineered bacteria as a promising new tool.

Methodology

To this aim, gold nanoparticles functionalized with SpyCatcher were orthogonally assembled on the surface of *Escherchia coli* cells expressing the SpyTag fused to the intimin display system[6]. As a proof-of-concept, the targeting and bioimaging capabilities of the reprogrammed bacterial biohybrid bearing synthetic adhesins[6] against the human epidermal growth factor receptor (EGFR) tumor biomarker were assessed in cultured cells *in vitro* by SERS.

Results and discussion

Initial results showed that gold nanoparticles has been successfully functionalised with SpyCatcher. The complex AuNP-SpyCatcher was able to bind bacteria that express Spytag. For future work it will be done assays with bacteria that express Spytag and anti-EGFR nanobody and test this system in eucaryotic cells that express EGFR. Our results pave the way for a new class of bacterial biohybrid for bioimaging and cancer therapy.

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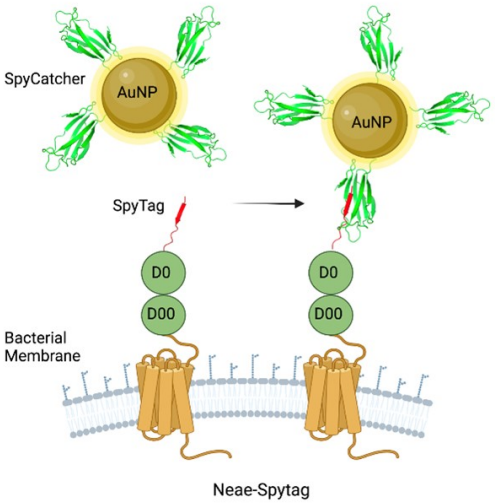


Figure 1.jpg

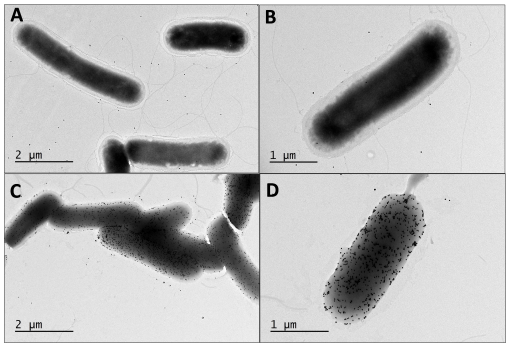


Figure 2.jpg

The importance of nanoparticle coating structural design in the development of nanovaccines

Tuesday, 16th January - 17:24: Nano-immunology - I (Room 507) - Oral - Abstract ID: 45

Dr. Maria Morbidelli [1], ***Dr. Ander Eguskiza Bilbao*** [2], ***Dr. Federico Luchi*** [2], ***Dr. Samuel Tanner Pasco*** [3], ***Dr. Cinzia Franchin*** [1], ***Prof. Giorgio Arrigoni*** [1], ***Prof. Juan Anguita*** [3], ***Prof. Roberto Fiammengo*** [2], ***Prof. Regina Tavano*** [1], ***Prof. Emanuele Papini*** [1]

1. *University of Padova,* ***2.*** *University of Verona,* ***3.*** *CIC bioGUNE*

Homogeneity/dis-homogeneity of the nanoparticle surface may deeply influence the formation of the protein corona (serum proteins that recognize and bind the surface of a nanoparticle). In fact, innate immunity may intercept nanoparticles whose coating mimic Pathogen Associated Molecular Patterns or Damage Associated Molecular Patterns, leading to Antigen Presenting Cells targeting via Pattern Recognition Receptors or complement receptors, and immune maturation. Knowing this, we decided to characterize the effect of different types of -COOH or -OH terminated polyetilenglycol (PEG) coatings, that differ in length and charge, (Fig.1) with the aim of finding candidates for the design of an anti-cancer nanovaccine.

Biochemical techniques, proteomic approach and cell capture experiments using primary derived human and mouse macrophages and dendritic cells were used to characterize these nanosystems.

We found an impressive difference in the protein corona composition of these systems: the differential presence of opsonins, like Platelet Factor 4 and Serum Amyloid P, and complement cascade proteins translates in a different recognition and uptake by phagocytic cells (Fig.2). Since the nanoparticle coated with the shorter PEG, and all negatively charged (100EG) showed the best behavior in terms of uptake and immune recognition and uptake, we decided to functionalize it with cancer MUC1 peptide, generating a potential therapeutic anti-cancer nanovaccine. This nanosystem was tested in comparison with the nanoparticle coated with the 100PEG nanoparticles conjugated with cancer MUC1 (tested in a previous immunization campaign).

Cell capture experiments and proteomic characterization of the protein corona revealed that the addition of the peptide to the coatings alters the protein corona composition, inducing a reduction in the capture by phagocytic cells *in vitro* (Fig3).

To test the antibody production induced, an immunization campaign using C57BL/6 female mice was performed. According to what observed *in vitro*, the 100EG nanovaccine induces a lower antibody titer compared to the positive control (Fig4). For this reason, we plan to change the core of the nanovaccine to add the MUC1 peptide inside the nanosystem. In this way we intent to preserve the surface properties of the 100EG nanoparticle, and therefore the preferable uptake, to induce an augmentation in the antibody titers against MUC1.

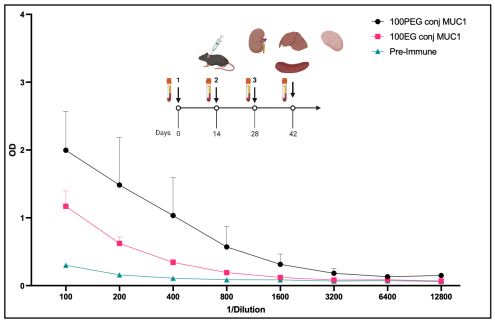


Fig.4: Anti MUC1 antibody production after 2 boosters at the day 42 of the immunization campaign, before the sacrifice.

Fig.4.png

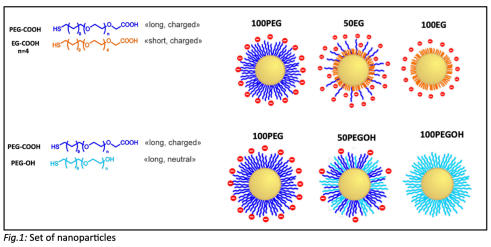


Fig.1: Set of nanoparticles

Fig.1.png

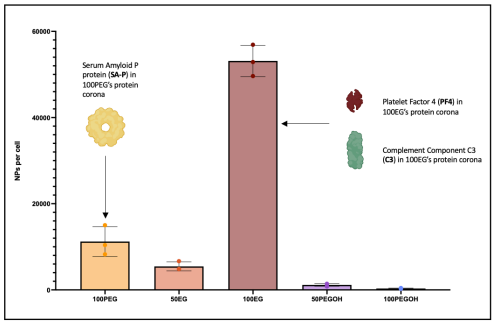


Fig.2: Representative cell capture experiment and interesting proteins found in the protein corona of 100PEG and 100EG nanoparticles that could have a role in the recognition and uptake by human primary macrophages.

Fig.2.png

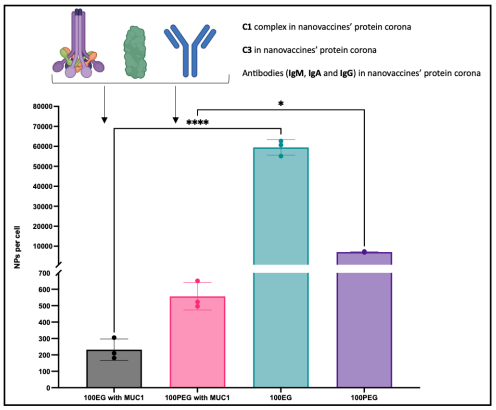


Fig.3: Representative cell capture experiment and interesting proteins found in the protein corona of the two proposed nanovaccines (100PEG and 100EG conjugated with MUC1) that could have a role in the recognition and uptake by human primary macrophages.

Fig.3.png

Beyond DDS: immunomodulatory layered double hydroxides NanoAlum for cancer metalloimmunotherapy

Tuesday, 16th January - 17:41: Nano-immunology - I (Room 507) - Oral - Abstract ID: 89

Dr. Lingxiao Zhang [1]

1. *Aarhus University*

Magnesium aluminum layered double hydroxides (LDH) is a kind of two-dimensional nanomaterials widely used in biomedical field. As an ideal drug delivery system (DDS), LDH efficiently delivers genes, peptides, proteins and small molecules against major diseases include cancers and neurodegenerative diseases. It is worth noting that LDH has been clinically used as an antigastric drug (Talcid, Bayer) since the 1960s, and our recent study also found that LDH can also be obtained from a commercial aluminum adjuvant (Imject Alum, Thermofisher) by hydrothermal treatment, thus defined as a new type of nano adjuvant (NanoAlum).

As DDS, LDH have efficiently deliver antigens or immunomodulators to lymph nodes, spleen and tumors to evoke potent cytotoxic T lymphocyte (CTL) response against solid tumors. Besides, LDH with appropriate surface modification also successfully crossed blood-brain barrier and delivered small molecules and genes to relieve the symptom of Alzheimer's disease mice.

Beyond DDS, the inherent antacid properties of LDH and the variability of its metal ions allow it to be used directly as drug-free nanoparticles for disease therapy. For example, to effectively remodel the suppressive tumor immune microenvironment (TIME). Our recent study found that peritumoral injection of LDH can neutralize the acidic TIME while supplementing Mg^{2+}, which greatly promotes the recruitment and activation of peripheral CTLs into the tumor to inhibit the growth of solid tumors. Upon LDH is taken up by tumor cells, it can neutralize intracellular acidic lysosomes to block the tumor autophagy pathway, thereby inducing tumor apoptosis.

Interestingly, partial replacement of Mg^{2+} in LDH by nutritional metal ions (M) such as Zn^{2+} results in nutritional NanoAlum (NanoMAlum) Zn-LDH, which can not only activate anti-tumor immune cells in TIME, but also induce tumor immunogenic death by activating the tumor cGas-STING signaling pathway and down-regulating the expression of tumor immune checkpoints.

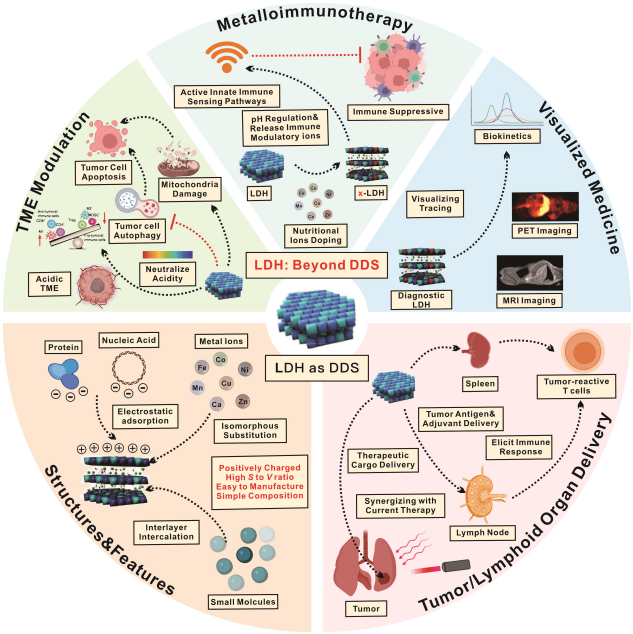


Figure 1 jpg.jpg

Interaction of Hollow Gold Nanoshells with NK cells

Tuesday, 16th January - 17:58: Nano-immunology - I (Room 507) - Oral - Abstract ID: 328

Mr. Noé Escareño [1], ***Dr. Antonio Topete*** [1], ***Prof. Adrián Daneri Navarro*** [1], ***Prof. Pablo Taboada*** [2]

1. *CUCS, Universidad de Guadalajara, Guadalajara, 44340, México; Grupo de Física de Coloides y Polímeros, Departamento de Física de la Materia Condensada Universidad de Santiago de Compostela, 15782, Spain,* ***2.*** *Grupo de Física de Coloides y Polímeros, Departamento de Física de Partículas Universidad de Santiago de Compostela; Instituto de Materiales (IMATUS), Santiago de Compostela, 15782, Spain*

Since the appearance of CAR T cells for the treatment of lymphomas, the study and development of cellular immunotherapies has acquired great relevance for the treatment of difficult-to-manage cancers. Natural Killer (NK) cells are part of the innate immune system repertoire and, like cytotoxic T cells, possess cytotoxicity mechanisms against cancer and infected cells, and are being used for the development of cancer immunotherapies. Here, we will address the study of the interaction of NK cells with therapeutic hollow gold nanoshells, functionalized with antibodies to target the cytotoxic granules of NK cells. The interaction of NK cells with these nanoparticles was studied through physicochemical and microscopic techniques, as well as cell uptake, expression of activation and inhibition receptors, and specific cytotoxic capacity of nanoshell-loaded NK cells against breast cancer cell lines.

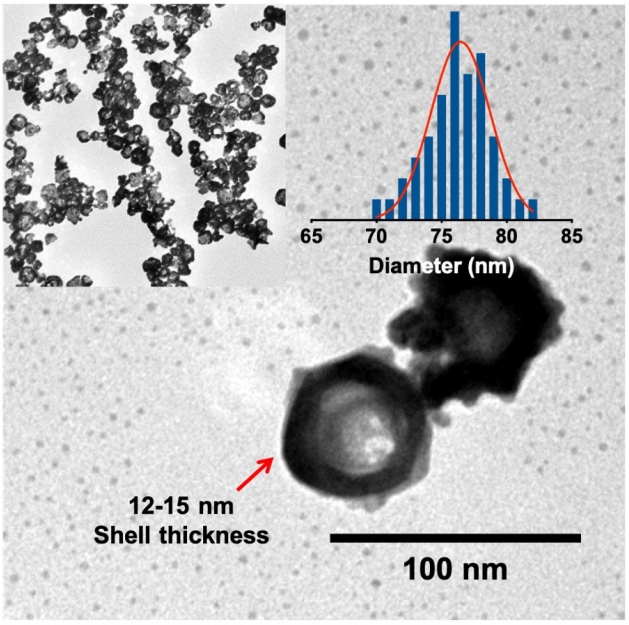


Imagen 1.jpg

A pre-targeted nanomedicine strategy for in vivo CD8+ T-cell engineering

Tuesday, 16th January - 18:15: Nano-immunology - I (Room 507) - Oral - Abstract ID: 85

Ms. Justine Aelvoet [1], ***Prof. Bruno De Geest*** [1]

1. *Ghent University*

Introduction

CD8+ T-cells are pivotal in anti-tumor immunity, as they possess the ability to recognize and eliminate infected or malignant cells through their T-cell receptors and cytolytic activity. Many cutting-edge immunotherapies, including cancer vaccines and CAR T-cells, rely on the effectiveness of CD8+ T-cells. Unfortunately, immune suppression in the tumor environment, as seen in cancer, hampers CD8+ T-cell activity, necessitating pharmaceutical interventions. Using nanoparticles for precise RNA drug delivery to CD8+ T-cells presents a potential solution to invigorate CD8+ T-cell activity. In this context, ligand-decorated nanoparticles have been widely explored. Surface-functionalization of nanoparticles with macromolecular ligands, however, is prone to causing off-target accumulation by excessive protein adsorption in serum.

Here we demonstrate targeted RNA delivery to CD8+ T-cells through a pre-targeting strategy, using tetrazine(Tz)-functionalized anti-CD8 nanobodies that home to CD8+ T-cells after intravenous administration, followed by administration of PEGylated lipid nanoparticles (LNP) functionalized with the complimentary trans-cyclooctene (TCO) reaction partner. The ultrafast inverse electron demand Diels-Alder click chemistry between Tz and TCO results in the conjugation of TCO-LNPs to CD8+ T-cells, facilitating specific RNA delivery (Fig. 1).

Methods

LNPs were formulated by mixing and aqueous solution of RNA and an ethanolic solution containing ionizable lipid, phospholipid, cholesterol and DSPE-PEG-TCO. LNPs were characterized by dynamic light scattering and electrophoretic mobility for their size and zeta-potential. RNA encapsulation efficiency was measured by RiboGreen assay. Mouse anti-CD8 nanobodies were produced by recombinant synthesis in *P. pastoris* and functionalized with tetrazines through either random lysine modification or C-terminal site-specific Sortase A-mediated conjugation. Binding of Tz-nanobodies to CD8 was tested by biolayer interferometry (BLI) and flow cytometry. Mice were injected with Tz-nanobodies followed by TCO-LNP one hour later. Cellular association of LNP was measured by flow cytometry.

Results and discussion

LNPs exhibited a sub-200 nm size, a slightly negative zeta-potential and nearly complete mRNA encapsulation efficiency. BLI data confirmed preservation of binding affinity between Tz-nanobody and its CD8 target. We demonstrated, *in vitro* on isolated mouse CD8+ T-cells (Fig. 2) and *in vivo* in the bloodstream and spleen of mice, that CD8+ T-cells efficiently became decorated with Tz-nanobodies and could subsequently be targeted by TCO-LNPs.

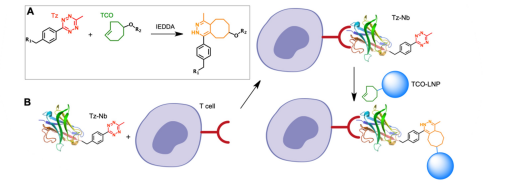
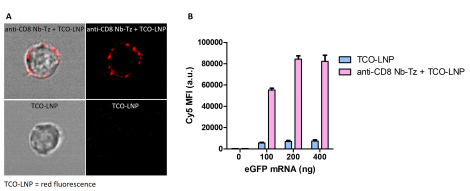


Fig. 1 The ultrafast inverse electron demand Diels-Alder (IEDDA) click chemistry between tetrazine (Tz) and trans-cyclooctene (TCO) results in the conjugation of TCO-functionalized lipid nanoparticles (TCO-LNPs) to CD8+ T-cells. (A) Reaction scheme of IEDDA between Tz and TCO. (B) Schematic representation of pre-targeting CD8+ T-cells with a Tz-nanobody (Tz-Nb) ligand followed by IEDDA conjugation of TCO-LNPs.

Fig.1 iedda.jpg



TCO-LNP = red fluorescence

Fig. 2 Pre-target strategy with anti-CD8 Nb-Tz targets TCO-LNPs to isolated mouse CD8+ T-cells. (A) Confocal microscopy confirmed targeting of TCO-LNPs to isolated mouse CD8+ T-cells. (B) Flow cytometry data showing the effect of anti-CD8 Nb-Tz on the binding of TCO-LNP for three different eGFP mRNA doses.

Fig.2 confocal and flow cytometry data.jpg

A new approach to target Diffuse Midline Gliomas (DMG) cell receptor using the molecularly-imprinted nanosized synthetic antibodies (SA-NPs) and their in-vitro activities

Tuesday, 16th January - 16:50: Destination brain (Room 608) - Oral - Abstract ID: 78

Dr. Damla ULKER [1], ***Prof. Dmitry Pshezhetskiy*** [2], ***Mr. Adem Ozleyen*** [3], ***Prof. Chris Jones*** [4], ***Prof. Sergey Piletsky*** [5]

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In this study, we have developed a new treatment approach for Diffuse Midline Glioma (DMG) based on molecularly imprinted polymeric nanoparticles referred to as “synthetic antibodies” (SAs). These SAs have numerous advantages when used as targeted therapies over natural antibodies, such as the ability to cross the Blood-Brain Barrier (BBB). SAs are very small, nano-sized, particles engineered to contain a region, known as a “binding site” [1, 2].

SA-NPs were designed using different amino acid sequences that only bind to a protein, known as the interleukin receptor (IL13Rα2), found at the surface of DMG cancer cells rather than brain cells [3, 4].

Herein, the six epitopes were selected using our unique epitope-mapping model to identify the possible sequences for IL-13Rα2-receptor protein, This selection was invented considering their electron densities, surface locations, nearness to the active binding sites, and similarity/diversity to known epitope sequences. In addition, the four epitopes were determined to compare our strategy to known active binding sides for the IL-13Rα2 receptor.

The binding affinity and selectivity of SA-NPs for IL13-Rα2 were assessed using Octet® biolayer interferometry and their morphology, size distributions, surface properties determined by Zetasizer and TEM. SA-NPS were selected considering their K_D (between 0.1 and 10nM) for further studies.

The cell viability assay was conducted to determine the activities of the SA-NPs on SU-DIPG-4N, HSJD-DIPG-007 and SU-DIPG-36 cell lines with H3.3K27M mutations. Western-blotting has been used for cell-signaling pathways. According to the results, our approach has important potential to target and treatment for DMG without any drug molecules.

This new approach lies in introducing new technologies to the field of brain tumours, and will bring new expertise in synthetic and bioanalytical chemistry to brain tumour treatment, which goes beyond the usual small molecule library screening and drug repurposing seen in standard therapy pipelines.

Acknowledgments: This study has been financially supported by Children’s Cancer and Leukemia Group UK. (GN: 2021BLPT37)

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Anti-glioblastoma nanochemotherapy via PLGA nanoparticles for extended survival rate in animal model

Tuesday, 16th January - 17:07: Destination brain (Room 608) - Oral - Abstract ID: 154

Mrs. Fatemeh Madani [1], ***Dr. Masood Khosravani*** [1], ***Dr. Mahdi Adabi*** [1]

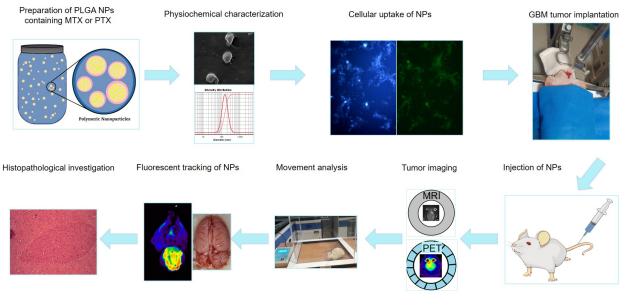
1. *Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*

Brain tumors, especially glioblastoma (GBM), are known for their poor prognosis, which mainly results from the aggressive nature of tumor cells, drug resistance, and hindrance of drug transfer to the brain because of the existence of the blood-brain barrier (BBB). The application of nanoparticles (NPs)-based drug delivery is a promising approach, especially when combined with surface modification. It is stated that poloxamer188-coated nanoparticles can circumvent the BBB and reach the brain tumor. In this study, we prepared PLGA (poly(lactic-co-glycolic acid)) NPs loaded with chemotherapeutic agents, MTX and PTX, separately, and the surface of the NPs was covered with PVA/Poloxamer188. The effectiveness of the combinational therapy of NPs was assessed on the GBM model.

PLGA NPs were prepared via the nanoprecipitation method. After physiochemical characterization, their effectiveness was investigated on C6-derived GBM tumors. The tumor size was checked via MRI and PET scans. The brain concentration of NPs was investigated by ex-vivo fluorescent tracking. Moreover, the immunohistochemical assay was performed to check the apoptosis, angiogenesis, and inflammation states of the brain. Finally, the health state of the liver, spleen, heart, lungs, kidneys, and testis was analyzed via H&E.

The mean diameter of both NPs was below 200 nm, and 17± 0.6% of NPs were concentrated in the brain after 48 h of intravenous injection. The tumor size decreased in the group treated with the combination of NPs, and two rats survived for more than 56 days. Finally, the quantitative results of the tissue H&E assays revealed that the number of ruined cells and epithelium thickness were increased in the liver and testis sections, respectively, and other organs were intact. It seems that these NPs could be a promising tool in the treatment of GBM in the future.

Keywords: Glioblastoma, PLGA nanoparticles, poloxamer188, methotrexate, paclitaxel



Graphical abstract.jpg

Nanoparticle-based approach for blood-brain-barrier crossing and glioblastoma treatment

Tuesday, 16th January - 17:24: Destination brain (Room 608) - Oral - Abstract ID: 310

Ms. Júlia German Cortés [1], Ms. Raquel Herrero [1], Dr. Diana Rafael [1], Dr. Ibane Abasolo [1], Dr. Fernanda Andrade [1]

1. *Clinical Biochemistry, Drug Delivery & Therapy, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain*

Introduction

Glioblastoma is an aggressive type of cancer presenting a poor prognosis and a high mortality rate. Nowadays there are no effective treatments, highly correlated to the presence of the blood-brain-barrier (BBB) hindering drugs at effective concentrations from reaching the brain. This work aims to develop polymeric micelles able to cross the BBB and improve the efficacy of drugs against glioblastoma cells.

Methods

Micelles composed of different amphiphilic polymers (Pluronic®F127 and Soluplus®) were produced by the film-hydration technique and loaded with lomustine. The surface of micelles was modified with antibodies against CD147 and insulin to improve their BBB crossing capacity. The developed systems were characterized regarding size, surface charge (DLS), drug release (UV-Vis), BBB crossing capacity (hCMEC-D3 cells), internalization, and efficacy in glioblastoma cell cultures and spheroids (U87-MG and U-251-MG cells).

Results

Were obtained micelles with a mean diameter of around 20-40 nm and 70-80 nm for Pluronic®F127 and Soluplus®-based micelles, respectively, a neutral to slightly negative surface charge and drug encapsulation efficiencies ≥90%. The drug release profile was pH-dependent, with a higher release rate at pH 6.4 compared to pH 7.4 (Figure 1). Regarding biological validation, the micelles were shown to be safe (empty micelles) and presented biological efficacy (drug-loaded micelles) both in cell culture and in spheroids (Figure 2-3), where the micelles seemed to internalize and penetrate (Figure 4), respectively. Concerning BBB crossing, the modification of the micelles' surface with antibodies against CD147 or insulin improved the crossing capacity of the micelles up to almost 50% (Figure 2).

Discussion

Polymeric micelles allowed the solubilization of drugs in aqueous media, increasing the safety profile of the drugs by eliminating deleterious excipients. The pH-dependent release profile of drugs demonstrated their preferential release at the tumor microenvironment over the bloodstream, which could lead to improved safety and efficacy. The micelles also showed the capacity to be internalized by cells, and penetrate into spheroids, improving drug therapeutic efficacy. Importantly, the modified micelles present a higher capacity for BBB crossing. The results obtained so far indicate that the developed micelles could serve as a platform for BBB crossing and treatment of glioblastoma.

A

Formulation	Size d(nm)	PdI	ZP (mV)	EE (%)
PM	27.95 ± 1.32	0.26 ± 0.11	-16.17 ± 1.51	NA
PM-L	26.91 ± 0.151	0.28 ± 0.001	-10.68 ± 2.83	97.00 ± 1.49
SM	74.15 ± 0.078	0.04 ± 0.013	-3.86 ± 0.47	NA
SM-L	70.85 ± 0.277	0.04 ± 0.009	-2.89 ± 0.55	90.23 ± 1.30

B

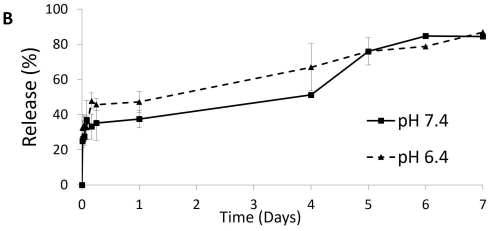


Figure 1 A. Summary of the physic-chemical characteristics of the micelles. Results are expressed as mean±SD, n=3. B. Release profile of Lomustine from SM over time (until 7 days) at different pH. Results are expressed as mean±SD, n≥3.

Figure 1.jpg

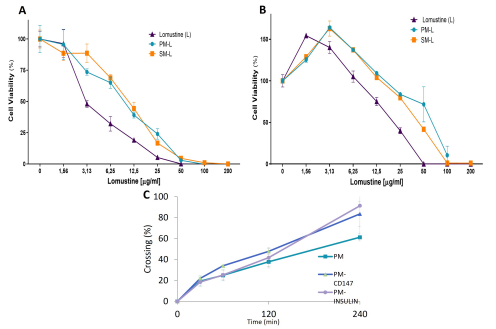


Figure 2. Cell viability curves for U87-MG (A) and U251-MG (B) treated with the lomustine loaded formulations and free Lomustine in adherent conditions. Results are expressed as mean±SD, n≥3. C. BBB crossing ability of PM-CD147, PM-Insulin and PM in hCMEC-D3 cells. Results are expressed as mean±SD, n=3.

Figure 2.jpg

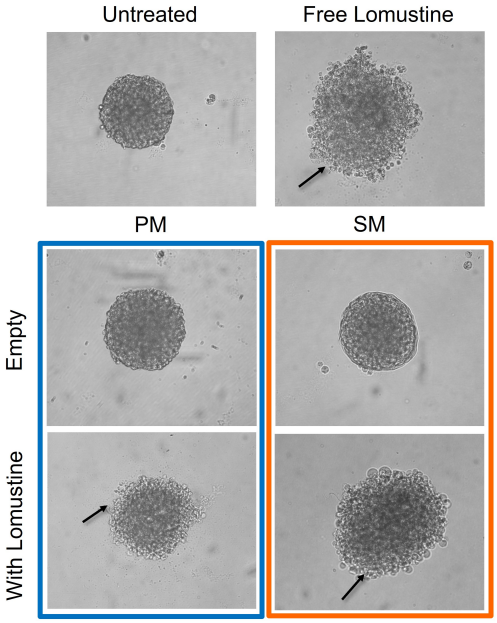


Figure 3. Efficacy of empty micelles, free lomustine, and lomustine-loaded micelles in U87-MG spheroids.

Figure 3.jpg

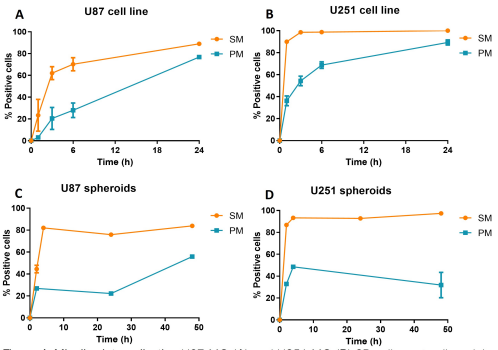


Figure 4. Micelles internalization U87-MG (A) and U251-MG (B) 2D-adherent cell models. Micelles penetration and internalization capacity U87-MG (C) and U251-MG (D) 3D-spheroids cell models. Results presented as percentage DTAF-fluorescent cells at different time points. Results are expressed as mean±SD, n≥3.

Figure 4.jpg

Precision radiotherapy by Thera -cHANPs to obtain novel therapeutic vulnerabilities in Glioblastoma Multiforme (GBM)

Tuesday, 16th January - 17:41: Destination brain (Room 608) - Oral - Abstract ID: 274

Mrs. ANGELA COSTAGLIOLA DI POLIDORO [1], ***Prof. Paolo Antonio Netti*** [2], ***Prof. Enza Torino*** [3]

1. *Interdisciplinary Research Centre on Biomaterials (CRIB), University of Naples Federico II, P.le Tecchio 80, Naples, 80125, Italy,* ***2.*** *University of Naples Federico II, Department of Chemical, Materials and Production Engineering (DICMaPI), P.le Tecchio 80, 80125, Naples, Italy,* ***3.*** *Department of Chemical, Materials and Production Engineering, University of Naples Federico II, 80125 Naples, Italy*

INTRODUCTION: Radiotherapy is the gold standard non-surgical treatment for many solid tumours. Precision radiotherapy aims to maximize the radiobiological effect in tumor cells while minimizing exposure of the surrounding healthy tissues. This approach turns particularly relevant in radioresistant tumours as Glioblastoma Multiforme (GBM) where local dose maximization might improve the overall outcome of the treatment. High Z-metals have been proven to boost the physical effect of radiotherapy. This reflects in a boosted production of Reactive Oxygen Species (ROS) and a stronger biological effect. In this framework, crosslinked hyaluronic acid nanoparticles (cHANPs) encapsulating Gd-DTPA are exploited as a theranostic probe for GBM, bearing both improved diagnostic capabilities and boosted therapeutic effect. cHANPs hydrogel network increases the hydration degree of the CA with consequent 12-fold boosting of relaxometric properties by the effect of *Hydrodenticity*. In the theranostic perspective, Gd high Z number makes it suitable as radiosensitizer. Furthermore, the engineering of the surface of cHANPs with the synthetic peptide Angiopep-2 (ANG-cHANPs) guarantees specificity towards glioma cells by the interaction with LRP-1 receptors overexpressed at BBB endothelial layer and on cancer cell membrane.

METHODS: Microfluidic production of NPs allows the tight control of their synthetic identity. Physicochemical characterization of the particles is performed by Nanoparticle Tracking Analysis and Electron Microscopy. Minispec benchtop relaxometer (1.5 T) is used to measure the relaxometric properties of cHANPs and a clinical accelerator (6MV) is used to irradiate cells.

RESULTS AND DISCUSSION: cHANPs and ANG-cHANPs synthetic identity and improved relaxometric propertied are presented in Figure 1a, b, c. ANG-cHANPs improved accumulation in GBM patient derived cells is presented in Figure 1d. Results on U87 cells demonstrate that cHANPs incubation strongly reduces clonogenic survival of cells and their surviving fraction proving NP ability of altering proliferation of irradiated cells. Moreover, cHANPs loaded with Gd-DTPA are able to alter the metabolic activity of cells as free Gd-DTPA at 20-folds higher doses. Accordingly, cells pre-treated with cHANPs determine a ROS-enhancement of 1.28-folds *in-vitro* (Figure 2d, e). In the perspective of precision radiotherapy, ANG-cHANPs could further confer a transport advantage to the formulation, with selective transport into tumoral cells.

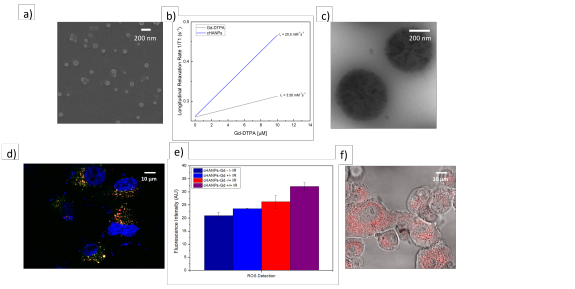


Figure 1. Theranostic cHANPs for boosted imaging and improved radiotherapy of GBM. a) Morphological characterization of cHANPs by Scanning Electron Microscopy (SEM) imaging; **b)** Hydrodenticity of cHANPs showing a relaxivity boosting of 5.7 times with respect to free Gd-DTPA ; **c)** Morphological characterization of Angiopep-2 engineered cHANPs (ANG-cHANPs) by Trasmission Electron Microscopy (TEM) imaging; **d)** Cellular localization of ANG-cHANPs after 6h of incubation. Hoechst 33342, nuclei; Green: Lysotracker Green, lysosomes; Red: ATTO633, ANG-cHANPs; **e)** Boosted production of Reactive Oxygen Species (ROS) in irradiated cells when pre-incubated with cHANPs for 24h; **f)** Visualization of boosted ROS production in cells pre-incubated with cHANPs by conversion of dihydrorhodamine (123-DHR);

Figure 1. theranostic chanps for boosted imaging and improved radiotherapy of gbm.png

Identification of Peptides Selectively Retained on Brain Endothelial Cells as Artificial Targets for Specific Brain Delivery

Tuesday, 16th January - 17:58: Destination brain (Room 608) - Oral - Abstract ID: 265

Ms. Giulia Maria Porro [1], ***Dr. Daniel Gonzalez-Carter*** [1], ***Prof. Giuseppe Battaglia*** [2], ***Mr. Italo Lorandi*** [1]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia (IBEC), Catalan Institute for Research and Advanced Studies (ICREA)*

The Blood-Brain Barrier (BBB) is an impermeable barrier composed primarily of specialised brain endothelial cells (BECs). It represents an obstacle for the treatment of neurological disorders by preventing the delivery of therapeutics into the brain. Current brain strategies involve the transport of drugs across the BBB targeting proteins associated with, but not exclusively expressed on BECs such as LRP1/TfR1, SLC7A5, or Glut1. Hence, such strategies result in off-target drug delivery to peripheral organs, thereby limiting brain specificity and clinical translation of biopharmaceutical therapies. Therefore, new approaches for neurological diseases able to overcome the BBB are needed.

The aim of the project is to generate artificial targets by identifying peptides selectively retained on the surface of BECs to enhance brain delivery specificity. This is achieved by harnessing the impermeability of the BBB as BECs are characterised by a lower internalisation rate compared to peripheral ECs. The generation of artificial targets labelling BECs could represent another way to direct drugs to the BBB.

Using phage display technology on primary brain, liver, and lung ECs we selected, based on time, a binding peptide population and a retained peptide population for each organ. The selection of the binding population is based on the removal of unspecific peptides; while the retained population represents peptides that have not been subjected to endocytic internalisation within an 8-hours period.

The results showed that the binding population displays heterogeneity within the same ECs type, but high similarity among the various ECs. In particular, in BECs a unique brain-specific peptide was not identified. On the contrary, the retained population is highly homogenous, and BECs displayed at least three specific-brain peptides; thus, indicating specific selection based on peptide retention on BECs. Binding studies and internalisation assays suggested CFAG peptide as the best “artificial” target as it showed solid binding indistinctly on the three different types of ECs and its retention on the BECs over time.

Overall, the results demonstrated the potentiality of exploiting the lower internalisation rate of the BBB to generate specific targets on the membrane of BECs, paving the way for designing specific brain drug delivery strategy.

Defect-free graphene enhances enzyme delivery to fibroblasts derived from the patients with lysosomal disorders

Tuesday, 16th January - 18:15: Destination brain (Room 608) - Oral - Abstract ID: 282

Dr. Sandra Vranic [1], ***Dr. Ana Jovanovic*** [2], ***Prof. Simon Jones*** [3], ***Prof. Kostas Kostarelos*** [1], ***Prof. Cinzia Casiraghi*** [4]

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Conventional enzyme replacement therapy (ERT) shows remarkable clinical improvement in treating many lysosomal disorders (LDs). However, this therapeutic approach is limited by insufficient enzyme delivery to cells and tissues. There is, therefore, an urgent and unmet clinical need to develop new strategies for the enhanced delivery of the enzymes to diseased cells.

Graphene-based materials (GBMs), due to their dimensionality and specific pattern of interaction with cells, represent a promising platform for loading of therapeutic cargos. Herein, the potential use of GBMs, including defect-free graphene (Gr) flakes with positive or negative surface charge and graphene oxide (GO) with different lateral dimensions, were investigated in healthy and primary fibroblasts derived from the patients with Mucopolysaccharidosis VI (MPS VI) and Pompe's disease. We report excellent biocompatibility of all GBMs up to the concentration of 100 ug/mL in the three cell lines studied. In addition, a noticeable difference in the uptake profile of the materials was observed. Neither type of GO was taken up by any of the studied cell lines to a significant extent. In contrast, the two types of defect-free Gr were efficiently taken up. In particular, we demonstrate that cationic defect-free Gr can be used as arylsulfatase B (ARSB) carriers: the Gr:ARSB complex retained not only a higher enzyme activity, which offers a better chance of delivering the effective dose of the enzyme to the cells, but also exerted a biological effect almost twice as effective than ARSB alone in the clearance of the substrate in MPS VI derived fibroblasts.

This study lays the groundwork for the potential use of GBMs as carriers for ERT in a range of LDs.

Supramolecular Broad-Spectrum Antivirals.

Wednesday, 17th January - 09:00: Plenary Session (Auditorium) - Oral - Abstract ID: 339

Prof. Francesco Stellacci [1]

1. *Institute of Materials, Bioengineering Institute, and Global Health Institute, EPFL, Lausanne*

Viral infections are a great threat for modern society, there are thousands of people that die every year because of them (mostly in under-developed countries) and many more have a lower quality of life because of them. Furthermore, it is become apparent that pandemic infection can have enormous consequences on global health as well as on the economy of the entire world. In this talk I will summarize a decade-long effort in my laboratory to develop broad-spectrum antivirals. The approach that will be presented is different from most biological approach as it is focused on an extracellular mechanism that affect the structural integrity of the virus rendering them non infective irreversibly. I will discuss the development of the compounds that we are investigating and their putative mechanism. In vitro, ex vivo, and in vivo example of the efficacy of such compounds will be discussed.

Nanoengineering for the Detection and Treatment of Cancer

Wednesday, 17th January - 09:40: Plenary Session (Auditorium) - Oral - Abstract ID: 334

Prof. Daniel Heller [1]

1. *Weill Cornell Medicine*

We develop nanotechnologies to accelerate the research, diagnosis, and treatment of cancer and allied diseases. To build better cancer therapeutics, we investigate the potential to improve the therapeutic index of precision medicines via nanomedicine-based strategies to localize drugs to tumors using vascular targets. We developed machine learning processes to facilitate the encapsulation of diverse drug classes into these nanoparticles, based on drug molecular structure, resulting in the rapid synthesis of many, diverse, targeted nanotherapeutics. We found that P-selectin, expressed endogenously on activated endothelium in tumors, can be used as a nanotherapeutic target improve the efficacy of kinase inhibitors and abrogate dose-limiting toxicities, to improve therapeutic index. P-selectin can also be induced via ionizing radiation, enabling the enhancement of the target. We also found that endothelial targeting can improve delivery across intact blood-brain barrier for the treatment of intracranial tumors and metastases, via activating transendothelial transport. We also develop optical nanosensor technologies using carbon nanotubes to facilitate longitudinal detection of cancer biomarkers, and to build new assays for cancer drug development. These technologies employ the bandgap fluorescence of single-walled carbon nanotubes (SWCNTs) which emit in the near-infrared “tissue transparent” window and can respond to analytes down to the single-molecule level. We have developed new sensors for the detection of metabolic changes in live cells and tissues, disease biomarkers in situ via implants, and overall disease states, aided by machine learning processes.

New Approaches for Therapeutic Pulmonary RNA Delivery.

Wednesday, 17th January - 10:45: Plenary Session (Auditorium) - Oral - Abstract ID: 337

Prof. Olivia M. Merkel [1]

1. *Ludwig-Maximilians-Universität München*

TBD

Rapid Amyloid-Beta Clearance through Blood Brain-Barrier in Alzheimer's Disease.

Wednesday, 17th January - 11:25: Plenary Session (Auditorium) - Oral - Abstract ID: 341

Prof. Xiaohe Tian [1]

1. *West China Hospital of Sichuan University*

Alzheimer's disease (AD) remains a leading cause of dementia, with current treatments offering limited efficacy and significant side effects. This study advances AD treatment by introducing an angiopep-functionalised polymersome that targets the LRP1 receptor on the BBB, facilitating the rapid clearance of Aβ. Our approach not only significantly reduced Aβ deposits but also swiftly altered the AD phenotype, with enhanced LRP1-mediated transcytosis observed. Behavioral tests confirmed that our treatment improved spatial learning and memory in AD mice, suggesting a promising direction for AD therapy that modifies disease presentation through targeted Aβ removal.

Stimuli-responsive Supramolecular Polymers based on benzene-1,3,5-tricarboxamide hydrophobic core as promising self-assembled nanocarriers for drug delivery

Wednesday, 17th January - 14:00: Desing of novel nanomedicines - II (Auditorium) - Oral - Abstract ID: 158

Dr. Mónica Martínez-Orts [1]***, Dr. Edgar Fuentes*** [2]***, Mr. Yeray Gabaldon*** [3]***, Dr. Lorenzo Albertazzi*** [4]***, Dr. Silvia Pujals*** [3]

1. *Institute for Advanced Chemistry of Catalonia (IQAC),* ***2.*** *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST),,* ***3.*** *Institute for Advanced Chemistry of Catalonia (IQAC-CSIC),* ***4.*** *Eindhoven University of Technology*

Controlled release of drugs remains a challenge for cancer therapeutics. Supramolecular polymers (SPs), that are ordered self-assemblies held together by weak interactions, have emerged as potential candidates in drug delivery[1]. Due to their non-covalent nature, a marvelous property of SPs is their ability to assemble/disassemble in response to external stimuli. Multiple anomalies occurring in tumor microenvironment, allow for the triggered release of anticancer drugs carried by stimuli-responsive SPs. In recent years, the development of SPs responsive to two or more stimuli have attracted considerable interest[2].

In chemistry, a common strategy in the design of water-soluble SPs involves the use of a hydrophobic core, such as BTA (benzene-1,3,5-tricarboxamide), followed by the introduction of hydrophobic amino acid residues. Terminal, covalently attached hydrophilic side chains in the synthetic pathway of SPs enhances water-solubility. The dual nature of these scaffolds guarantees the formation of self-assembled structures in water.

In our research, we exploit the synthesis of novel and stimuli-responsive C_3-symmetrical discotic amphiphiles that self-assemble into SPs[9,10]. Notably, our group synthesized for the first time a multi-sensitive polymer (Figure 1) that responds to four stimuli (light, temperature, pH and ionic strength)[9]. The design was based on a BTA core with three identical wedges synthetized by the Solid Phase Peptide Synthesis which leads to the incorporation of stimuli-responsive moieties and specific amino acids to grant water solubility and self-assembly. In drug delivery, SPs must remain stable from the administration until they reach the target. When they carry drugs, SPs drive through the bloodstream and resist adverse conditions. In our group, the supramolecular stability of different BTA-azobenzene polymers in biological media has recently been studied by monitoring self-assembly upon dilutions in phosphate-buffered saline and by increasing concentrations of bovine serum albumin[10].

Our investigation is currently focused on the structural modifications of BTA-based SPs including the introduction of specific peptide sequences that are known substrates for key enzymes related to cancer.

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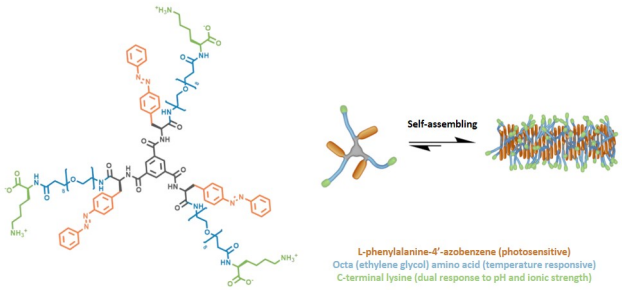
Self-assembling
L-phenylalanine-4'-azobenzene (photosensitive)
Octa (ethylene glycol) amino acid (temperature responsive)
C-terminal lysine (dual response to pH and ionic strength)

Figure1.jpg

An inline microfluidic-based real-time screening approach for enhancing bench-to-bedside translation of biopharmaceuticals

Wednesday, 17th January - 14:17: Desing of novel nanomedicines - II (Auditorium) - Oral - Abstract ID: 188

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Biologics have garnered significant interest over time, due to their exceptional attributes, offering different advantages compared to small-molecule drugs. In fact, biopharmaceuticals showed great potentialities in various medical fields, including oncology, dermatology, hematology, and the treatment of autoimmune diseases (Superson et al., 2019). Unfortunately, scientists and pharmaceutical companies are still facing problems in the bench-to-bedside translation. One of the reasons is that aggregation and structural modifications of biologics significantly impact the quality of biopharmaceuticals, posing challenges in terms of immunogenicity risk and reduced specificity (Boehncke & Brembilla, 2018; Wang & Ohtake, 2019). Consequently, the formulation of biopharmaceuticals includes excipients aimed at mitigating destabilizing effects (Ohtake et al., 2011).

In this work (Fig. 1), we introduce an innovative microfluidic-based screening approach that combines a custom-made microfluidic physico-chemical stimulation platform with an in-line analytical one. The goal is to study and characterize, rapidly, inline and in real-time, the behavior of different biopharmaceuticals in response to mechanical shear stress.

Three different high-concentrated formulations of a therapeutic nAb (provided by Merck Serono S.p.A.) were processed with the custom-made microfluidic platform. Their stability upon different extent of shearing was characterized by means of real-time inline ATR-FITR spectroscopy. The baseline of spectra was corrected prior to their normalization; respective placebos were subtracted, and second derivative was computed. Squared Pearson's metric ($R^2=[(\sum(x_i-x)(y_i-y))/\sqrt{((\sum(x_i-x)^2)(\sum(y_i-y)^2))}]$) in the Amide I spectral range (1680-1600cm^{-1}) was evaluated as similarity score.

The role of the excipient composition in mitigating the effect of the shear stress on nAb secondary structure modifications stands out from the 2nd derivative of the absorbance spectra. In particular, Trehalose-to-Arginine ratio (decreasing from formulation 1st to 3rd) appears to play a fundamental role in the preservation of the secondary structure upon mechanical shearing, with the Arginine acting as stabilizer at every shear-stress level tested (*i.e.*, weak, medium, and strong).

This approach advances the field of high-throughput screening methods in biopharmaceutical development, taking advantages from the superior environmental control offered by the microfluidics. Plus, it is ready to be integrated with machine learning, thus representing a novel and powerful tool able to revolutionize biologics stability assessment and formulation optimization in a Quality-by-Design-oriented framework.

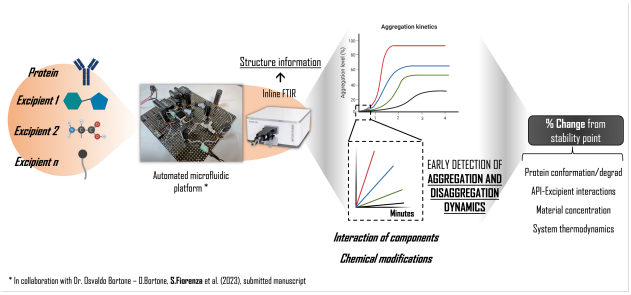

Fig.1 - aim of the work - microfluidic-based inline real-time characterization of injectable biologics.png

Pressure-based Microfluidics for the high-throughput isolation and loading of small Extracellular Vesicles (sEVs).

Wednesday, 17th January - 14:34: Desing of novel nanomedicines - II (Auditorium) - Oral - Abstract ID: 303

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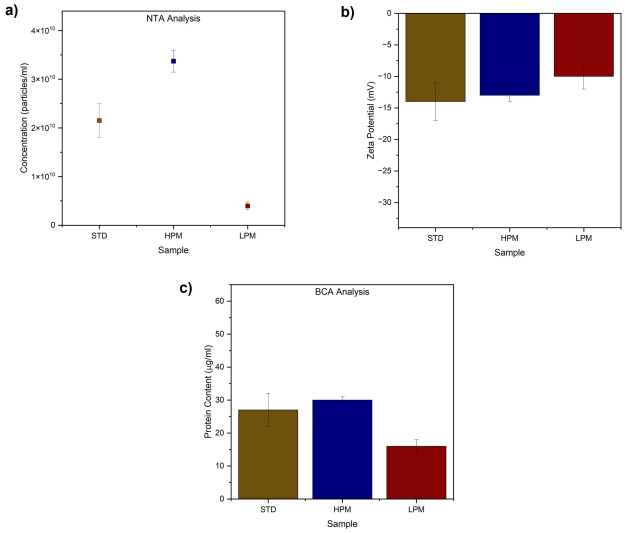
Small extracellular vesicles (sEVs) emerge from the wide class of nanomedical lipid-based systems due to their endogenous nature that provide them with incomparable physico-chemical characteristics.
However, their enforceability is still facing with critical technical challenges, spanning from the low and heterogenous yield of secretion to the tricky purification steps, and the high membrane impermeability against the majority of molecules.
So, there is an urgent need of innovative technologies to speed-up vesicles' release and increase their encapsulation efficiency.
The use of high- and low- hydrodynamic pressures on biological entities is here proposed as an approach to reversibly manipulate phospholipidic membranes to rise the yield of release of sEVs and their loading.
We recently demonstrated that high hydrostatic pressure microfluidics (HPM) could expose sEVs previously co-encapsulated with different molecules to a complex force field. As a results, temporary membrane permeabilization increases their encapsulation efficiencies up to 45-50% compared to 8-12% from standard (STD) techniques, while preserving both biological and physico-chemical characteristics, as well as the integrity of the molecules.
NTA, BCA, Zeta Potential, TEM and Proteomic analyses were conducted for sample characterization.
Then, the design and fabrication of microfluidic devices allowed us to stimulate biological entities with low hydrostatic pressure (LPM), thus investigating the effects of a wider spectrum of possible conditions.
While the stimulations have demonstrated to preserve normal characteristics, it showed to impact on the vesicles' biogenesis by determining an increase in the concentration release.
Therefore, we advise the investigation and exploitation of controlled and selected high- and low- pressures as an approach to handle, stimulate, and manipulate EVs, in order to improve and widen their industrial and clinical application.



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Lipo-polymers with shielding properties as PEG alternatives for Lipid Nanoparticle (LNP) components.

Wednesday, 17th January - 14:51: Desing of novel nanomedicines - II (Auditorium) - Oral - Abstract ID: 208

Dr. Josep Garcia Garcia [1], ***Mr. Rafael Miravet-Martí*** [1], ***Dr. Silvia Alonso de Castro*** [1], ***Ms. Inés García*** [1], ***Ms. Paula Martínez Colomina*** [1], ***Ms. Gloria Sogorb*** [1], ***Dr. Lidia Herrera*** [1], ***Dr. Sergio Esteban*** [1], ***Dr. Aroa Duro-Castaño*** [1], ***Dr. Vicent J. Nebot*** [1]

1. *Curapath S.L.*

Polyethylene glycol (PEG) has been widely used to enhance solubility and biocompatibility of various biomolecules and different carriers. In general, PEG acts as shielding to reduce immunogenicity and enhance blood circulation times. Particularly, lipo-PEG moieties are commonly applied in the formulation of the most successful nucleic acid delivery systems: lipid nanoparticles (LNPs). Indeed, these PEGylated vehicles have positioned as key non-viral vectors for gene delivery and a clear example are the two most distributed COVID-19 vaccines produced by BioNTech/Pfizer and Moderna/NIAID.

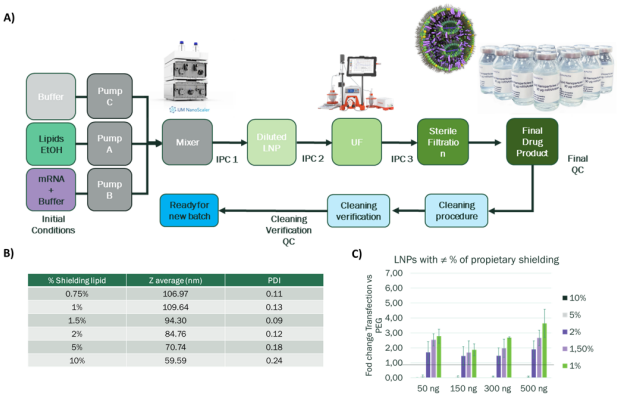
However, the extensive use of PEGylated products has raised concerns related to immunogenicity and loss of efficiency of PEG-containing medicines due to the production of anti-PEG antibodies which clear PEGylated excipients from the bloodstream (Accelerated Blood Clearance, ABC). This effect has been aggravated by the exposure of the population to PEG by the worldwide vaccination campaign against COVID, attracting the interest in PEG-free alternatives by pharma Industries.

Many strategies have been reported in order to overcome the secondary effects produced by PEG derivatives and Curapath is specifically working with a portfolio of proprietary bioinspired alternatives to PEG based on lipo-polypeptides and polypeptoids. Examples are the PSar-alpha tocopherol conjugate or diol modified PGA-lipid conjugates that have similar properties compared to PEG. In addition, our proprietary PEG alternatives have been produced and scaled up in technical batches with demonstrated batch-to-batch reproducibility and QC compliance.

The incorporation of these compounds into LNPs have been demonstrated; forming stable formulations with adequate encapsulation efficiencies, suitable physic-chemical properties and good reproducibility once the % of lipid-shielding is optimized.

In this case, our PEG-free alternatives have shown superior transfection efficiency in immortalized and primary cell cultures, lower immunogenic profile and *comparable in vivo* transfection to that of benchmark PEG-containing formulations.

These results make these PEG-free alternatives ideal shielding component for the delivery of biologics and nucleic acids. Envisaging what is ahead in the current nucleic acid delivery landscape, Curapath has engineered a library of different PEG replacement alternatives with the aim to not only overcome PEG drawbacks but to confer different surface features to help build the next generation LNPs.



% Shielding lipid	Z average (nm)	PDI
0.75%	106.97	0.11
1%	109.64	0.13
1.5%	94.30	0.09
2%	84.76	0.12
5%	70.74	0.18
10%	59.59	0.24

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CD300f derived F7 peptide: a powerful tool to facilitate drug delivery systems to cross biological barriers

Wednesday, 17th January - 15:08: Desing of novel nanomedicines - II (Auditorium) - Oral - Abstract ID: 248

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Drug delivery systems (DDS) are known to improve the bioavailability and tumor accumulation of many drugs and therapeutic compounds. However, biological barriers such cell membrane or blood brain barrier (BBB) crossing are often impeded or slowed down due to the size and surface composition of the nanomaterials. Herein we propose the use of F7 peptide, derived from the amino-acid sequence of CD300f immune receptor and specifically binding sphingomyelin, to increase the efficacy of different DDS (Figure 1). F7 was incorporated to distinct types of synthetic and natural DDS and the cell internalization rate, biological efficacy, and BBB crossing capacity were evaluated. On the one hand, Pluronic F-127 based polymeric micelles (PM), solid lipid nanoparticles (SLN) and polymeric nanoparticles (PNP) composed by chitosan-PEI-PEG were chemically conjugated with F7. On the other hand, both methods, the recombinant expression or the direct conjugation of the peptide on extracellular vesicles (EVs) was also explored using HEK293 cells previously overexpressing a therapeutic enzyme.

Regarding the cell uptake, a significant and selective increase of cell internalization capacity of PM, SLN and EVs conjugated with the F7 peptide was observed (Figure 2). Such increase was higher than the one observed for the gold standard among cell penetrating peptides, the TAT, and increased with the presence of sphingomyelin on the membrane of target cells. In terms of biological efficacy, EVs decorated with F7 induced an increase in the efficacy of the therapeutic enzyme directly proportional to the increase observed in the cellular uptake. Also, F7-PNPs demonstrated a higher efficacy in terms of plasmid DNA transfection than the non-functionalized PNPs (Figure 3). Finally, the ability of F7-functionalized PM and SLN to cross the blood brain barrier was evaluated using organ-on-a-chip models for the BBB. Results show that the F7 peptide facilitates also the surmount of more complex biological barriers, such the BBB (Figure 4).

Overall our results demonstrate that F7 acts as a first-class cell penetrating peptide, favoring in the cell internalization, BBB-crossing and subsequently increasing the efficacy of different types of drug delivery systems.

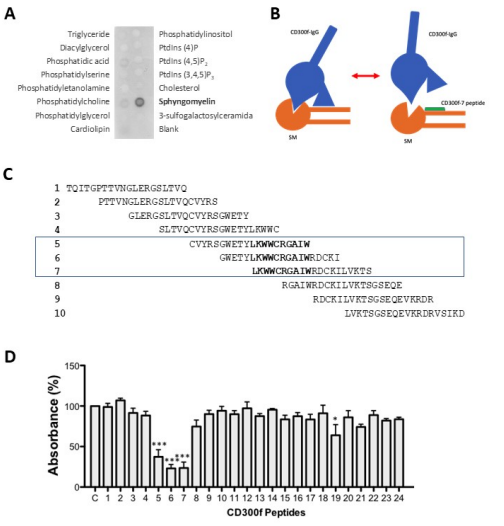

Figure 1. Interaction of CD300f with lipids and identification of F7 peptide. A) Extracellular domain of hCD300f was incubated with membrane strips containing different lipids. Strong positive signal was obtained for sphingomyelin. **B)** Schematic representation of CD300f binding inhibition to sphingomyelin. **C)** Sequence of the first 10 peptides tested, showing the common LKWW sequence in peptide fragments 5, 6 and 7. **D)** Inhibition of the binding between CD300f and sphingomyelin by different peptides(1-24) derived from the original CD300f sequence.

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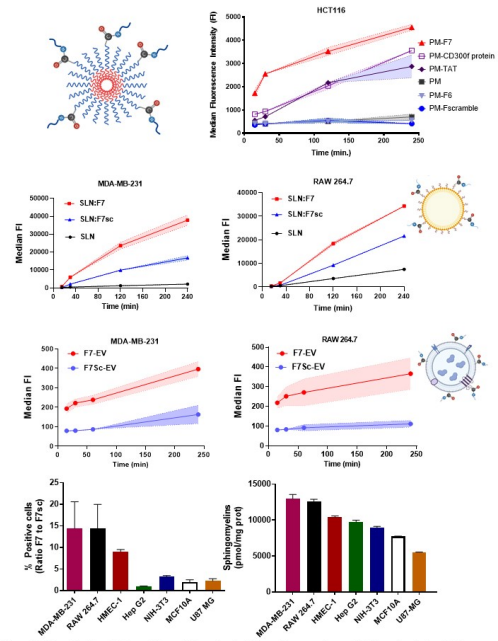

Figure 2: Internalization of F7 peptide and F7-conjugated drug delivery systems. A) Polymeric micelles (PM) were conjugated with the CD300f protein as well with the F7, F6 and Scramble Peptides. The internalization efficiency of each formulation was compared with the internalization of PM conjugated with the gold standard internalization peptide (TAT) in HCT116 colon cancer cells. B) Internalization of F7-functionalized solid lipid nanoparticles (SLN) vs. F7 scrambled (F7sc)-conjugated ones in MDA-MB-231 and RAW 264.7 cells, representative of breast cancer cells and macrophages, respectively. C) Uptake of extracellular vesicles (EVs) conjugated with F7 or F7sc in ones in MDA-MB-231 and RAW 264.7 cells. D) Internalization of F7 peptide as related to F7sc in different cell cultures and the content in the sphyngomyelin in the same cells. Results are presented as Mean ± SEM, n ≥ 3.

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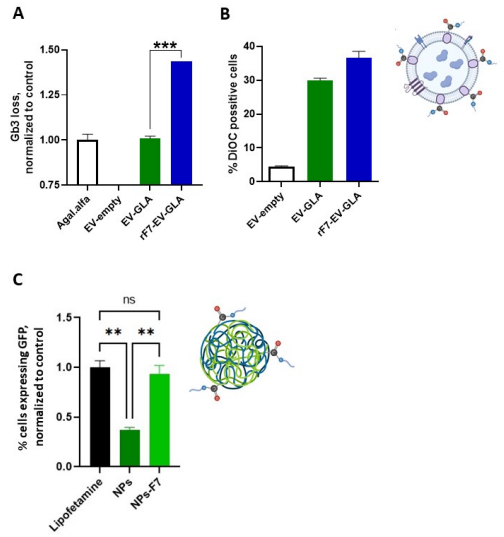

Figure 3. Efficacy of F7-decorated drug delivery systems (DDS). A) Efficacy of extracellular vesicles (EV) loaded with GLA protein and expressing recombinant F7 linked to the Lamp2A lysosomal protein (rF7-EV-GLA) reducing the Gb3 deposits vs. non-functionalized EV-GLA or empty EVs. Results were referred to clinically approved GLA enzyme, agalsidase alpha. **B)** Internalization of in HMEC-1 endothelial cells, 3 h post-incubation . **C)** Efficacy of Chitosan-Polyethylenimine-Polyethyleneglycol (CS-PEI-PEG) Polymeric Nanoparticles (PNPs) functionalized with F7 Peptide in the delivery of GFP coding cDNA into HEK-293 cells. Results are presented as Mean ± SEM, n ≥ 3.

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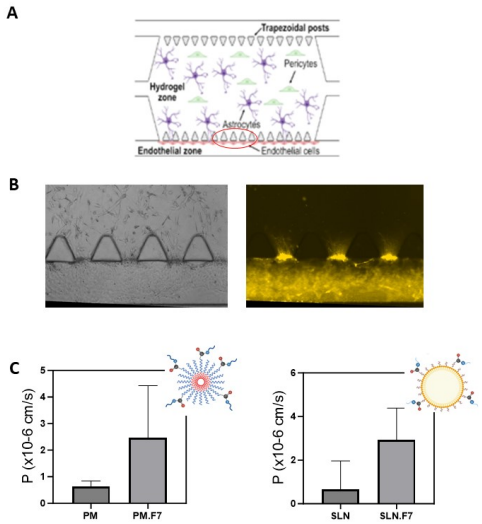

Figure 4: Blood brain barrier (BBB) crossing assays of F7-decorated drug delivery systems. A) Scheme of the microfluidic device with the organo-on-a-chip model of BBB. B) Bright field and fluorescence images of an area similar to the one circled in red in A. The endothelial barrier is formed with endothelial cells between trapezoidal posts. Solution with fluorescently labelled SLN (DiL) is applied in the bottom chamber and images are taken at different time points to record the signal in the central chamber. C) Quantification of fluorescence in the central chamber after 30 min, in polymeric micelles (PM) and SLN with and without F7 peptide.

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Hybrid nanoassemblies for neuroblastoma therapy

Wednesday, 17th January - 14:00: Nano-Oncology - III (Room 507) - Oral - Abstract ID: 32

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Introduction: Neuroblastoma (NB) is the most frequent extracranial solid tumor in children which present the highest mortality rate in pediatric oncology.[1] The current treatment of this disease employs chemotherapeutic agents which were discovered more than 40 years ago, and it has failed to increase the survival rates. Clearly, novel strategies to address this pathology are required.[2] The development of multifunctional hybrid nanoassemblies composed by mesoporous silica nanoparticles coated with lipid bilayers (known as Protocells) and polymeric enzyme nanocapsules provides the capacity to act cooperatively with different cell populations inducing a potent antitumoral response.

Results: A set of protocells capable to house therapeutic agents with diverse nature (hydrophilic and hydrophobic small molecules, proteins, and enzymes) were synthesized. The external surface of these nanocarriers were decorated with synthetic targeting moieties to provide selectivity towards their specific cell target (NB cells and macrophages). Furthermore, polymeric nanocapsules which contained therapeutic enzymes (glucose oxidase and catalase) have been anchored on the nanocarrier surface to improve the efficacy by synergistic effects. Selective uptake and therapeutic efficacy were evaluated *in vitro*. First, we have screened the capacity of these protocells to recognize each target cell population. The attachment of specific 4-aminobenzylguanidine analogues induced 10-fold higher uptake in NB cells in comparison with naked protocells, whereas macrophage targeting was achieved employing a synthetic carboxymannose derivative. Protocells loaded with cytotoxic agents and enzyme-loaded polymeric nanocapsules were capable to induce neuroblastoma cell death with extremely low dosages. Parallelly, protocells loaded with cocktails of macrophage-depletion drugs showed selective uptake and depletion capacity of macrophages.

Conclusion: Multifunctional Protocells decorated with polymeric nanocapsules exhibit great capacity to transport multiple therapeutic agents with different nature. The combined action of the released drugs and the catalytic action of the enzymes housed in the polymeric nanocapsules provide a significative improvement of the therapeutic efficacy by synergistic effects, being capable to interact with different cell populations within the tumoral tissue.

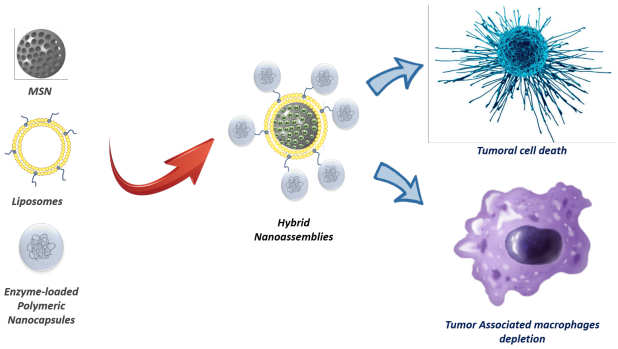

Figure 1.png

A new ML/AI proposed approach for the identification of transport delivery efficiency of nanoparticles according to the targeted tumor

Wednesday, 17th January - 14:17: Nano-Oncology - III (Room 507) - Oral - Abstract ID: 272

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

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Once injected in blood, nanoparticles face sequential biological barriers, whose transport properties change with the pathophysiology of the tumor, thus posing obstacles to nanoparticles' on-target biodistribution. This suggests that the design of nanoparticles must be informed by the patient.

However, there is a lack of integration between the data available about the clinical characterization of the tumors and the data collected from *in vitro* and *in vivo* studies of nanoparticles.

Here, we propose a Machine Learning/Artificial Intelligence (ML/AI) algorithm to predict Nanoparticle Delivery Efficiency (NDE), a parameter introduced to model the relationship between nanoparticles' features and their transport properties in tumors.

To compute NDE, "synthetic datasets" for vascular permeability (*P*) and Extracellular Matrix (ECM) diffusivity (*D*) of nanoparticles were generated. The datasets aimed at incorporating, in the computation of *P* and *D* values, both the effect of the pathophysiological degree of the tumors and the impact of the different nanoparticles' features. Particularly, in the computation of *P* a clinical differentiation between tumors was achieved by using different values of surface area of the vessels occupied by the pores, size of the pores, thickness of the vessel walls and k_{trans}; similarly, for *D,* different values of cell density, volume fraction of the ECM components, fiber radius and Apparent Diffusion Coefficient (ADC) were exploited. *P* and *D* values were further corrected to include the effect of size, charge and stiffness of the nanoparticles. The values of *P* and *D* were compared against arbitrary thresholds to model *"efficient nanoparticles", "moderate efficient nanoparticles"* and *"not efficient nanoparticles"* (i.e., NDE). The modeled efficiency of nanoparticles, thus, stems from the transport properties of the nanoparticles, according to the tumor type. Finally, different ML/AI classification algorithms have been implemented to predict NDE, with Support Vector Machines (SVM) offering the best discriminative power. In *Figure1* an overview of the implemented strategy is reported.

Through the implemented approach, we show the potentiality of ML/AI in finding relationships in nanoparticles' related datasets; at the same time, we evidence the lack of clinical characterization of animal models to move this approach in a real-case scenario.

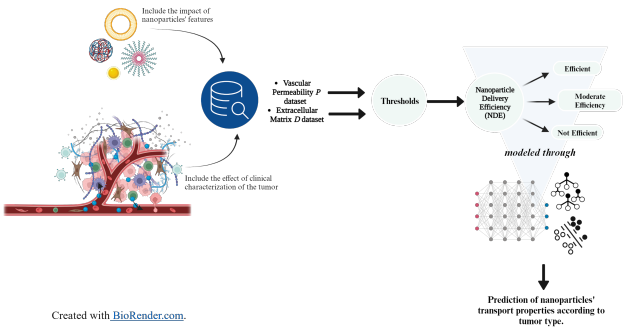


Figure1 - iconan2024.png

Targeted anti-cancer drug delivery using apoferritin-based protein nanocages

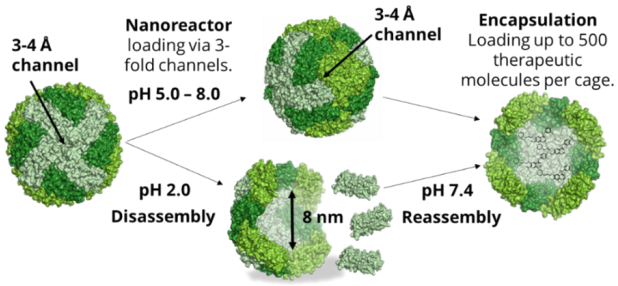
Wednesday, 17th January - 14:34: Nano-Oncology - III (Room 507) - Oral - Abstract ID: 69

Prof. Neil Thomas [1], ***Ms. Isobel Holden*** [1], ***Dr. Lei Zhang*** [1], ***Dr. Kaouthar Bouzinab*** [2], ***Dr. Tracey Bradshaw*** [2], ***Dr. Lyudmila Turyanska*** [3]

1. *University of Nottingham, School of Chemistry,* ***2.*** *University of Nottingham, School of Pharmacy,* ***3.*** *University of Nottingham, Engineering*

Introduction: Apoferritin is a spherical (12 nm diameter) self-assembling protein nanocage composed of 24 subunits whose natural role is the storage and transportation of iron ions (as ferritin) in humans and many other species. The nanocage can be formed from both heavy and light chain ferritin subunits in any ratio. The heavy chain ferritin possesses both peroxidase activity and a recognition sequence for the transferrin receptor (TfR1) that allows it to be endocytosed and undergo transcytosis through the epithelium of the blood-brain barrier. The apoferritin nanocage disassembles below pH 4.0 into its 24 subunits and this property can be utilised to encapsulate drugs, proteins or nanoparticles less than 8 nm in diameter as it reassembles when the pH is adjusted back above 4.0 (figure 1). We have exploited horse spleen and recombinant human forms of apoferritin to deliver near-infrared fluorescent PbS quantum dots and MRI active nanoparticles into cells for imaging. We have also encapsulated anticancer drugs including gefitinib and temozolomide (TMZ) (up to 500 drug molecules/nanocage) and determined their activity on breast cancer and glioma cells. Encapsulation of TMZ in apoferritin has been found to re-sensitize cancer cell lines that are resistant to the drug on its own. Through protein engineering of the exterior of the apoferritin nanocage, we have redirected the apoferritin nanocage to target biomarkers other than TfR1 such as EGFR and HER2 that are expressed at highly elevated levels on some breast or brain cancer cell surfaces. Data on these studies will be presented.

Methods: Recombinant human apoferritins have been produced in *E. coli* and functionalised using a variety of chemical and enzymatic conjugation methods. The cages encapsulating drugs including TMZ have been characterised using both DLS and TEM and the biological activity of these materials have been examined in a variety of cell lines including SKBR3 (HER2 +ve), MBA-MD-231(triple negative) breast cancer and U87MG glioma cell lines through cell viability and clonogenic assays as well as flow cytometry cell cycle analysis. The uptake pathway(s) for the different apoferritins has been investigated using super-resolution fluorescence microscopy and their *in vivo* behaviour imaged in xenograft models.



Picture1.png

Nanoparticle-based drug delivery to brain tumor to overcome standard of care resistance

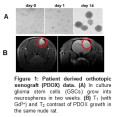
Wednesday, 17th January - 14:51: Nano-Oncology - III (Room 507) - Oral - Abstract ID: 152

Dr. Meser Ali [1], ***Dr. Tobias Walbert*** [1], ***Dr. James Snyder*** [1], ***Dr. James Ewing*** [1]

1. *Henry Ford Hospital*

The uniform lethality of glioblastoma (GBM) with a survival of less than 2 years despite best available therapy is attributed to treatment resistance due to DNA repair mechanisms that drive disease relapse and tumor heterogeneity. One prognostic factor identified as a reliable biomarker for GBM sensitivity to temozolomide (TMZ) and radiotherapy (RT) is the overexpression of O^6-methylguanine-methyl-transferase (MGMT) enzyme. Patients with active MGMT were found to receive little benefit from TMZ and RT and represent a group of great unmet need with no treatment options that significantly improve survival. Recently, several preclinical and clinical studies suggest that alcohol aversion drug, disulfiram (DSF), inhibited MGMT and improved the efficacy of TMZ in GBM when combined with copper (Cu). However, phase II trial showed that there was no survival benefit from oral Cu/DSF. Nevertheless, the major limitation of oral Cu/DSF has been delivery of fragile DSF within the *in vivo* system.

We have developed nanoformulation of Cu/DSF that addresses major drawbacks of the Cu/DSF: easy degradation in the blood and non-specific interactions with cells and serum proteins and lack of tissue specific delivery. Nanoparticle (NP) providing stability of DSF is identified. *In vitro* cell culture study revealed that NP-Cu/DSF inhibited MGMT through the ubiquitin-proteasome pathway. Inhibition of MGMT activity in cell cultures vastly increased the alkylation-induced DNA double-strand breaks, cytotoxicity, and the levels of apoptotic markers like g-H2AX, JNK-P and cleavage of PARP-1. Preliminary intravenous delivery of NP-Cu/DSF in combination with TMZ in an MGMT-positive patient derived orthotopic xenograft (PDOX) model demonstrated tumor size regression with prolonged survival. NP-Cu/DSF targets MGMT-145-cysteine and its unique cytotoxic mechanism circumvents MGMT-mediated chemo- and radiation resistance. The present work aimed at the development and application of nanomedicine containing Cu/DSF for targeted delivery of drug to GBM overcoming standard of care resistance.



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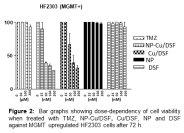

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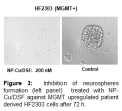

Figure 3: Inhibition of neurospheres formation (left panel) treated with NP-Cu/DSF against MGMT upregulated patient derived HF2303 cells after 72 h.

Slide3.png

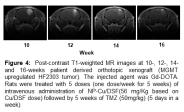

Figure 4: Post-contrast T1-weighted MR images at 10-, 12-, 14- and 16-weeks patient derived orthotopic xenograft (MGMT upregulated HF2303 tumor). The injected agent was Gd-DOTA. Rats were treated with 5 doses (one dose/week for 5 weeks) of intravenous administration of NP-Cu/DSF(56 mg/Kg based on Cu/DSF dose) followed by 5 weeks of TMZ (50mg/kg) (5 days in a week).

Slide4.png

Engineering of glioblastoma-derived biomimetic vesicles

Wednesday, 17th January - 15:08: Nano-Oncology - III (Room 507) - Oral - Abstract ID: 308

Ms. Noelia Hernandez Lobato [1]***, Dr. Marilena Hadjidemetriou*** [2]***, Dr. Neus Lozano Valdés*** [1]***, Prof. Kostas Kostarelos*** [3]

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In the last decade, the development of biomimetic delivery nanosystems has emerged as a new strategy to engineer vesicular transport systems with a variety of therapeutic applications. This 'biomimetic' concept relies on the incorporation of cell membrane components as integral parts of the vesicles, conferring them with biological properties originating from the cell of origin[1]. Among the different designs of biomimetic nanosystems, there can be distinguished essentially in two categories: i) cell-membrane wrapped (pre-formed) nanoparticles[2,3] and ii) cell-derived lipid vesicles, containing either mixture of all cell-membrane components[4,5] or selected protein components[6,7]. The majority of studies published today focus on the evaluation of the biological activity and function of the different biomimetic nanosystems, with limited engineering and formulation details of the methodology used.

This study focuses on the description of a general protocol for the engineering of biomimetic lipid vesicles by incorporation of murine glioblastoma cell (GL261) membrane proteins only, using classical liposome fabrication methodologies (lipid film hydration and extrusion). We evaluated different vesicle engineering parameters, including cholesterol content, degree of PEGylation, as well as, some processing aspects like purification and quantification. The cholesterol concentration played an important role in the size and monodispersity of the biomimetic vesicles, while PEGylation influenced their stability over time. The biomimetic GL261-derived vesicles were purified using size exclusion chromatography in combination with other solid phase purification techniques to ensure the separation between the unbound proteins and the GL261-derived vesicles. After obtaining the purified systems, the GL261-derived vesicles were characterized by EM and differential scanning calorimetry (DSC), while their protein profile was evaluated using SDS-PAGE and mass spectroscopy (LC-MS/MS). We elucidate valuable insights on the importance of various molecular and methodological parameters in the fabrication of monodisperse and colloidally stable GL261-derived biomimetic vesicles, and their impact on the resultant vesicle morphology, thermal properties and protein profile.

1. *Adv. Mater.* **30**, 1–34 (2018)
2. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 10980–10985 (2011)

3. *Adv. Healthc. Mater.* **12**, 1–23 (2023)
4. *Nano Lett.* **13**, 3248–3255 (2013)
5. *Nanomedicine Nanotechnology, Biol. Med.* **49**, 102663 (2023)
6. *Nat. Mater.* **15**, 1037–1046 (2016)
7. *ACS Nano* **15**, 6326–6339 (2021)

Polymersomes Regulating Immune Microenvironment Reduces Inflammation and Alleviates Idiopathic Pulmonary Fibrosis (IPF) by Phenotypic Targeting

Wednesday, 17th January - 14:00: Nano-immunology - II (Room 608) - Oral - Abstract ID: 242

Ms. Jiangqi Feng [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Department of Chemistry, University College London,* ***2.*** *Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC)*

Idiopathic Pulmonary Fibrosis (IPF) is a chronic interstitial lung disease caused by excessive collagen deposition, ultimately leading to progressive pulmonary sclerosis, causing patients to lose the ability to regulate gas exchange[1]. There are only two FDA-approved drugs for the treatment of IPF, and neither can stop or reverse disease progression or reduce mortality[2].

Previous studies have suggested that macrophages are innate immune cells that can regulate injury and repair in lung fibrosis models[3]. However, clinical therapies that directly suppress the M1 macrophage response are not effective, suggesting that instead of directly targeting the traditional activated pro-inflammatory phenotypes, it may be more useful to multivalently target different immune cell subgroups and modulate the pulmonary immune microenvironment.

Significant differences exist in the expression of surface markers on bone marrow-derived interstitial macrophages (IM), monocytes, and resident alveolar macrophages (AMs). For example, human AMs highly express mannose receptor CD206[3]. We propose a pH-responsive polymer, poly (2-(methacryloyloxy) ethyl phosphorylcholine)-poly (2-(diisopropylamino) ethyl methacrylate) (PMPC-PDPA) polymersomes, that can target the scavenger receptor class B member 1 (SRB1) and scavenger receptor class B member 3 (CD36)[4]. Different expressions of surface markers on different immune cell subgroups, and different particle radius could make the multivalently targeting of a subgroup of immune cells successful. We can interfere with innate immune activation and improve the pulmonary immune microenvironment to treat IPF, providing a new approach for developing anti-fibrotic drugs.

The antifibrotic effects of PMPC-PDPA polymersomes have been proved using a bleomycin (BLM)-induced mouse model of PF. Multicolour flow cytometry was also used to reveal changes in the pulmonary immune microenvironment under the intervention of PMPC-PDPA polymersomes with the disease progression, especially regarding the number of immune cells. However, cell spatial position is closely related to cellular functions, and therefore, the locations of different types of cells may affect their overall pro-inflammatory or anti-inflammatory activity. The spatial interactions between PMPC-PDPA polymersomes and potential immune cells during early inflammatory and late fibrotic pathological states have been demonstrated by tissue-clearing techniques.

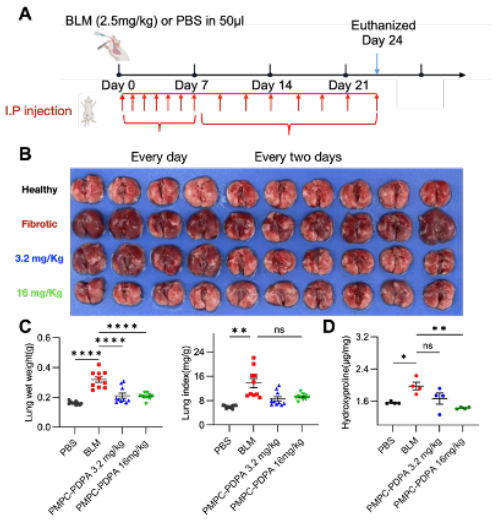

Fig.1 pmpc-pdpa polymersomes alleviate bleomycin-induced pulmonary fibrosis mice model.png

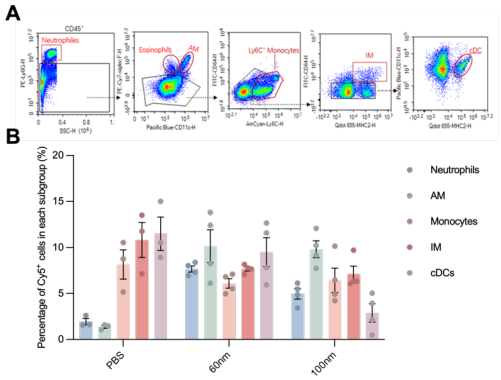

Fig.2 the interactions between five immune cell subgroups and pmpc-pdpa polymersomes.png

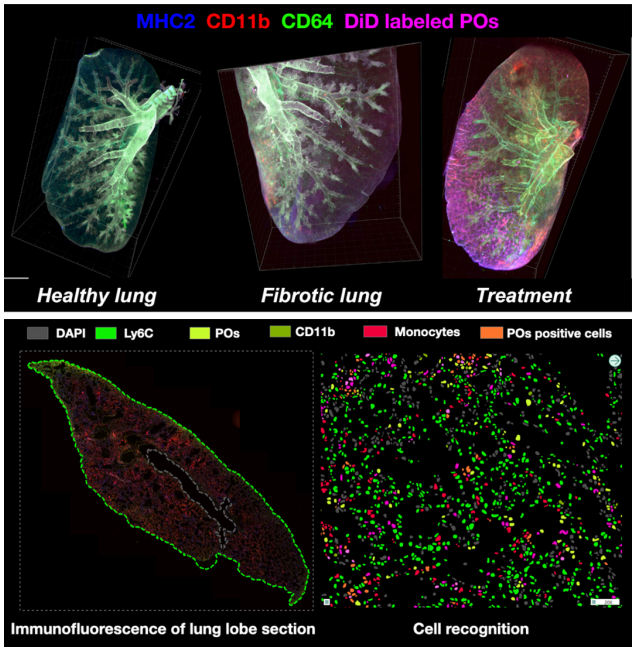

Fig.3 visualize the microenvironment of the lung. .png

Calcium phosphate nanoparticles as potential carriers for vaccines

Wednesday, 17th January - 14:17: Nano-immunology - II (Room 608) - Oral - Abstract ID: 41

Ms. Anshika Maheshwari [1], ***Dr. Rebecca Dookie*** [1], ***Prof. Birgitta Henriques-Normark*** [1], ***Prof. Georgios Sotiriou*** [1]

1. *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm*

Introduction: Subunit vaccines have emerged as a promising strategy for the prevention of infectious diseases owing to their safety profile, scalability, and cost-effectiveness. However, they can suffer from poor immunogenicity and stability issues. Therefore, nanoparticles (NPs) can be used as vaccine delivery platforms to overcome these challenges. Calcium phosphate (CaP) NPs are one of the promising candidates for vaccine delivery. They are biocompatible and biodegradable and well tolerated by the body and can easily load a variety of proteins/peptides. Moreover, they have the potential to illicit both cell-mediated and humoral immune responses, thereby, overcoming the poor immunogenicity associated with recombinant protein/antigen vaccines.

Methods: CaP NPs were produced using flame spray pyrolysis (FSP), a scalable nanofabrication technique. Dynamic light scattering (DLS) was used to estimate the hydrodynamic size of NPs. Ovalbumin (OVA) was employed as a model antigen to assess immunomodulatory effects using murine bone-marrow derived dendritic cell (BMDC) culture.

Results: We have synthesized amorphous CaP NPs with varying silica (0%, 10%, 25%, and 50%) content for screening. CaP NPs with ≥25 wt% silica content yielded a NPs suspension of 100-200 nm mean hydrodynamic diameter. None of these NPs exhibited any cytotoxicity against human lung epithelial cells. Upon loading OVA, we achieved loading capacity values of up to 60 µg/mg NPs in case of 25 wt% silica CaP particles. These particles also display pH-dependent solubility with more than 50% dissolution in 2 hrs at pH 4. After screening, we used 10% & 25% SiO_2 containing CaP NPs to study the OVA uptake and the immunomodulatory effects on BMDCs. Preliminary studies demonstrate an upregulation of CD80 and CD86 expression on BMDCs in the case of NP:OVA conjugates, as compared to water and ovalbumin alone thus indicating immunomodulation via DC activation.

Discussion: FSP can be used for scalable production of CaP NPs with good control over the NPs properties. It was possible to load a model antigen on the NP surface and help in the internalization by BMDCs. The NP-antigen conjugate also resulted in the activation of dendritic cells. Thus, CaP NPs are suitable vaccine nanocarriers for protein-based vaccines.

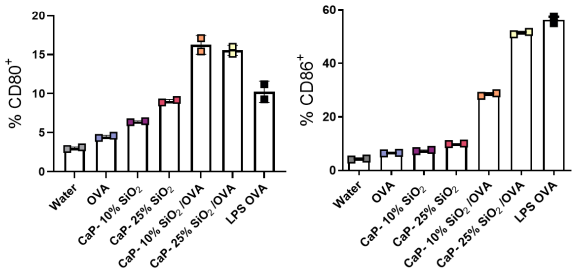

Figure- percentage of bmdc expressing cd80 and cd86 when incubated with 10 and 25 silica containing cap nps with and without ova .png

Inexpensive antibacterial nanofibrous wound dressing with flame-made Ag/SiO2 nanoparticles

Wednesday, 17th January - 14:34: Nano-immunology - II (Room 608) - Oral - Abstract ID: 132

Dr. Reshma Vasantha Ramachandran [1], ***Prof. Georgios Sotiriou*** [1]

1. *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm*

Introduction

Wound healing is an intricate biological process within the body aimed at repairing damaged tissue and restoring its structure and function. Infections in wounds occur when bacteria or other microorganisms infiltrate the wound and proliferate, thereby hindering the healing process and posing potential health risks, such as septicemia, or severe infections in the deep tissues and bones. Antibacterial dressings are designed to combat this issue by reducing bacterial colonization. While many contain antibiotics, there's a growing need for antibiotic-free options to curb antimicrobial resistance. One approach is using antimicrobial nanomaterials like nanosilver (Ag). However, despite their promising antimicrobial activity in lab settings, Ag-based dressings often fall short in clinical efficacy due to undefined Ag^+ ion release that can promote resistance. For these dressings to be more than a marketing tool, they must offer scalability, reproducibility, and precise nanoparticle characteristics. Here, we present a solution by integrating two industrial manufacturing processes- flame spray pyrolysis (FSP) and electrospinning, to develop an antibacterial wound dressing using silver-silica nanoparticles (Ag/SiO_2 NPs).

Methods

Ag/SiO_2 NPs are produced by FSP using silver acetate and a mixture of acetonitrile and 2-ethylhexanoic acid as precursors. These particles are then incorporated into polyvinylalcohol/chitosan (PVA/CS)-based electrospun fibers. Here, a mixture of PVA and CS with PVA:CS ratios of 100:0, 95:5, 90:10 and 80:20 is used for electrospinning.

Results

The nanoparticles are 2-10 nm in size and the electrospun dressings have a fiber diameter of 100-400 nm (fig 1). These dressings provide sustained release of silver ions which result in antibacterial activity with Methicillin-Resistant Staphylococcus Aureus (MRSA)-a pathogen known to cause wound infections (fig 2).

Discussion

In this study, we showcase an economical method for producing wound dressings utilizing PVA/CS nanofibrous membranes infused with Ag/SiO_2 nanoparticles. These dressings exhibit potent antibacterial properties against MRSA, at PVA:CS ratios of 90:10 and 80:20. Additionally, the minimal release of Ag^+ ions reduces the likelihood of bacteria developing resistance to silver ions.

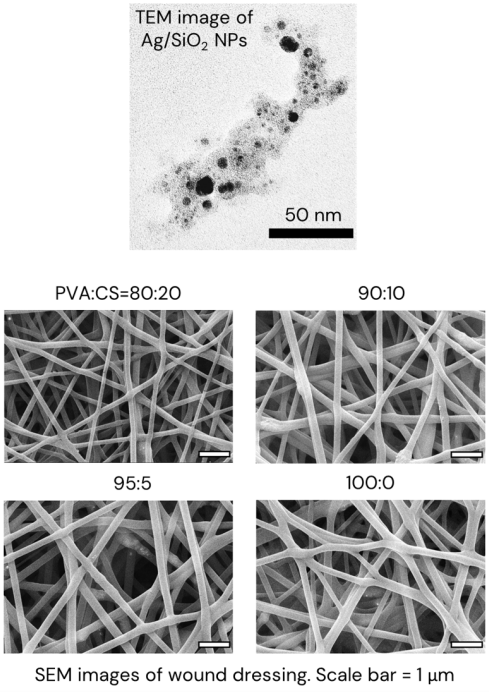

Fig 1 tem and sem images.png

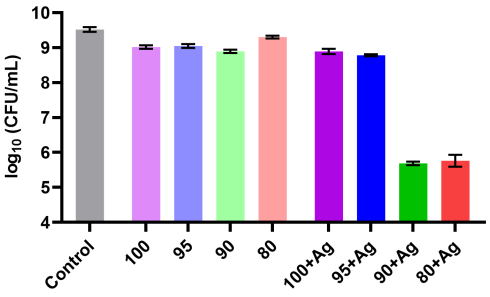

Fig 2 antibacterial activity.png

Click chemistry-based bioconjugation of iron oxide nanoparticles for diagnosis of inflammatory bowel disease

Wednesday, 17th January - 14:51: Nano-immunology - II (Room 608) - Oral - Abstract ID: 33

Ms. Shno Asad [1]

1. *Uppsala University*

Introduction: Superparamagnetic iron oxide nanoparticles (SPIONs) are promising for targeted drug delivery and bioimaging. They can be functionalized with ligands, exhibit hyperthermia for triggered drug release and are active as contrast agents for magnetic resonance imaging (MRI). We engineer SPIONs for oral administration to serve as diagnostic biosensors for detection of inflammatory bowel disease (IBD). We have developed a single-step flame process for encapsulating SPIONs with SiO_2-coating that facilitates further modification *via* click chemistry. By functionalizing SPIONs with ligands that target overexpressed biomarkers in the inflamed intestinal tissue, we aim to develop biosensors for noninvasive monitoring of IBD progression using MRI.

Methods: SiO_2-coated iron oxide nanoparticles were made through flame spray pyrolysis and characterized with transmission electron microscopy and X-ray diffraction analysis. SPIONs were modified through silanization reaction and analyzed using Fourier Transform Infrared (FTIR) spectroscopy. Organic linkers with a terminal alkyne were either purchased or synthesized and installed on the particle surface. ICAM-1 antibodies were modified with terminal azides using SiteClick™ Antibody Azido Modification Kit, followed by conjugation with alkyne-modified SPIONs through click chemistry. The antibodies were tagged with fluorophores and visualized using fluorescence microscopy. Thermogravimetric analysis (TGA) was used to compare amount of organic material on SPION surface after each functionalization step. Targeting capability of bioconjugated SPIONs are evaluated in inflammation-induced Caco-2 cells, visualized with confocal microscopy and quantified using inductively coupled plasma optical emission spectroscopy.

Results: SiO_2-coated SPIONS were successfully produced. The synthesized organic linker was obtained as a white solid (94 % yield), confirmed with nuclear magnetic resonance. Silanization and subsequent linker conjugation was confirmed with FTIR and TGA. Antibodies were attached onto SPIONs *via* click chemistry and visualized with fluorescent microscopy. Confocal crossectional imaging of inflamed cells showed apically expressed ICAM-1 and presence of functionalized SPIONs both apically and internally, suggesting cell internalization of the targeted particles.

Discussion: Click chemistry enables control of the antibody orientation on the particle surface, ensuring exposure of the active site. Our findings suggest that click chemistry allows simple and controlled bioconjugation of ligands onto SiO_2-coated SPION surfaces, which could enable the development of efficiently targeted MRI-contrast agents for IBD.

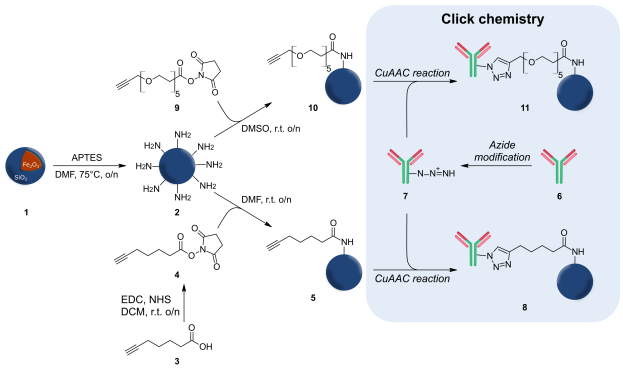

Figure1 new linker.png

Layer-by-Layer-modified lipid nanoparticles for miR-181a delivery in glioblastoma treatment

Wednesday, 17th January - 15:08: Nano-immunology - II (Room 608) - Oral - Abstract ID: 304

Mr. Victor Passos Gibson [1], ***Dr. Houda Tahiri*** [2], ***Dr. Chun Yang*** [3], ***Dr. Xavier Banquy*** [4], ***Dr. Pierre Hardy*** [2]

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Introduction: Glioblastoma multiforme (GBM) is the most common and lethal primary brain cancer (2). Current pharmacological interventions marginally increase the 12-month overall survival of patients with GBM. Among the novel therapeutic strategies being pursued, micro-RNAs, a class of non-coding RNAs, are receiving considerable attention for their regulation of several pathways implicated in tumorigenesis and survival. Notably, microRNA-181a-5p (miR-181a) has consistently been reported to be downregulated in GBM clinical samples, and its overexpression negatively affects tumor growth both *in vitro* and *in vivo* (2). To improve the delivery of miR-181a to GBM cells, we sought to develop a modified lipid-based nanocarrier capable of encapsulating and delivering miR-181a to GBM cells *in vitro* and *in vivo*.

Methods: Optimized ionizable lipid-containing lipid nanoparticles (LNPs) were constructed by covering themiR-181a-loaded LNPs with hyaluronic acid. Hyaluronan-decorated LNPs (HA-LNP) were characterized by DLS, encapsulation efficiency, and Atomic Force Microscopy. Targeting of GBM cells was identified after Flow Cytometry and Confocal Microscopy. Transfection efficiency was evaluated by GFP silencing after incubation of HA-LNPs/siGFP with HeLa and U251 GFP cells. The effect of miR-181a on U87 cells was assessed *in vitro* (viability assay) and *in vivo* (tumor growth, subcutaneous model).

Results: The resulting HA-LNPs targeted GBM cells more efficiently than non-modified LNPs and mediated siRNA and miRNA transfection *in vitro*. Finally, delivery of miR-181a by HA-LNPs induced significant cellular death of U87 GBM cells *in vitro* and delayed tumor growth in an *in vivo* subcutaneous tumor model.

Conclusions/Impact: In summary, we report on the surface modification of lipid nanoparticles using the LbL process for improved siRNA and miRNA delivery. We add more evidence that miR-181a is downregulated in GBM and rescuing its expression by exogenous gene transfer impairs tumor development *in vivo*. A better understanding of the miR-181a intracellular mechanism in GBM context will help researchers and clinicians to propose a combined strategy to alleviate the yet dismal prognosis of this malignancy.

Authors Index