Program& Abstract Book

SFNan
FRENCH SOCIETY FOR NANOMEDICINE

Montpellier, Le Corum
December, 4-6th, 2023

SCIENTIFIC PROGRAM

DECEMBER 4TH, 2023

12:30 Registration - Poster installation - Session A

13:30 **Opening session**
Dr TSAPIS Nicolas, President of SFNano - Welcome words

13:45 **Prof Gaurav SAHAY,** Department of Pharmaceutical Sciences, College of Pharmacy at Oregon State University, USA.
The quest for next generation lipid nanoparticles for cell-specific delivery of mRNA.

SESSION 1 – NANOMATERIALS FOR THERAPY

Chairpersons: Prof Sylvie Begu, Dr Cédric Chauvierre

14:20 **Prof Iwona CICHA,** University Hospital Erlangen, Germany.
Nanosystems for cardiovascular imaging and therapies

14:50 *Jaspe CHEN - Potentiality of lipid nanocapsules to deliver amiodarone for the treatment of atrial fibrillation*

15:05 *Guillaume JACQUOT - Chitosan-based hydrogel formulation for subcutaneous administration of monoclonal antibodies*

15:20 *Thibault DE LA TAILLE - Functionalized polysaccharide nanoparticles for a targeted treatment of ischemic strokes*

15:35 Coffee break

16:00 *Antonin MARQUANT - Biochemical Approach using Lipid Anchors to Associate Proteins to Extracellular Vesicles for Intracellular Drug Delivery*

16:15 *Riccardo PINOTTI - Synthesis of CuS nanoparticles using BSA as a scaffold for combined chemo/photothermal anticancer therapy*

SESSION 2 – ORGANIC NANOMATERIALS

Chairpersons: Prof Sylvie Begu, Dr Cédric Chauvierre

16:30 **Prof David VIRIEUX & Prof Philippe LEGRAND,** ICGM, Montpellier, France.
A shared story between medicinal chemists and galenists

17:00 Sponsors: Inside Therapeutics, Malvern, Nanoscale Metrix, Sanofi, Nano FCM

17:30 **General Assembly of SFNano** (French Society for Nanomedicine)

18:00 *Poster (Session A) and Cocktail*

20:30 Corum closure

DECEMBER 5TH, 2023

8:00 Registration

SESSION 3 – NUCLEIC ACIDS DELIVERY

Chairpersons: Dr Jeanne Chain, Dr Nicolas Tsapis

8:30 **Prof Olivia MERKEL,** LMU Munich, Germany.
Drugging the undruggable with pulmonary RNA delivery

9:00 *Hussein GENEDY - Chitosan-based nanoparticles for the delivery of micro-RNA for regeneration of the cardiac muscle*

9:15 *Karidia KONATE - WRAP family: Polyvalent cell-penetrating peptides for nucleic acids delivery*

9:30 *Qinglin WANG - In vitro and in vivo delivery mechanisms of ionizable lipid nanoparticles encapsulating TNFa siRNA and their efficacy on inflammatory models*

9:45 *Arianna RINALDI* - Hybrid polymer-lipid nanoparticles for siRNA delivery in glioblastoma therapy

10:00 *Sébastien ULRICH - Dynamic Covalent Polymers for RNA Delivery*

10:15 Coffee break et Poster Session A

SESSION 2 (PART 2) – ORGANIC NANOMATERIALS

Chairpersons: Dr Elisabeth Garanger, Prof Yohann Corvis

10:45 *Guillaume HERLEM - Nanostructuration of ePTFE by direct defluorinative amination for antibacterial activity*

11:00 *Thi Hong Van NGUYEN - Biodegradable nanoconjugates for precision lung delivery of anti-tuberculosis drug*

11:15 *Nasir ARAFATH - Preparation and characterization of DNase-functionalized antioxidant cerium oxide nanoparticles for stroke treatment*

11:30 **Prof Efie KOKKOLI,** John Hopkins University, Baltimore, USA.
Design of DNA and RNA nanotubes for selective targeting of different cancers

12:00 *Sponsors:* Izon, Wyatt, Serlabo, Microtrac, Formulation

12h15 Lunch and Poster Session A (end) and installation poster session B (from 13h45)

14:15 *Julie MOUGIN - Modelling and prediction of the fate of anticancer polymer prodrugs after subcutaneous administration*

14:30 *Feras OYOUN Preparation and physico-chemical characterization of eutectic-based nanoparticles functionalized by biocompatible co-polymers for cancer treatment*

14:45 *Hervé COTTET - Size-based characterization of nanoobjects in health applications by Taylor dispersion analysis*

15:00 *Julien DRAUSSIN - Heterodimeric peptide-based building blocks for nanoparticle functionalization*

SESSION 1 (PART 2) – NANOMATERIALS FOR THERAPY

Chairpersons: Dr Elisabeth Garanger, Prof Yohann Corvis

15:15 **Prof Claus-Michael LEHR,** Helmholtz Institute for Pharmaceutical Research.
Drug Delivery across Biological Barriers for combatting and preventing infectious diseases

15:45 Coffee break and Poster session B

YOUNG SESSION

Chairpersons: Dr Ghadir Kalot, Younès Louaguenouni

16h15 **Young Session and PhD award**

16h20 **Raghavendra PALANKAR** associate Editor, Nature Nanotechnology – An insider's view of *Nature* journals

16h50 *Clément LINGER - Labeling Solid Lipid Nanoparticles with Tuned BODIPY for Photoacoustic Imaging*

Pierre SARFATI - Hybrid particles for the physical treatment of thrombotic diseases

Lila LOUADJ - TROP2: a promising new therapeutic target in breast cancer

Zhihang ZHANG - A deep comparison of microwave-assisted synthesis methods of luminescent Carbon Dots

Daniel TRUCHAN - Controlling photothermal properties in cellular niche – synthesis and functionalization of novel molybdenum oxide nanocolloids

17h30 ***PhD Award – Best PhD in Nanomedicine year 2023***

17h45 Posters (session B)

18h30 Bus departure for Gala Dinner at Chateau Puech Haut

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DECEMBER 6TH, 2023

8:30 Registration

SESSION 4 – NANOSYSTEMS FOR IMAGING AND DIAGNOSTICS

Chairpersons: Dr Lucie Sancey, Dr Andrey Klymchenko

9:00 **Dr Jean-Luc COLL,** Institute for Advanced Biosciences, Grenoble, France.
Theranostic treatment of cancer base on optically active nanosystems.

9:30 *Ophélie DAL PRA - Two-Photon Dye-based Fluorogenic Organic Nanoparticles: Surface functionalization for Hyperbright Biosensors or Biomarkers*

9:45 *Mohamed BENDELLAA - Contrast agent for photoacoustic imaging of tumor: from aza-ZrDIPY molecule to nanoformulation*

10:00 *Tomas ETRYCH - Stimuli-sensitive polymer probes intended for fluorescence-guided surgery*

10:15 Coffee break and Poster session B

10:45 **Dr Massimo ALFANO,** Extracellular microenvironment Unit, Milan, Italy. Detect the undetectable: gold nanorods-assisted imaging and thermal treatment of bladder cancer lesions smaller than 1 mm.

11:15 *Catherine AMIENS - A nanostructured polymeric contrast agent for 19F-based Magnetic Resonance Imaging*

11:30 *Robin KUHNER - Ultrasensitive detection using luminescent nanoparticles*

12h00 Lunch and Poster session B

SESSION 5 – INORGANIC AND HYBRID NANOMATERIALS

Chairpersons: Prof Sylvie Begin, Dr Frédérique Cunin

14h00 **Dr Magali GARY-BOBO,** IBMM, Montpellier, France.
Therapeutic potential of photoactivable nanoparticles

14h30 *Joëlle BIZEAU – Controlling the silica shell growth of core-shell iron oxide @ stellate mesoporous silica nanoparticles: effects on MRI, magnetic hyperthermia and NIR-photothermia properties*

14h45 Gautier FELIX - Radical release induced by Magnetothermia

15h00 *Mélody PERRET Intracellular proteins targeting with bi-functionalized magnetic nanoparticles*

15h15 SFNano Awards and Final conclusion

15h45 **Meeting closure**

16h30 **Corum closure**

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SFNano
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Montpellier 4-6th December, 2023

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SFNano
FRENCH SOCIETY FOR NANOMEDICINE
Montpellier 4-6th December, 2023

Local organizing committee

Prof Sylvie Begu, ICGM

Dr Frédérique Cunin, ICGM
Prof Jean Marie Devoisselle, ICGM
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Dr Anne Aubert Pouessel, ICGM
Dr Laurianne Simon, ICGM

SFNano
FRENCH SOCIETY FOR NANOMEDICINE
Montpellier 4-6th December, 2023

General informations

Venue : Le Corum Convention Center,
Registration : Level 1 Joffre 1
Le Corum, Allée des Républicains, 34000 Montpellier
*Please use the left-side entrance**, not the main entrance of the Corum***

Poster sessions schedule:

- Session A : from 4th December to 5th december (13:45 pm)
- Session B : 5th December to 6th december

Social events

Welcome dinner cocktail

will be offered to all participants on the first evening.

- Date & time: December 4th – from 18h00 to 20h30
- Venue: Corum Convention Center, Joffre 1, Montpellier

Gala dinner

The **gala dinner** will be organized at the Chateau Puech Haut

The evening will start with wine tasting of a selection of wines from the Domain, followed by dinner.

- Date & time: December 5th – 19:00
- Venue: Château Puech Haut

2250 route de Teyran 34160 Saint-Drezery

- Access: Buses transportation at 18:30 from the Corum
- Price: 30€ (not included in registration fees)

Welcome letter

SFNan

Dear Colleagues, dear Friends,

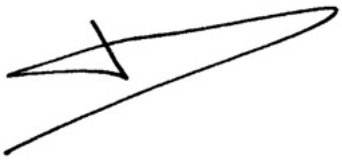
It is my great pleasure to welcome you to the 9th annual meeting of the French Society for Nanomedecine. SFNano is coming back to Montpellier for its annual meeting. We are particularly happy to be back here again five years after the 2018 meeting and hope this one will be as successful and fruitful in scientific exchanges and discussions. SFNano is grateful to the local committee for the thorough organization of this annual meeting. Many thanks to Prof Sylvie Begu and to the local committee.

Great speakers, well known in their fields, have been invited for all sessions and I would like to thank them for accepting our invitation. We are grateful to our sponsors for their continuous support over the years: Fujifilm, Izon, Malvern, Nanoparticle Metrix, SANOFI, Wyatt, Excilone, Dispertech, CordouanBrightSens Diagnostics, Serlabo, Servier, SOTAX, NanoFCM, Inside TX, Medincell, Phospholipid Research Center, Microtrac Formulaction, University Montpellier LUM, The Royal Society of Chemistry and Pole Chimie Balard.

SFNano strongly supports young scientists by awarding prizes for best poster, best oral presentation and best PhD award. In 2016, the Young SFNano group was launched and this group started a specific session of short talks. We continue to strongly support this initiative notably via a special price for the “3 min Short Talks”. The SFNano young group has been very active this year with the organization of two seminars dedicated to young scientists, one in person and one virtual. These events have been a great success. To all young PhD students, post-docs and young researchers, do not hesitate to join them and propose new ideas.

I would also personally like to thank colleagues from the SFNano board for their constant involvement. We hope that our commitment and dedication will be of great help for our community.

Dr Nicolas Tsapis, President of SFNano



The quest for next generation lipid nanoparticles for cell-specific delivery of mRNA

Prof Gaurav SAHAY
Department of Pharmaceutical Sciences, College of Pharmacy
Oregon State University, USA
contact: sahay@ohsu.edu

The field of nanomedicine is moving from an age of renaissance towards industrial revolution, in part, due to the ability of lipid nanoparticles (LNP) to package and deliver antigenic mRNA against SARS-CoV2, leading to the development of a powerful vaccine against COVID. The next horizon for our field remains tissue/cell-specific mRNA delivery that can lead to treatment of several diseases. Our lab has worked extensively in LNP design, synthesis, structure, and its impact on intracellular delivery of mRNA. I will talk about our labs pursuit onto understanding the journey of an LNP-mRNA within the cellular interior. These fundamental insights led us to design nanoparticles that can deliver mRNA to different tissues for the treatment of cystic fibrosis, retinal degeneration, and as COVID-19 therapeutics. We have further shown that novel peptide guided LNPs can deliver mRNA selectively in the retina of non-human primates and we plan to use mRNA-based genome editors for gene correction for treatment of blindness. Evolution of a cell-selective carrier for mRNA delivery through approaches will be discussed. Such platform technologies with capability to precisely deliver cargo to manipulate cells, correct diseases, with minimal off-target effects, will transform modern medicine.

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INVITED SPEAKER

Nanosystems for cardiovascular imaging and therapies

Prof Iwona CICHA
University Hospital Erlangen, Germany
Contact:

INVITED SPEAKERS

A shared story between medicinal chemists and drug formulation pharmacist

Pr Philippe LEGRAND & Pr David VIRIEUX
ICGM, UMR 5253 Montpellier, France
Contact : david.virieux@enscm.fr, philippe.legrand@umontpellier.fr

The development of a drug candidate requires a combination of skills that an individual alone cannot possess. While, in the early stages, only a medicinal chemist and a biologist are needed to establish an in vitro proof of concept, the preclinical studies on animals demand new expertise, particularly in setting up an administration scheme that must also be operational for clinical trials in humans.

This presentation focuses on a new class of glycomimetics in which the phosphorus atom acted as an excellent bioisostere for the anomeric carbon. These easily synthesizable compounds have been evaluated on various types of cancer cells. While some of the resulting compounds were perfectly soluble in aqueous media, those that proved most active both in vitro and in vivo turned out to be the most lipophilic. Being highly soluble only in organic solvents, it was necessary to develop a stable nanosuspension of these molecules allowing an IV early preclinical formulation following an oral administration one.

This collaborative effort between a group of medicinal chemists and drug formulation pharmacists has recently enabled this family of active molecules to reach Phase 1 clinical trial.

Potentiality of lipid nano capsules to deliver amiodarone for the treatment of atrial fibrillation

Jaspe Chen [1,2], Guillaume Lefebvre [1], Emilie Martinez [1], Sophie Tamareille [3], Clara Rapenne [2], Brice Calvignac [1], Patrick Saulnier [1,2]

[1] Micro et Nanomédecines Translationnelles – Université d'Angers, Institut National de la Santé et de la Recherche Médicale, Institut de Chimie du CNRS, France.

[2] Centre Hospitalier Universitaire d'Angers – PRES Université Nantes Angers Le Mans – France

[3]MitoVasc - Équipe CarME (Cardiovascular Pathophysiology) MitoVasc - Physiopathologie Cardiovasculaire et Mitochondriale, Université d'Angers, CNRS, INSERM – France

Introduction: Amiodarone (AMD) is used in patients with atrial fibrillation. As a lipophilic rug, it has a large but variable volume of distribution in the body and it accumulates in highly perfused organs such as liver and lungs. This pharmacokinetic profile explains why long-term treatments with AMD are associated with multiple side effects. One potential strategy to mod- ify the biodistribution of AMD is the use of nanomedicines. By encapsulating AMD inside nanovectors, its physico-chemical properties are replaced by the ones of the nanovectors. More- over, decorating the surface of these vectors with peptides confers them the ability to recognize specific cells. Therefore, encapsulation of AMD in functionalized lipid nanocapsules (LNC) to target actively the heart represents a promising strategy to limit its toxicity. This work focused on LNC-AMD nano-formulations and their toxicity studies.

Methods: LNC-AMD were prepared by a patented low-energy process using microfluidic and were purified (separation between LNC and wasted micelles) by a tangential flow filtration (TFF) process. They were characterized in terms of size and polydispersity (PDI) by dynamic light scattering and Nanoparticle Tracking Analysis (NTA), zeta potential by electrophoretic mobility, drug loading (DL) and encapsulation efficiency (EE) by UPLC coupled to UV detector. *In vitro* cytotoxicity of LNC-AMD was evaluated on different cell types (H9C2 cardiomyoblasts, HepG2 hepatoma cells and A549 human lung cancer cells). Viability was measured by MTT cell viability assay and lactate dehydrogenase (LDH) release. *Ex vivo* cardiotoxicity was evaluated on Langendorff-perfused rat hearts (recording of left ventricular developed pressure)

Results: LNC-AMD of 50 and 100 nm were obtained. They were slightly positive (zeta potential of +18mV) whereas blank LNC (without AMD) were nearly neutral. After purification, DL of LNC-AMD-50nm was of 0.4mg/ml of suspension of LNC with an EE of 60%. In H9C2 cells, TFF-purified LNC-AMD-50nm reduced cell viability at 20μM at both 24h and 48h compared to free AMD. Similar results are observed on HepG2 cells which viability is affected by treatment with LNC-AMD-50nm at 10μM. In A549 cells, MTT assay revealed more toxicity of LNC of 50nm whether they contained or not AMD compared to free AMD. This toxicity is reversed when LNC are purified by TFF. Finally, in *ex vivo* study, preliminary results showed that per- fusion with

LNC-BLK-50nm induces instability in left ventricular developed pressure and heart rate whereas perfusion with LNC-AMD-50nm does not affect left ventricular developed pressure but reduces heart rate starting 7μM.

Conclusion: *In vitro* studies showed different toxicity profile, with H9C2 and HepG2 cells being more sensitive to treatment with LNC-AMD-50nm, whereas toxicity could be reversed in A549 when formulations are purified by TFF. *Ex vivo* preliminary results give an insight on cardiac tolerance to LNC system. Short-term perspectives are the surface functionalization of LNC to establish proof-of-concept on Langendorff-perfused rat hearts.

Keywords: atrial fibrillation, cytotoxicity, targeting, lipid nanocapsule

Chitosan-based hydrogel formulation for subcutaneous administration of monoclonal antibodies.

Guillaume Jacquot * [1,2,3], Thomas Grea [4,5], Olivier Tillement [4], Sébastien Harlepp [1,3], Xavier Pivot [1,3], Alexandre Detappe [1,3,6]

[1] Institut de Cancérologie Strasbourg Europe (ICANS) – France
[2] Nano-H –France
[3] Strasbourg Drug Discovery and Development Institute (IMS) – France
[4] Institut Lumière Matière, UMR 5306, Université Claude Bernard Lyon1-CNRS – France
[5] Université Claude Bernard Lyon 1, INSA Lyon, Université Jean Monet, CNRS, UMR 5223 Ingénierie des Matériaux Polymères (IMP) – France
[6] Equipe de Synthèse Pour l'Analyse, Institut Pluridisciplinaire Hubert Curien (IPHC), UMR 7178 CNRS/Université de Strasbourg – France

Subcutaneous (SC) administration of monoclonal antibodies (mAbs) is an established approach for enhancing therapeutic outcomes and patient adherence to treatment. The FDA/EMA-approved enzymatic approach using recombinant human hyaluronidase (rHuPH20) for mAbs delivery involves the degradation of crosslinked sodium hyaluronate to increase tissue per- meability, compared to the free SC administration. However, this approach lacks tunable release properties and necessitates individual optimization for each mAb. To overcome this drawback, physical polysaccharide hydrogels, which are soft, water-swollen gels formed by physical inter- chain interactions, are possible alternatives because they exhibit favorable tunable physicochem-ical and biodegradability properties. But, to date, none were found to exhibit simultaneously biocompatibility, biodegradability, and controlled release properties for large protein delivery (≥150kDa). Here, we report the development of a novel two-component hydrogel comprising two polymers (chitosan and chitosan@DOTAGA) that can be minute-mixed with sterile mAbs formulations initially developed for intravenous (IV) administration to repurpose them as novel tunable SC formulations. We validated this hydrogel formulation in mice with several mAbs (trastuzumab, trastuzumab biosimilars, rituximab) and in nonhuman primates (NHPs) studies with clinically-relevant volumes of injection confirming the biodegradability and biocompati- bility of this material. Further, pharmacokinetic studies in mice demonstrated the ability to achieve several weeks of treatment from a single SC administration, a feat currently unattainable with rHuPH20. Finally, in the pharmacokinetic study conducted NHPs, we confirmed the possibility of obtaining comparable pharmacokinetic parameters to that of the rHuPH20+mAbs formulation. These results suggest the potential for rapid translational application to humans and open possibilities for the clinical development of this novel formulation for subcutaneous (SC) biosimilars.

Hybrid particles for the physical treatment of thrombotic diseases

Pierre Sarfati*[1], Thibault De La Taille[1], Louise Fournier[1], Claire Wilhelm[1], Yoann Lalatonne[1], Cédric Chauvierre[1]

[1] Laboratoire de Recherche Vasculaire Translationnelle Institut National de la Santé et de la Recherche Médicale, Université Paris Cité, Université Sorbonne Paris nord – France

Thrombosis is responsible for most strokes and heart attacks, which are the two leading causes of death worldwide (1). Reperfusion is commonly performed with thrombolytics such as recombinant tissue plasminogen activator (rt-PA) but these present numerous limitations (severe adverse effects, few eligible patients, low recanalization rate). With the objective of designing a safer and efficient treatment, we propose here to have a targeted physical action on the thrombus. This non-pharmaceutical thrombolysis would be a combination of thermal and mechanical action, through light and magnetic stimulation, and would be targeted to the occluded vessel by an external permanent magnet (2,3). To achieve this strategy, we designed hybrid organic/inorganic particles that are stable, intravenously injectable, and have great photo thermal and magnetic properties. We confirmed the hybrid structure by electronic microscopy and FT-IR spectroscopy. Then, we characterized the size and charge of these new microsystems. Additionally, we studied their stability in saline solution and in time. Magnetization measurements and particle counting were performed to compute the magnetic moment of these hybrid particles. Also, their response to magnetic stimulation was verified. The magnetic evaluation was completed with photothermal tests to estimate their ability to increase temperature when irradiated by a near-infrared laser. Finally, we were able to develop fluorescent hybrid particles and showed that they could efficiently target thrombi in vitro, even under venous or arterial flow, through a magnetic field. In the coming months, we will study the thrombolytic effect of these photo- and magneto-stimulated hybrid particles on ex vivo blood clots. When the therapeutic dose of these microsystems is determined, we will carry out cytotoxicity tests in this range.

References:

(1) The top 10 causes of death. https://www.who.int/news-room/fact-sheets/detail/the-top-10- causes-of-death.

(2) Tasci, T. O. et al. Enhanced Fibrinolysis with Magnetically Powered Colloidal Microwheels. Small 13, 1700954 (2017).

(3) Fromain, A., Perez, J. E., Van De Walle, A., Lalatonne, Y. & Wilhelm, C. Photothermia at the nanoscale induces ferroptosis via nanoparticle degradation. Nat. Commun. 14, 4637 (2023).

Keywords: hybrid particles, non-pharmaceutical treatment, photothermia, magnetism, mechanical action, targeting, thrombolysis

Biochemical Approach using Lipid Anchors to Associate Proteins to Extracellular Vesicles for Intracellular Drug Delivery

Antonin Marquant [a], Anne Aubert-Pouessel [a], Claudia Muracciole-Bich [c], Guillaume Cazals [a], Jade Berthelot [a], Julie Constanzo [d], Jean-Marie Devoisselle [a], Joel Chopineau [a], Pierre Martineau [d], Laurence Guglielmi [d], Marie Morille [a,b]

[a]Institut Charles Gerhardt Montpellier (ICGM), UMR 5253-CNRS-UM-ENSCM , Montpellier,
[b] Institut Universitaire de France.
[C] Institut des Biomol´ecules Max Mousseron (IBMM), UMR 5247-CNRS-UM-ENSCM, Montpellier,
[d]Institut de Recherche en Cancérologie de Montpellier (IRCM), Inserm U1194, Montpellier

Intrasic natural properties of Extracellular Vesicles (EVs) enable them to deliver any type of biomolecules from nucleic acids to proteins. Therefore, EVs are interesting tools to deliver pro- teins in cell cytoplasm and reach intracellular targets. Indeed, when systemically administered, therapeutic proteins can be degraded by plasma proteases, eliminated by renal clearance and, even if they reach target cells, are poorly able to cross cellular membrane and to reach their molecular target. Nevertheless, even if EVs are natural protein delivery systems, loading of exogeneous proteins inside these vesicles still complex. This is achieved either by working on genetic engineered producing cell or by using pharmaceutical engineering of already isolated EVs [(1).]

In this context, our team aims at using a new biochemical approach to associate an exogeneous protein to EVs. We conjugated a lipid anchor to the exogeneous protein by using the maleimid-thiol michaelis addition in order to confer it an hydrophobic EV-association driving force. To establish our proof of concept, we first selected Horse Radish Peroxidase (HRP) as model protein and mMSC-EVs. EVs and biochemically-modified HRP were incubated following our established protocol, before Steric Exclusion Chromatography separation to allow calculation of biofunctional protein association to EV. Different parameters of association were tested, such as type of lipid and linker, concentration of HRP and temperature of incubation. EVs concentration and size (NanoTracking Analysis) as well as protein biologic activity (enzymatic assay) were measured. Interestingly, a 20-fold increase in the association of biochemically-modified HRP to EVs as compared to the non-modified HRP was observed, without losing its enzymatic activity and a yield of 34% of HRP associated to EVs was reached. Based on these promising results, we are investigating if this association can led to an actual intracellular delivery. In a subsequent step, we will investigate if this driving force can increase the loading of protein inside EVs in association with physical membrane-permeabilisation protocol onto EVs.

Finally, our objective is to establish the versatility of our method and to apply these results to other proteins, especially to intrabodies, which could represent a powerfull therapeutic tool.

References :

(1) Le Saux et al. Advanced Drug Delivery Reviews 176 (2021) 113837. doi : 10.1016/j.addr.2021.113837

Keywords: Biochemistry, Drug delivery, protein, drug loading, Extracellular Vesicles

Synthesis of CuS nanoparticles using BSA as a scaffold for combined chemo/photothermal anticancer therapy

Riccardo Pinotti*[1,2], Leonardo Dias[2], Ana Hortelão[3], Ali Abou Hassan[2,4], Cecilia Ménard-Moyon[1]

[1] CNRS – Laboratory CNRS I²CT/UPR3572 Immunology, Immunopathology and Therapeutic Chemistry, Strasbourg Drug Discovery and Development Institute (IMS), Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France – France

[2] Sorbonne Université – Laboratoire de Physico-chimie des Electrolytes et Nanosystèmes InterfaciauX (PHENIX UMR 7195, UPMC-CNRS), Université Pierre et Marie Curie, 4 place Jussieu, 75252 Paris –France

[3] Sorbonne Université – Laboratoire de PHysico-chimie des Electrolytes et Nanosystèmes InterfaciauX (PHENIX UMR 7195, UPMC-CNRS), Université Pierre et Marie Curie, 4 place Jussieu, 75252 Paris –France

[4] Institut Universitaire de France

Mainstream cancer therapies, such as chemotherapy and surgery, are effective but riddled with inconvenient side effects. Photothermia (PT), being a non-invasive and localized treatment, is steadily gaining attention in the medical field. (1) One of the main challenges in this field is to provide safe and low-cost materials with high efficiency in PT. For the latter is important to un- derstand the relationship between the synthesis conditions, the structure, and the properties of the final nanoparticles (NPs). Copper sulfide is one of the best thermal nanoplasmonic agents in this field. (2) In this context, we investigated the synthesis of copper sulfide nanoparticles (CuS NPs) with an average size of 10 nm by taking advantage of bovine serum albumin (BSA) both as sulfur source and dispersing agent, obtaining CuS NPs with a protein shell (BSA@CuS). (3) Various synthesis parameters were investigated, including the copper source, temperature, and copper concentration. To understand the link between these parameters and the NP properties, characterization was performed using various techniques such as scanning transmission electron microscopy, UV-Vis-NIR spectroscopy, circular dichroism, Raman spectroscopy, and inductively coupled atomic emission spectrometry (ICP-AES). The NPs showed high absorbance in the bio- logical windows (NIR-I & II) and were tested for photothermal activity using two different lasers with a low power density (1064 nm and 808 nm at 0.3 W/cm^2), demonstrating a concentration- dependent photothermal effect. The NPs were also tested for the production of reactive oxygen species. Finally, the optimized NPs were tested *in vitro* using U87MG glioblastoma cell line coupled with NIR irradiation at 1064 nm, offering interesting perspectives for the treatment of cancer.

References:

(1) Li, X.; Lovell, J. F.; Yoon, J.; Chen, X.. *Nat. Rev. Clin. Oncol.* 2020, *17* (11), 657–674.

(2) Curcio, A.; Silva, A. K. A.; Cabana, S.; Espinosa, A.; Baptiste, B.; Menguy, N.; Wilhelm, C.; Abou-Hassan, A.. *Theranostics* 2019, *9* (5), 1288–1302.

(3) Sheng, J.; Wang, L.; Han, Y.; Chen, W.; Liu, H.; Zhang, M.; Deng, L.; Liu, Y. *Small* 2018, *14* (1), 1702529.

Keywords: Cancer Therapy, Phototermal Therapy, Copper Sulfide, Bovine Serum Albumine, NIR, ROS, Optimization

DECEMBER 5TH, 2023

INVITED SPEAKERS

Drugging the undruggable with pulmonary RNA delivery

.

Prof. Olivia MERKEL
LMU Munich, Germany

The rise of RNA medicines, including mRNA vaccines and other therapeutic approaches like short interfering RNA (siRNA) and antisense oligonucleotide (ASO)-based drugs, has been remarkable. However, the primary obstacle faced is efficient delivery beyond the liver. While successful intramuscular delivery of mRNA vaccines has been proven effective in billions of individuals, most RNA therapeutics are confined to treating a limited number of conditions.

To address this limitation, our research aims to establish a platform for spray-drying RNA nanoparticles (NPs), improving storage, transport conditions, and expanding delivery to the lungs through inhalation. By creating dry powder formulations, we aim to extend shelf-life and improve targetability beyond the liver.

Regarding lung cell targeting, our research focuses on enhancing the specificity and effectiveness of RNA nanocarriers. We've developed shielded nanocarriers to decrease nonspecific interactions with mucus and modified carriers with targeting ligands for specific cell uptake in the lung for the treatment of target, which are currently considered undruggable in asthma, cystic fibrosis, idiopathic fibrosis, lung cancer, and respiratory viral infections. Furthermore, we're investigating biodistribution and pharmacokinetic experiments to understand local bioavailability in the lung after pulmonary administration. Our goal is to develop dosing regimens based on lung retention and to understand potential side effects in off-target tissues for further testing in models of disease for the development of inhaled RNA therapeutics.

Engineering lipid-based micro-RNA therapeutics for treatment of intervertebral disc degeneration

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Low back pain (LBP) stands as a global health burden, afflicting almost 80% of adults at some point in their lives, with substantial disability and socioeconomic consequences. Remark- ably, around 40% of all LBP cases are attributable to intervertebral disc degeneration (IVDD), a condition that unfortunately lacks a definitive cure. Hence, there is a dire need for innovative therapeutics that not only offer symptomatic relief but also have the capacity to stop or even reverse the IVDD process. The past decade has witnessed an increased interest in harnessing RNA interference (RNAi) mechanisms for therapeutic applications, going all the way to the clinic and meeting the regulatory agencies' criteria. Given the accumulating evidence that multiple micro-RNAs (miRs) are dysregulated during disc degeneration, they could have a huge potential as biomarkers, targets, or active principles against this debilitating condition. Our miR of choice, miR-155, is downreg-ulated in IVDD and is known to be involved in protecting the IVD from apoptosis, decreasing degradation, and controlling inflammation (1). However, miRNA delivery encounters extracellular and intracellular barriers. Extracellularly, they would be rapidly degraded by endogenous nucleases, and intracellularly, their uptake will be hindered by their relatively high molecular weight and negative charge. A promising technique to address this challenge is the vectorization of miRNAs within lipid nanocapsules (LNCs), providing both protection from degradation and improving their uptake within the scarce target cells, called nucleus pulposus (NP), of the degenerated IVD (1,2).

Henceforth, in this study, we developed miR-155-loaded LNCs to be safely injected for the treatment of IVDD. These LNCs were formulated using the phase inversion process by adding cooled miR-155-lipoplexes in the phase inversion zone (PIZ) of a mixture of caprylic-capric triglycerides and hydroxy-stearate-PEG. The LNCs were fully characterized to be 78 nm in hydrodynamic diameter, 0.08 of polydispersity index, and +11 mV in zeta potential. Finally, surface modifi- cation of the LNCs with targeting peptides was proposed. Nucleus pulposus (NP) cell affinity peptides such as short Link-N (s-Link-N) peptide have a direct anabolic effect on the NP cells and could be utilized in LNCs surface decoration either covalently or by adsorption techniques. Loading the LNCs with both miR-155 and s-Link-N could offer a huge potential in terms of synergetic pro-regenerative effects with enhanced ability to target the NP cells in situ and in vivo.

Keywords: Intervertebral disc degeneration (IVDD), Lipid nanocapsules (LNCs), microRNA (miR), RNA interference (RNAi) delivery, and for stratified medicine.

WRAP family: Polyvalent cell-penetrating peptides for nucleic acids delivery

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The WRAP family are short tryptophan and arginine rich amphipathic Cell-Penetrating Peptides (CPPs) originally designed for delivering short interfering RNA (siRNA) (1). By a non-covalent strategy, they are able to form peptide-based nanoparticles (PBNs) that exhibit efficient siRNA delivery both *in cellulo* and *in vivo*. This highly effective vector family generates at a peptide/siRNA molar ratio of 20/1 stable small PBNs around 80-100 nm in size. These nanoparticles are able to achieve an important knock-down (KD) until 70-80% of luciferase over- expressed in a wide variety of cell lines such as HT29, Neuro2a, U87 and even in xenografted tumor mouse models (2).

In the field of gene therapy, siRNAs are promising therapeutic tools with five FDA-approved entities since 2018 and they have gained recognition in developmental biology research. How- ever, for the WRAP-based PBNs, the challenge lies in transfecting other nucleic acids such as plasmids (pDNA) and more recently mRNA. These nucleic acids differ from siRNA due to their larger and more flexible structures, posing a challenge for WRAP-based PBNs to promote their transfection.

We optimized the formulation and internalization conditions for both pDNA and mRNA to achieve successful transfection using the same peptide. This fine tuning between PBNs forma- tion and delivery involves optimizing the ratio of charges, a crucial parameter adapted to their high molecular weight, and determining the appropriate quantities of these nucleic acids to pre- vent any cytotoxic effects while still ensuring effective delivery. Both pDNA or mRNA-loaded PBNs exhibit size between 50 nm to 80 nm, smaller than those get with siRNA. Using nucleic acids encoding the GFP protein (either pDNA or mRNA), we demonstrate the transfection ef-ficiency of those PBNs in cells by microscopy and western blotting.

In conclusion, WRAPs are polyvalent and very easy to handle CPPs which can form PBNs by simply mixing them to different kinds of nucleic acids, giving rise to a biological response.

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Dexamethasone-loaded DSPE-PEG(2000) micelles as a drug delivery system to treat dysregulated inflammatoryresponse

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Sepsis arises from a systemic infection that triggers an uncontrolled inflammatory response which may worsen into multi-organ failure and death1. Currently, other than antibiotic regimens in combination with cardiovascular/respiratory supports, there is no established cures for sepsis. Among the therapeutic approaches, glucocorticoids, notably dexamethasone (DXM), have been widely used to ameliorate the excessive inflammation associated with sepsis. This work presents the formulation and efficacy assessment of DXM-micelles, a drug delivery system based on DSPE-PEG(2000) micelles encapsulating DXM.

First, we comprehensively investigate the physicochemical properties, stability, *in vitro* release kinetics, and cytotoxicity of DXM-micelles on both human and murine monocytes. Additionally, we investigate the *in vivo* therapeutic efficacy of DXM-micelles using two mouse models, namely the endotoxemia model and the caecal ligation and puncture (CLP) model for sepsis. Further- more, the targeted delivery and preferential accumulation of DXM-micelles within immune cells were explored, shedding light on the potential immunomodulatory benefits of this innovative drug delivery approach. The results indicate that DXM-micelles have the potential to revolu-tionize anti-inflammatory therapy, offering a safer and more effective alternative to conventional treatment.

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Keywords: Sepsis, dexamethasone, micelles, immunomodulation

Hybrid polymer-lipid nanoparticles for siRNA delivery in glioblastoma therapy

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Small interfering RNA (siRNA) holds great potential to treat many difficult-to-treat diseases. However, developing safe and efficient delivery systems to exploit siRNA in therapy remains challenging.1 This study aimed at investigating the suitability of polymer-lipid hybrid nanoparticles (HNPs) as a novel siRNA delivery platform for application in glioblastoma therapy. 2 Two HNP formulations, consisting of a FDA-approved polymer (PLGA) and a cationic lipid (DC-Chol or DOTAP), were developed. After a characterization of HNPs, a model siRNA was complexed onto their surface to form siRNA/HNP complexes. The physicochemical properties and siRNA binding ability of complexes were assessed over a range of nitrogen-to-phosphate (N/P) ratios to optimize the formulations. At the optimal N/P ratio, complexes effectively bound siRNA and protected it from enzymatic degradation. As proof-of-concept, uptake and bioefficacy of complexes were assessed in vitro on U87MG human glioblastoma cell line expressing luciferase gene. Complexes were able to deliver anti-luciferase siRNA and induce a remarkable suppression of gene expression. Noteworthy, the effect of DOTAP-based formulation was not only three-times higher than DC-Chol-based ones, but also comparable to lipofectamine transfection reagent. Using NIH3T3 mouse fibroblast cell line, this formulation was shown to possess high cytocompatibility in vitro up to a dose 15 times higher than the effective one. These findings set the basis to exploit this nanosystem for targeting relevant GB-related genes in further *in vitro* and *in vivo* studies.

Keywords: small interfering RNA (siRNA), nanomedicine, glioblastoma, lipid, polymer hybrid nanomedicines, DOTAP, DC, Chol

Dynamic Covalent Polymers for RNA Delivery

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Living matter is made of dynamic assemblies of biomolecules. Viruses also exploit abiotic dynamic self-assembly processes to build and dissociate on demand the capsids that serve to transport their viral genetic material. We thus believe that the introduction of dynamic covalent chemistry in the design of artificial gene delivery vectors is very promising since it will enable control over the complexation and release of therapeutic nucleic acids.(1). We explore the potential of dynamic covalent polymers (DCPs) for nucleic acids recognition and delivery. We first reported the formation of cationic DCPs and their unprecedented ability to effectively complex DNA through multivalent interactions, while also being susceptible to depolymerization at acidic pH.(2) We then developed a second generation of DCP vectors made of modified amino acids and showed an initial successful application in siRNA delivery on cells.(3) More recently, we reported an in situ approach whereby the DCPs are formed directly in aqueous media through a dynamic covalent polymerization templated by the siRNA.(4) More recent studies by our group have extended the concept toward the effective complexation of mRNA.

In this talk, I will present our approach and the main results that show how RNAs can template the fabrication of their own vectors.

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Keywords: RNA delivery, dynamic covalent polymers

INVITED SPEAKER

Design of DNA and RNA nanotubes for selective targeting of different cancers

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Self-assembly of amphiphilic molecules is an attractive method to engineer supramolecular materials for biomedical and other applications. In my group, we focus on the design of DNA- and RNA-amphiphiles and evaluate how different building blocks of the amphiphiles affect their tendency to self-assemble spontaneously into different nanostructures, as well as their potential to be used for different applications. In this presentation, I will discuss aspects of the molecular design of nucleic acid amphiphiles that control the formation of functional nanotubes, along with their use as targeted delivery vehicles for glioblastoma and triple negative breast cancer.

Nanostructuration of ePTFE by direct defluorinative amination for antibacterial activity

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Expanded polytetrafluoroethylene (ePTFE, aka gore-texTM) is commonly used for implantable medical devices due to its low-fouling property, thermal and mechanical stabilities as well as their chemical inertness. The downside is its challenging chemical nanofunctionalization to retain these remarkable bulk properties by modifying only its surface for further grafting an- tibacterial molecules to combat antibiotic resistance.1

Herein, we report lithium alkylamides as a new class of reagents allowing direct amination of ePTFE. This one-pot experimental process performed under mild conditions quickly leads to both the reduction of C-F bonds and their amination by the alkylamide moiety, following aSN1 mechanism, as shown by full ab-initio molecular dynamics (AIMD) calculations. Experimental proofs of the direct nano-etching of the ePTFE surfaces come first from eyes and microscopy observations. Indeed, upon chemical treatment there is a drastic change in colors and rugosity as evidenced by SEM, AFM, and optical microscopy. The new morphology of the surface, compared to pristine one, reveals not only a modification of the color but also the apparition of spherulite-like structures. Defluorination and amination of ePTFE are confirmed by IR-ATR and Raman, in agreement with XPS analysis. For instance, the IR-ATR -CF2 bands of pristine ePTFE decrease in in- tensity upon modification with LiEDA (the reaction between lithium and ethylenediamine). Characteristic IR-ATR bands of -NH2, are visible for chemically modified ePTFE. Carbons sp2 and sp revealed by XPS confirm the drastic rearrangement of the modified ePTFE surface, low- ering contact angle, and indicating raise of the surface free energy. Grafting of antibacterial chitosan, branched polyethylenimine (b-PEI), or polyvinylpyrrolidone iodine, was evaluated towards dermal cell cytotoxicity and against six main strains causing nosocomial diseases for non-cytotoxicity and antibacterial efficacy, respectively. The treatments were effective on at least 3 bacteria. One treatment stood out with an antibacterial effect on all the bacteria. These treatments can be considered as a proof of concept that can be applied to PTFE or ePTFE based surgical prostheses and to fight against antibiotic resistance in nosocomial infections.

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Keywords: Nanostruturation, ePTFE, bacteria, antimicrobial, cytotoxicity, SEM, XPS, IR, Incucyte

Biodegradable nanoconjugates for precision lung delivery of anti-tuberculosis drug

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Tuberculosis (TB) is an infectious disease mainly affecting the lungs, which remains a global health problem, causing millions of deaths worldwide. The bacilli accumulate in alveolar macrophages, leading to the formation of granulomas. Conventional oral therapies fail to deliver therapeutic levels of drugs to the bacteria at the target sites. Thus, direct administration of anti-TB drugs into the lungs has been proposed to achieve higher drug concentrations close to the lesions. This work aims to develop microparticles (MPs) suitable for inhalation containing pyrazinoic acid-loaded nanoconjugates (NCs). The NCs will be dispersed in MPs and undergo degradation to release the active moieties on the target sites, thereby enhancing the efficacy of anti-tuberculosis drug. The conjugates are composed of pyrazinoic acid (POA) grafted to a biocompatible, biodegradable, and highly functionalizable polymer: poly (malic acid). Different spacers were considered to link the POA to the polymer to control the release rate. The conjugates can self-assemble into monodisperse NCs of ~140nm diameter with POA contents ranging from 25 to 75 wt%. The release rate could be tuned from 2 to 7 days by varying the spacer chemistry and the percentage of coupled POA. Hydrolytic degradation was followed by NMR to study the degradation mechanism. Their efficacy against Mycobacterium tuberculosis in vitro was tested. Preliminary formulations into MPs using spray drying resulted in powder with a geometric diameter of around 4 μm. This new system, which provides a high loading rate and controlled drug release, has proved to be an attractive option for the treatment of tuberculosis.

Keywords: nanoconjugates, microparticles, tuberculosis, poly(malic acid), controlled release

Preparation and characterization of DNase-functionalized antioxidant cerium oxide nanoparticles for stroke treatment

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Ischemic stroke, a disease resulting from the occlusion of a cerebral artery by a thrombus is a major global health concern. Although thrombolysis with recombinant tissue plasminogen activator (r-tPA) is considered the gold standard of pharmacological treatment for stroke, only 1/3 of patients experience complete reperfusion of the occluded vessel. This resistance to r-tPA has recently been linked to the presence of Neutrophil Extracellular Traps (NETs) at the surface of the thrombus. DNA composing these NETs are thus a potential therapeutic target to improve post-stroke reperfusion. However, increasing reperfusion might increase oxidative stress known to alter vessels and lead to drastic cerebral hemorrhages.
In order to improve stroke treatment by both increasing reperfusion and reducing oxidative stress, we propose to functionalize antioxidant cerium oxide nanoparticles (CNPs) with DNase I to degrade NETs. CNPs were first coated with PEG co-polymers to enhance their stability, biocompatibility, and facilitate functionalization with DNase I. DLS, UV-spectroscopy, TGA, SLS, and fluo-rescamine assay allowed characterizing coated nanoparticles. Successful DNase grafting onto coated CNPs was performed and confirmed by Bradford assay. The antioxidant activity of DNase-functionalized CNPs was assessed through the evaluation of their superoxide dismutase (SOD) and catalase-like activity. The biological activity of grafted DNase was tested by its ability to degrade plasmid DNA and then further confirmed and quantified on fibrillar DNA. Coated CNPs had a hydrodynamic diameter of 40 nm and a neutral charge as determined by DLS and electrophoretic mobility. TGA and SLS allowed determining the number of poly- mers per particle. DNase grafting was optimized by studying the influence of DNase and particle concentration as well as activation time and change of pH from activation step to coupling step. Following DNase functionalization, CNPs were able to retain their SOD- and catalase-like ac- tivity. Moreover, the biological activity of grafted DNase was preserved as it could efficiently degrade both plasmid and fibrillar DNA.
In conclusion, DNase could be successfully grafted onto CNPs. DNase-grafted nanoceria retained their SOD-, catalase-like activities as well as DNA-degrading ability. This study thus highlights the potential of DNase-functionalized CNPs as a promising strategy to improve ther- apeutic outcomes after ischemic stroke.

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Keywords: Cerium oxide nanoparticles, Antioxidant, Functionalization, DNase

Modelling and prediction of the fate of anticancer polymer prodrugs after subcutaneous administration

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Conventional anticancer chemotherapy is usually administered *via* intravenous (IV) injec- tion. However, this route is invasive and has a number of disadvantages, resulting in patient discomfort, impaired compliance and important healthcare costs. Subcutaneous (SC) injection may overcome many of these limitations, resulting in increasing use for drug administration as this method is less invasive, may allow self-administration, requires minimal preparation and is less costly, making it much more convenient for the patient and the healthcare system. Nonetheless, SC injection is only possible for a limited amount of anticancer drugs, as most of them are irritant or vesicant and therefore, not suitable for this administration route. (1, 2)

We recently developed a novel approach consisting in the design of hydrophilic polymer pro- drugs, using paclitaxel (Ptx) as model drug due to its strong hydrophobic and vesicant nature, and polyacrylamide (PAAm) as a biocompatible and hydrophilic polymer, to allow subcutaneous administration of irritant/vesicant chemotherapies. (3) SC injection of these polymer prodrugs to tumor-bearing mice demonstrated a sustained release of Ptx in the bloodstream and a superior anticancer efficacy compared to Taxol, without inducing any local toxicity, thus confirming the suitability of this strategy.

With the aim of extending this approach to other polymers and drugs, we felt the need to rely on an *in vitro* tool to predict the SC fate of the designed polymer prodrugs and identify the best candidates for further *in vitro* and *in vivo* studies. We present here the method we developed to fill this knowledge gap, using the Subcutaneous Injection Site Simulator (Scissor) to simultaneously establish the diffusion profile of the polymer prodrugs through the SC compartment, as well as to detect a potential early drug release at the injection site.

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Keywords: Subcutaneous, cancer, chemotherapy, polymer, prodrug

Preparation and physico-chemical characterization of eutectic-based nanoparticles functionalized by biocompatible co-polymers for cancer treatment

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The involvement of organic solvents in the nanoparticle preparation process can lead to harmful effects on the environment and human health. Over the past decades, therapeutic deep eutectic solvents (TheDESs) were deeply studied as potential alternatives to organic sol- vents due to their potential low toxicity, low cost, high adjustability, biodegradability, and easy preparation. Therefore, previous studies have demonstrated the interest of using these eutectic systems for various pharmaceutical applications. In this work, we prepared two novel TheDESs comprising active pharmaceutical ingredients (APIs) with potent anticancer activity. The prepared eutectic systems were characterized using different techniques such as Differential scanning calorimetry (DSC) and Fourier-transform infrared (FTIR) spectroscopy. Then, the obtained TheDESs were used to produce original eutectic-based nanoparticles (ENPs) function- alized by biocompatible polymer stabilizing agents using two different bottom-up preparations: the eutectic solvent manual injection and a microfluidic tool. The physico-chemical character- istics of these nanoparticles were evaluated by using different techniques such as dynamic light scattering (DLS) and transmission electron microscope (TEM). In order to enhance the thera- peutic outcomes of these ENPs, several attempts have been made to graft the hyaluronic acid polymers on the surface of the nanoparticles owing to its high affinity to the CD44 receptors which are highly expressed in tumor cells (Figure 1). Finally, *in vitro* tests were performed in order to evaluate and compare the therapeutic activity of the most promising formulations for further *in vivo* experiments.

Keywords: Therapeutic deep eutectic solvents, nanoparticles, drug delivery systems, targeted therapy, polymers, microfluidics, cancer

Size-based characterization of nanoobjects in health applications by Taylor dispersion analysis

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Taylor dispersion analysis (TDA) is a new promising technique for the determination of dif- fusion coefficients and hydrodynamic radii of a myriad of nanoscale objects. The principle of this method is based on the band broadening of a solute plug injected in a miniaturized Poiseuille flow. It allows determining the hydrodynamic radius of virtually any mixture of solutes, on a range of size ranking between 0.1 and 300 nm. In this presentation, after a brief introduction about the principle of the method supported by a motion design, different applications will be presented on various pharmaceutical/biomedical topics including, mRNA loaded lipidic nanoparticles and their formulations (LNP) (1), proteins and vaccine antigens (2), drug deliv- ery nanoparticles or nanogels (3), cubosomes and liposomes (4), microemulsions, beta-amyloïd peptide mixtures and their aggregates (5), and antigen/adjuvant interactions in vaccines (6). Through these examples of applications, the advantages and limits of TDA will be presented. TDA is insensitive to the presence of dusts (contrary to scattering techniques), and leads to a fair size distribution of the sample generally based on the weight-average of the constituents. With very small injected volumes (nL), application to ultra-small nanoparticles (below 5 nm), straightforward implementation, the absence of calibration, no filtration of the sample, TDA is a method of choice for the size characterization of solutes in health applications.

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Heterodimeric peptide-based building blocks for nanoparticle functionalization

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Currently, twelve nanoparticles have been clinically approved in oncology, either as drug delivery systems or imaging agents. However, it has been demonstrated that only 0.7% of the injected dose effectively reaches tumor cells upon systemic administration. As an alternative solution, the development of targeted nanoparticle systems, akin to what has been achieved with antibody-drug conjugates, is gaining momentum. In this study, we present a novel strategy for functionalizing nanoparticles using a modular approach, allowing for precise biofunctionalization with tumor-associated antigens and immune-cell-associated antigens. This strategy aims to overcome the challenges associated with site-specific conjugation processes, maintaining con- trolled ratios of nanoparticles to antibodies, and ensuring batch-to-batch replicates with limited variations. In this proof-of-concept study, we validated the in vitro targeting specificity of five different nanoparticles (liposomes, PLGA nanoparticles, Gd-based/Tb-based/Eu-based ultrasmall nanoparticles) designed to target HER2, CD38, and NKG2D antigens. Furthermore, in healthy mice, our findings reaffirmed the outcomes of our previously published meta-analysis study, con- firming that the pharmacokinetic and pharmacodynamic properties of functionalized nanoparticles are primarily influenced by the nanoparticle itself. Finally, using a HER2+ mouse cancer model, we demonstrated that targeted nanoparticles improved tumor uptake from approximately 4% of the injected dose per gram of tumor when using liposomes alone to as high as 8% when employing HER2-targeting moieties. Additionally, these targeted nanoparticles exhibited enhanced tumor retention over time. This study underscores the potential of our platform to generate easily implementable targeted nanoparticles capable of addressing a wide range of targets, including both immune-based and tumor antigens. This versatility opens up a broad spectrum of theranostic applications.

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INVITED SPEAKER

Drug Delivery across Biological Barriers for combatting and preventing infectious diseases

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Urgently needed anti-infective drugs and vaccines must reach their targets safely and efficiently. Not only the body's outer epithelia, like e.g., gut, skin and lung, but also the bacterial cell envelope as well as the polymer matrix of bacterial biofilms represent important biological barriers which may delimit the transport of anti-infectives to their site of action ("bacterial bioavailability").
To model the air-blood barrier of the peripheral human lung, our group was the first who published a protocol for growing monolayers of human alveolar epithelial cells in primary culture (hAEpC) to develop functional tight junctions and high transepithelial electrical resistance (TEER). Later we introduced a first polyclonal human alveolar epithelial (hAELVi) and just recently a monoclonal cell line (Arlo) with similar properties. These epithelial cells may be implemented in various micro-physiological systems, also to study the effect of breathing and co-cultivated with other cells types, like e.g., macrophages or endothelial cells. A particular challenge is the mixed culture with bacterial biofilms to model chronic lung infections, which can meanwhile be realized most elegantly by 3D bioprinting.
Such complex in-vitro models aim to reflect the (patho)physiology of specific organs or tissues either in healthy or reduce diseased state and to generate clinically meaningful readouts. They have been used for developing novel anti-infectives, like e.g., quorum sensing inhibitors, aiming to eradicate pathogens without inducing antimicrobial resistance. Aerosolizable nano-antibiotics are also being investigated to combat intracellular infections, such as e.g., tuberculosis or viral infections by Crispr/CAS-like approaches.

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INVITED SPEAKER

An insider's view of *Nature* journals

Raghavendra PALANKAR
Associate Editor, Nature Nanotechnology

Labeling Solid Lipid Nanoparticles with Tuned BODIPY for Photoacoustic Imaging

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Imaging the biodistribution of drug nanoparticles (NPs) at a targeted site could greatly improve the prediction of their therapeutic efficiency (1). Photoacoustic imaging (PAI) is an emerging biomedical imaging modality combining optical excitation and ultrasound detection to map optical-absorption at centimetric depth in sub-millimeter resolution (2). PAI provides a sensitive detection of molecular agents that can label NPs.

BODIPY dye is a very popular fluorescent scaffold with high optical absorption coefficient, tun- able absorption band and good photostability, but it usually absorbs in the visible range. To label NPs for PAI, we have synthesized a new BODIPY (3) dye with the following properties:

1) absorption in the near infrared region (max absorption at 760 nm), where biological tissues absorb and scatter less favoring the penetration depth for PAI, 2) a high extinction coefficient,

3) a low fluorescence quantum yield to increase the photoacoustic generation efficiency.

This BODIPY was covalently linked to a palmitate chain and formulated into solid lipid (SL) NPs by mixing in different proportions with dexamethasone-palmitate (prodrug of dexamethasone) and adding a PEGylated lipid. These nanoparticles are the first labelled SLNPs for PAI. High proportion of BODIPY-palmitate (up to 100%) were found stable and these NPs are therefore promising theranostic agents. Surprisingly, for proportions above 25% BODIPY-palmitate, NP spectra exhibit an absorption band with an 80-nm red shift, allowing allows better separation in PAI. We interpret this band as the signature of J-aggregates of BODIPY.

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Keywords: Photoacoustic imaging, BODIPY, Optical constrast, Theranostics, Solid lipid nanoparticles

Hybrid particles for the physical treatment of thrombotic diseases

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Thrombosis is responsible for most strokes and heart attacks, which are the two leading causes of death worldwide (1). Reperfusion is commonly performed with thrombolytics such as recombinant tissue plasminogen activator (rt-PA) but these present numerous limitations (severe adverse effects, few eligible patients, low recanalization rate). With the objective of designing a safer and efficient treatment, we propose here to have a targeted physical action on the thrombus. This non-pharmaceutical thrombolysis would be a combination of thermal and mechanical action, through light and magnetic stimulation, and would be targeted to the occluded vessel by an external permanent magnet (2,3). To achieve this strategy, we designed hybrid organic/inorganic particles that are stable, intravenously injectable, and have great photothermal and magnetic properties. We confirmed the hybrid structure by electronic microscopy and FT-IR spectroscopy. Then, we characterized the size and charge of these new microsystems. Additionally, we studied their stability in saline solution and in time. Magnetization measurements and particle counting were performed to compute the magnetic moment of these hybrid particles. Also, their response to magnetic stimulation was verified. The magnetic evaluation was completed with photothermal tests to estimate their ability to increase temperature when irradiated by a near-infrared laser. Finally, we were able to develop fluorescent hybrid particles and showed that they could efficiently target thrombi in vitro, even under venous or arterial flow, through a magnetic field. In the coming months, we will study the thrombolytic effect of these photo- and magneto-stimulated hybrid particles on ex vivo blood clots. When the therapeutic dose of these microsystems is determined, we will carry out cytotoxicity tests in this range.

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Keywords: hybrid particles, non pharmaceutical treatment, photothermia, magnetism, mechanical action, targeting, thrombolysis

TROP2: a promising new therapeutic target in breast cancer

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Despite undeniable progress in the prevention, screening and treatment of breast cancer, this- disease remains a major health problem with alarming incidence and mortality rates worldwide. While the majority of these cancers are curable at an early stage with locoregional treatments such as surgery and/or chemotherapy-radiotherapy, the main therapeutic weapons in situations at risk of recurrence (resistance to treatment) or in metastatic situations are targeted therapies. In this context, the recent emergence of the glycoprotein Trop-2 (Trophoblast Cell Surface Antigen 2) as a promising therapeutic target has led to new therapeutic paradigms for treat- ing patients with advanced or metastatic breast cancer(1).Recently, antibody-drug conjugates (ADC) have been developed and launched to the market for this purpose(2, 3). However, the production of ADCs is highly complex, time consuming and costly. Thus, an alternative must be developed to provide a new approach to breast cancer treatment. Indeed, this is what we are focusing on in our project. Using a completely new approach in the field of nanomedicine (4), we have developed fluorescent synthetic nanoparticles with a TROP2imprint (MIPs-TROP2), encapsulating active molecules (chemotherapies, targeted therapies) used in breast cancer treatment. The goal of the study is to use these MIPs-TROP2 nanoparticles to specifically target breast tumors overexpressing TROP2 protein and deliver the encapsulated therapeutic molecule precisely to the tumor site.To achieve this, first in vitro analyses using flow cytometry, NanoITC, confocal microscopy and cell viability were performed to validate the fluorescent nanoparticles imprinting and then to assess their toxicity and biocompatibility. We used four breast cancer cell models overexpressing the protein of interest (MCF7, T47D, MDAMB231 and SUM149) and one cell model not expressing the protein as a control (SUM159). The results showed specific targeting of TROP2-positive cell lines compared to the SUM159 control, confirming the affinity and accessibility of the imprint to its target. In addition, no decrease in viability was observed, confirming the cellular biocompatibility of the nanoparticles. Second, in vivo studies were performed in a mouse model. The accumulation and biodistribution of the nanoparticles in the different organs of the animal were evaluated by imaging. Preliminary results show that MIPs-TROP2 accumulate mainly in the liver. They also accumulate slightly in the kidneys and lungs of mice.
Taken together, our results confirm that the use of molecularly imprinted polymers as an alternative to ADC against cell membrane receptors represents a promising and innovative new line of nanomedicine.

Keywords: Molecular imprinting polymer, breast cancer, TROP2, tergeted therapy, drug delivery

A deep comparison of microwave-assisted synthesis methods of luminescent Carbon Dots

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Due to their biocompatibility and photostability, carbon-based nanomaterials, such as the emergent carbon dots (CDs), are gaining increasing attention in the biomedical field. The properties of CDs are dependent on many factors (size, edge effects, functional groups at the surface, synthetic methods & precursors, etc.). However, till now no rational has been established to better understand and control the impact of selected synthetic methods and conditions over the final structure and properties that present this new carbon nanomaterial, particularly in the bottom-up approach.

In addition, one of the weaknesses of CDs is their reduced spectral window (UV-VIS), which may restrict their applications in the biomedical field. To elucidate and expand this spectral window, the surface properties of CDs can be modified by doping, which could drastically modify their electronic characteristics and offer more active sites, thus producing new photoelectronic transitions towards the NIR. A greater understanding of the structure-property relationship can be attained by optimizing the synthetic process, undertaking a rational study under different conditions, and performing an exhaustive and in-depth characterization of the nanomaterial. Herein, we explored and optimized the methods (pyrolysis & hydrothermal) of synthesizing CDs via microwave-assisted processes using citric acid as the precursor. After that, an exhaustive characterization has been made using conventional methods such as UV-Vis, Dynamic light scattering (DLS), fluorescence spectrophotometry (FL), and new methodologies based on capillary electrophoresis (CE). In this sense, we have developed an electrokinetic method to help optimize the synthetic process thanks to the possibility offered by this separation method to visualize the number of populations of nanomaterial synthesized as well as the polydispersity and the yield of conversion. Till now, we have been able to identify two kinds of carbon-based materials depending on the synthetic process performed, such as distinguishing, before any purification step, the different fluorescence imaging windows of each synthesized nanomaterial. Thus, a complete comparison correlating synthetic process-structure-properties of both nanomaterials will be presented and discussed as well as the pros and cons of these two synthetic methods. This is the first step in the rational study of this new family of carbon materials, setting the basis to afford the next step of doping in view to enlarge the optical window towards the NIR region for biomedical applications.

Keywords: Carbon dots, Microwave, assisted synthesis, Physicochemical characterizations, Optical imaging

Controlling photothermal properties in cellular niche – synthesis and functionalization of novel molybdenum oxide nanocolloids

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Application of nanomaterials in living organisms imposes requirements on their stability in biological media, non-toxicity, and preserved activity. For photothermal nanomaterials, this translates to constant optical properties and high photothermal conversion in target cells. As for their low toxicity (1), optical tunability (2) and structural flexibility (3), molybdenum-oxides gained significant attention in the development of photothermal nanoplatforms (1, 4). In our previous work, we prepared photothermal MoOX nanocolloids by liquid-phase exfoliation (sonication) and observed their internalization by confocal Raman microscopy (5). Recently, we proved that microwave-assisted synthesis of molybdenum oxides provides control not only over particle size, but also their oxidation state (6). In this contribution, we explore in detail the link between parameters of the synthesis, photothermal properties of products, and cell interactions in relation to surface functionalization. In addition to physico-chemical characterization (TEM, UV-vis, pH-stability, XPS, photothermal conversion efficiency), biological tests for toxicity and photothermal activity of prepared products *in vitro* were performed. Complementing perfor- mance comparison of different microwave products and its explanation from structural point of view, obtained results bring focus to delicate interplay between coating and photothermal per- formance in cells. Whereas fully protected MoOX nanocolloids with constant optical properties provide stable photothermal response, MoOX nanocolloids without such conservative function- alization exhibit pH-dependent change in absorbance. In our case, it led to decreased efficiency, but in future it can provide a potent mechanism to selectively restrict photothermal activity to specific characteristics of cancer tissue.

This work was supported by the Slovak Research and Development Agency contract No. APVV- 20-0485 and by the grant contract No. VEGA 2/0117/22.

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Keywords: photothermal therapy/molybdenum oxide/functionalization/microwave, assisted syn- thesis

DECEMBER 6TH, 2023

INVITED SPEAKER

Theranostic treatment of cancer based on optically active nanosystems

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Our team is developing multifunctional theranostic nanoparticles (TN) or organic macromolecules capable of targeting tumor cells passively via the EPR effect and/or actively by grafting specific ligands [1] [2]. We also design nanosystems that are able to accumulate in the tumor microenvironment.
Our nanovectors can deliver drugs including RNAs, or inorganic atoms but most of our work is focused on the design of optical-based nanosystems emitting in the near-infrared spectrum for their detection [3] [4] [5] [6]and that can be activated on site(s) in order to become toxic.

Our nanosystems are based on scaffolds of organic and inorganic molecules and can deliver contrast agents with different drugs or pro-drugs. Using near-infrared imaging, we can track their distribution, monitor their function and therapeutic activity using non-invasive, non-radiative, real-time in vivo optical and photoacoustic imaging. Once they are on the tumor site, we activate them using near-infrared light (photodynamic therapy (PDT) or photothermal therapy (PTT)), X-Rays [7] [8]or neutrons [9].
These nanosystems can also be used intraoperatively for optical guided surgery of cancer [10].

In order to perform these investigations, we also develop the adapted imaging devices [11]and animal models. These different systems are then made accessible on our OPTIMAL small animal imaging facility.

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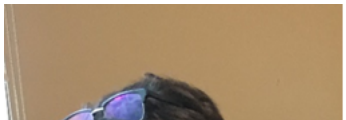

INVITED SPEAKER

Detect the undetectable: gold nanorods-assisted imaging and thermal treatment of bladder cancer lesions smaller than 1 mm

Dr Massimo ALFANO,
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INVITED SPEAKER

Therapeutic potential of photoactivable nanoparticles

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Currently, the development of in vitro and in vivo models according to the 3Rs (reduction, replacement, refinement) is of great importance. Indeed, research activities need these robust and easy-to-use techniques to demonstrate the imaging and therapeutic potential of innovative nanoparticles. Among several effective organic or inorganic nanoparticles, we are focusing on mesoporous organosilicon nanoparticles, which offer several advantages in terms of drug or genetic material loading capabilities, photoactivatable properties for imaging or photodynamic therapy, and personalization for targeting. In our team, the biological effects of these nanoparticles are studied first on human cancer cell lines in culture (in 2 dimensions or as spheroids) and then on zebrafish embryos. The robustness of these models enables us to provide rapid and reliable proof-of-concept for biocompatible and therapeutic nanoparticles.

Two-Photon Dye-based Fluorogenic Organic Nanoparticles Surface functionalization for Hyperbright Biosensors or Biomarkers

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Light-emitting nanoparticles are a unique class of optical nanomaterials combining improved brightness and photoresistance compared to standard dyes. Among the numerous existing lumi- nescent nanoparticles (QDs, UCNPs, P-dots...), the dye-based Fluorescent Organic Nanoparticles (dFONs) are less known, while they offer many promises. As they only consist of hydrophobic organic dyes aggregated in water, their optical and physico-chemical properties only depend on the structure of the dye building blocks. dFONS can be designed to be stealthy and used for *in vivo* single particle tracking or bioimaging. For these purposes, surface functionalization is essential to target the biomolecules of interest.

Today, *there is no option to directly functionalize the surface of such dFONs*. In this context, we propose an original approach based on the direct dye-functionalization through maleimide-thiol reaction. We synthesized a functional hydrophobic dye with maleimide functions, which upon nanoprecipitation in water, leads to ˜15nm nanoparticles bearing maleimide surface groups. Interestingly, the pristine dFONs-maleimide are not fluorescent and turns on upon thiol surface reaction (Michael addition). For the first time, we demonstrated that such dFONs could be used as intracellular thiol sensors, working in the μM range (1 or 2P excitation). In addition, we proved that dFONs can be functionalized with small molecules like biotin. The resulting dFONs-biotin show comparable size and brightness as QDs, while being metal- free. The dFONS-biotin successfully recognize streptavidin, opening up interesting prospects as ultra-bright biomarkers.

Keywords: Nanoparticles, Fluorescence, surface functionalization, bioimaging, biosensors

Contrast agent for photoacoustic imaging of tumor: from aza-ZrDIPY molecule to nanoformulation

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Aza-boron-dipyrromethenes (Aza-BODIPYs) are usually rigidified by a boron atom (B) to improve their optical properties. When this metalloid is replaced by zirconium (Zr), the fluorescent aza-ZrDIPY will display photoacoustic (PA) imaging properties.

Methods: aza-ZrDIPY was synthesized and its chemical and photophysical properties were determined in DMSO and in serum. The cytotoxicity of the compound was evaluated in cells before *in vivo* evaluation in mice-bearing subcutaneous tumors. To optimize tumor uptake, different strategies were developed including nanoformulations and coupling to biological ligand.

Results: In solution, aza-ZrDIPY had a weak fluorescent signal but a strong photoacoustic signature. *In vitro*, aza-ZrDIPY was non-toxic for endothelial cells. After intravenous injection, the tumor accumulation of aza-ZrDIPY was not reproducible. However, peritumoral injection allowed to obtain a strong signal within the tumor (figure). To enhance the tumor uptake after intravenous administration, different nanoformulations were tested and changed the photoacoustic signal of the contrast agent into fluorescent signal.

Conclusion: Aza-ZrDIPY is a valuable contrast agent for photoacoustic imaging, however it requires further optimizations such as for its formulation and binding to a biological ligand (work in progress). In addition, aza-ZrDIPY can also be synthesized with ^{89}Zr for PET imaging studies.

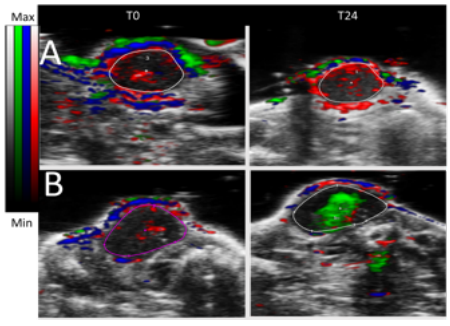

Figure: Photoacoustic imaging of a subcutaneous tumor before and 24h after intravenous (A) or peritumoral (B) injection of aza-ZrDIPY. Green, aza-ZrDIPY; Blue, Deoxyhemoglobin; Red, Oxyhemoglobin.

HPMA-based delivery systems conjugated with porphyrins used in photodynamic therapy and tumour imaging

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Photodynamic therapy (PDT) uses a light-sensitive photosensitizer (PS), such as tetraphenyl- porphyrin (TPP), in combination with illumination for the treatment of various malignant tu- mours. The light-activated PS reacts with oxygen in the tumour tissue leading to the formation of reactive oxygen species inducing cell death. (1) Unfortunately, the PS's possible use in PDT is restricted due to their limited solubility and/or low stability in physiological conditions or their lack of tumour selectivity.

Binding of PS to nano-carriers such as biocompatible, water-soluble, and non-toxic *N* -(2-hydroxypropyl)methac (HPMA) copolymers can improve its physico-chemical properties. Moreover, due to the Enhanced Permeability and Retention (EPR) effect, the nano-carriers are passively accumulated in tumour tissue amplifying the therapeutic outcome. (2)

This study presents the synthesis, physico-chemical and biological characterisation of HPMA- based conjugates with up to 6 wt.% TPP. PSs were bound by pH degradable hydrazone bond via either aliphatic or aromatic spacer. The structure-release rate dependency was studied in conditions mimicking the healthy tissue and the acidic lysosome environment of the tumour cells. Remarkable increase of *in vitro* cytotoxicity was observed after illumination with λ=420 nm in comparison with no illumination. Fluorescence of free TPP vs conjugated with HPMA was measured.

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Acknowledgement:

This work was supported by the Czech Academy of Sciences (project no. CNRS-22-01).

Keywords: polymer carrier, photodynamic therapy, octahedral molybdenum clusters

A nanostructured polymeric contrast agent for ^{19}F-based Magnetic Resonance Imaging

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Magnetic resonance imaging, MRI, relying on 19F nuclei has attracted much attention, be- cause the isotopes exhibit high gyromagnetic ratio (comparable to that of protons) and have 100% natural abundance. Furthermore, due to the very low traces of intrinsic fluorine in biologi- cal tissues, fluorine-labeling allows easy visualization *in-vivo* using 19F-based MRI.(1) However, one of the drawbacks of the available fluorine tracers is their very limited solubility in water. Here, we detail the design and preparation of a set of water compatible fluorine-rich polymers as contrast agents that can enhance the effectiveness of 19F-based MRI.(2) The agents are synthe- sized using the nucleophilic addition reaction between poly(isobutylene-alt-maleic anhydride) copolymer and a mixture of amine-appended fluorine groups and polyethylene glycol blocks. This allows control over the polymer architecture and stoichiometry, resulting in good affinity for water solutions and small hydrodynamic ratios (< 4nm). We further investigate the effects of introducing additional segmental mobility to the fluorine moieties in the polymer, by intro- ducing a PEG linker between the moieties and the polymer backbone. We find that controlling the polymer stoichiometry and introducing additional segmental mobility enhances the NMR signals and narrows the FWHM signatures. In particular, we assess the impact of the PEG linker on T2* and T1 relaxation times. We find that for equivalent concentrations, the PEG linker greatly increases T2*, while maintaining high T1 values, as compared to polymers without this linker.

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Keywords: 19F, MRI, diagnostics

Ultrasensitive detection using luminescent nanoparticles

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Rapid identification of pathogens (viruses, bacteria, toxins) or biomarkers in environmental (bioaerosols, water, food matrices,...) or biological samples is essential for prophylactic, diagnostic, and therapeutic measures. Existing methods to detect proteins are either sensitive (˜fM) but expensive and difficult to implement (e.g. SIMOA) or not sensitive enough to allow early detection of some biomarkers (p24, botulinum toxin...). We have demonstrated that the use of luminescent YVO4:Eu nanoparticles can reduce the limit of detection (LOD) for multiple tar- gets by an approach similar to conventional detection methods (ELISA), thanks to their optical properties (1). Rare- earth doped vanadate nanoparticles combine broad-band, high absorption coefficient, large Stokes shifts and narrow emission bands. The two latter characteristics allow efficient rejection of parasitic signals. These nanoparticles enabled highly sensitive detection at the same per test cost as ELISA when coupled to a transportable, home-made microplate reader. The detection of insulin (proof-of-concept analyte) was 25,000 times more sensitive than a commercial test using the same antibodies. In addition, at low cost and high sensitivity, we have extended this technology to the detection of nucleic acids to successfully detect the N1 gene of SARS-CoV-2

Keywords: lanthanide ions, nanoparticles, biomarker detection, DNA, RNA, ELISA, biomedical applications

Controlling the silica shell growth of core-shell iron oxide @ stellate mesoporous silica nanoparticles: effects on MRI, magnetic hyperthermia and NIR-photothermia properties

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Iron oxide nanoparticles (IO NPs) are of great interest as theranostic agents as they are very good T2 contrast agents for magnetic resonance imaging (MRI) (1), and also good heating agents under external fields such as alternating magnetic field (2) or NIR-light irradiation (3). Combined with a well-engineered mesoporous silica (MS) shell, their therapeutic action can be extended with drug delivery. In our lab, we have an expertise in such silica shell engineering. Indeed, we studied how to orient the MS morphology between stellate structure (STMS) and worm-like structure (WLMS) (4) and notably showed the great interest of synthesizing the STMS shell for drug delivery, whether it is" small" synthetic drugs (doxorubicin) (5) or large therapeutic biomolecules (proteins) (6-8).
However, to date, these core-shell nanostructures were synthesized only at a given final size (*ca*. 120 nm), and no work has reported in depth the growth mechanism of the STMS shell on IO NPs and its resulting properties. In this work, we investigated the growing kinetic of STMS shell on IO NPs core, studied the impact of the reaction time on structural properties and evaluated the influence of the shell thickness on MRI, MHT and photonic hyperthermia (PHT) properties. We found that we could control the STMS shell thickness by simply controlling the reaction time and that the higher this shell thickness, the lower the pore size and the specific surface area but the higher the colloidal stability. Regarding the response under external fields, IO@STMS with tuned shell thicknesses were shown to be relevant T2 contrast agents for MRI and good heating agents for MHT and PHT. The impact of the STMS shell thickness on cell internalization and survival was also evaluated on a pancreatic cancer cell line.

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Keywords: Design of core, shell iron oxide @ stellate mesoporous silica nanoparticles, Controlled growth of mesoporous silica shell, Magnetic Resonance Imaging, Magnetic hyperthermia, Near, infrared photothermia

Radical release induced by Magnetothermia

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Nanoparticle-assisted magnetothermia is a promising modern nanoscience technique that enables local heat activation through a remote and controlled external stimulus, an alternating current (AC) magnetic field. This concept is extensively utilized in nanomedicine for hyperthermia treatment, [1] and has recently found applications in catalysis[2] or polymerization processes.[3] In this presentation, a novel iron oxide-based nanoplatform will be presented. The platform comprises magnetite (FeO) with thermosensitive radical initiators, alkoxyamines RRNOR, anchored to its surface. The magnetic core exhibits a high intrinsic loss power of 4.73 $nHm^2.kg^1$ providing rapid heating of their surface under the action of an ac field. This causes the homolysis of the alkoxyamine C–ON bond and triggers the formation of radicals, independent of oxygen. The latter was demonstrated by electronic paramagnetic resonance spectroscopy, and the kinetics of homolysis has been investigated allowing a comparison of the temperature of alkoxyamine's homolysis with the one measured during the magnetothermia process.

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Keywords: Iron oxide, Nanoparticle, Magnetothermia, Radical release

Intracellular proteins targeting with bi-functionalized magnetic nanoparticles

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For some applications in nanomedecine, such as cancer therapy by magnetic hyperthermia or cellular engineering, it can be interesting for magnetic nanoparticles (MNPs) to target intracel- lular proteins or organites. However, as any other type of nanoparticles, MNPs are internalized into cells by endocytosis, and remain stuck inside small intracellular compartments called endo- somes. Some strategies have arisen to bypass this endosomal entrapment. Here we will present a strategy that relies on the original bi-functionalization of MNPs with poly(histidine) peptides (PPH), allowing the endosomal escape of the MNPs through proton sponge effect, and antibod- ies, allowing for the first time the targeting of specific proteins once MNPs are in the cytosol. In order to do that, γFe2O3@SiO2 MNPs with diameter smaller than 50 nm, were functionalized with zwitterionic moieties as well as with thiol groups at their surface. These SH groups were used to functionalize them with PPH through a labile link. This labile link between the peptide and the MNPs allows the peptide to be detached from the surface of the MNPs once in the cytosol, in order to avoid any interaction between these peptides and intracellular components, which could hinder the MNPs' intracellular mobility. A second functionalization of the MNPs with targeting antibodies through a non-labile link was then performed, so the MNPs can target specific intracellular proteins once the cytosol has been reached. In a first demonstration of this concept, MNPs functionalized with both PPH and anti-HSP27 antibodies were able to efficiently target intracellular HSP27 (figure 1), opening the door to new biomedical applications of MNPs.

Keywords: magnetic nanoparticles, intracellular targeting, peptide, antibody

SESSION A – Installation Monday 6th at 12:30 pm Removal Tuesday 7th at 13:30 pm

Nanomaterials for Therapy

A1. DNase-I functionalized cerium oxide nanoparticles (CNP) for degradation of neutrophil extracellular traps (NETs)

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Neutrophil Extracellular Traps (NETs) are DNA fibers containing a number of cytoplasmic proteins such as human neutrophil elastase (HNE). In a process called immuno-thrombosis, NETs are shed by neutrophils when stimulated by inflammatory mediators. They are present in the thrombus structure of ischemic stroke and induce thrombolysis resistance. Targeting NETs' DNA present in thrombi as adjuvant for thrombolytic therapy may thus represent a new opportunity for treating ischemic stroke. The use of DNase I grafted on cerium oxide nanoparticles (CNP) prepared as described by Md. N. Arafath (abstract submitted to SFNano 2023) was evaluated using artificial and native NETs.

Artificial NETs were prepared by complexing HNE to DNA at ratios similar to those found in their native counterparts. Native NETs were prepared by stimulating neutrophils isolated from healthy volunteer's blood. NETs were qualified by fluorescent microscopy after Hoechst staining. The analytical procedures for DNA quantification were in-house validated. The effect of soluble and CNP-grafted DNase I was assessed by fluorimetry on DNA alone and then on Artificial and Native NETs, whilst the content of HNE was measured photometrically using a chromogenic substrate.

Our results revealed that the CNP-grafted DNase I has similar kinetic behavior to the soluble form but with a slightly reduced activity on DNA, artificial and native NETs. The results were dependent on nanoparticle batch preparation as well as on the quality of neutrophils isolated from healthy donors. Based on the aforementioned studies, the team objective is to perform in vitro investigations on blood clots and thrombi extracted from patients. Grafted-DNase I stability, appropriate bead coatings and other biocompatibility criteria are crucial before moving to in vivo studies.

Keywords: Cerium oxyde nanoparticles, Ischemic stroke, neutrophil extracellular traps (NETs)

A2. Cytotoxicity and Antibacterial Effects of Essential Oils Synthesized Copper Oxide Nanoparticles

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This study investigated the biosynthesis approach for the preparation of copper oxide nanopar- ticles (CuO NPs) obtained by a biogenic route using essential oils derived from Cymbopogon citratus – Lemon Grass Essential Oil (CuONPs-LG) or Schinus terebinthifolia – Pink Pepper Essential Oil (CuONPs-PP) plants that have components with various pharmacological actions including anticancer, antifungal, antibacterial, antiviral, and antioxidant properties. CuONPs- LG and CuONPs-PP have a mean hydrodynamic size of 209.45 ± 28.65 nm and 242.73 ± 45.77 nm and PDI values of 0.260 ± 0.091 and 0.287 ± 0.058, respectively.

The cytotoxicity of both CuONPs were evaluated against malignant melanocyte cell lines stage III, derived from the lymph node of the metastatic (VMM39) and against the non-cancerous fi- broblast cells (FN1). The results demonstrate that both CuONPs-LG and CuONPs-PP nanopar- ticles have a concentration dependent toxicity against both cell lines at the tested concentrations (0.49 µg/mL to 250 µg/mL). Importantly, both nanoparticles were found to be more toxic to VMM39 compared to fibroblast. For instance, half maximal effective concentration (EC50) val- ues of CuONPs-LG were found to be 3.24 and 28.12 µg/mL for tumoral and non-tumoral cell lines, respectively. Similarly, EC50 values of CuONPs-PP were found to be 0.90 and 4.40 µg/mL for tumoral and non-tumoral cell lines, respectively. These results indicate higher toxicity of the essential oil synthesized CuONPs to melanoma cells, compared to fibroblast cells, suggesting a safe window for the potential use of these nanoparticles. Moreover, CuONPs-PP nanoparticles were found to be more toxic to both cell lines, compared to CuONPs-LG, indicating that the chemical nature of the essential oil used in the nanoparticle biosynthesis confer additional bio- logical effect due to the caping effect onto the nanoparticle surface.

The antibacterial effects of the nanoparticles were demonstrated against Gram-negative bacteria that have a thin layer of peptidoglycan in the periplasmic space and an outer membrane – a lipid bilayer that contains lipopolysaccharides/endotoxin (LPS), lipoproteins and porins. This outer membrane is what prevents the penetration of certain antibiotics, such as penicillin. The assay was based on fluorescence, resazurin assay, where the nanoparticles demonstrated toxic effects against the multidrug-resistant bacteria Acinetobacter spp. (MDR-A) and Klebsiella pneumo- niae (KPC). We observed that both nanoparticles have an antibacterial effect against MDR-A, even at the lowest tested concentration (4 µg/mL). For KPC, CuONPs-PP proved to be effective from 16 µg/mL and CuONPs-LG from 32 µg/mL in killing bacteria. Therefore, these results demonstrated that essential oils can be used in the biosynthesis of CuO NPs with significant toxicity against cancer cells and bacteria, including resistant strains.

References

Keywords: copper oxide nanoparticles, green synthesis, biomedical applications

A3. Photothermal Therapy Potential of Manganese-doped Prussian Blue Nanoparticles in vitro and in Zebrafish Embryos

Lamiaa M. A. Ali [1], Ma¨elle Cahu 2, Saad Sene [2], Jerome Long [2], Franck Camerel [3], Mathieu Ciancone [3], Fabrice Salles [2], Jo¨el Chopineau [2], Jean-Marie Devoisselle [2], Gautier Felix [2], Nicolas Cubedo [4], Mireille Rossel [4], Christophe Goze-Bac [5], Yannick Guari [2], Nadir Bettache [1], Joulia Larionova [2], Magali Gary-Bobo [1]

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In the fight against cancer, researchers are tending to find new ways to detect and treat early cancers. In the field of nanotechnology, several cancer-related nanomedicines have been approved for clinical use. This encourages many scientists to develop effective nanoplatforms for cancer theranostics. In our work, we synthesized Prussian blue nanoparticles doped with different percentages of manganese by a one-step self-assembly reaction, a synthetic strategy that yields nanoparticles with similar size and shape. Thanks to manganese ions, these nanoparticles showed interesting longitudinal nuclear magnetic resonance relaxivity. Further, due to the presence of Prussian blue, they showed low toxicity, good internalization and excellent photothermal therapy (PTT) properties against triple negative human breast adenocarcinoma (MDA-MB-231) cells under continuous and pulsed laser irradiation (808 nm). Investigating the PTT effect of these nanoparticles in zebrafish embryos xenografted with human breast cancer cell line under continuous laser irradiation showed a decrease in the tumor size in embryos treated with nanoparticles and exposed to only one irradiation.

Keywords: Cancer, Nanoparticles, Photothermal therapy, Prussian blue, Zebrafish embryos

A4. Formulation of nanoemulsions-loaded alginate microbeads for the oral delivery of tofacitinib in the treatment of inflammatory bowel diseases

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Inflammatory bowel diseases (IBDs) are characterized by persistent inflammatory episodes of the gastrointestinal tract. The quality of life of patients is very impaired, with severe abdominal pain, bleedings and extreme fatigue (1). Current therapeutic solutions aim at reducing intestinal inflammation, but they are not efficient in the long term and confer heavy side effect mainly due to systemic exposure. Thus, there is an urgent need to develop an effective treatment in the long term avoiding adverse effects (2). In recent years, promising approaches for IBDs therapy aim at avoiding the systemic effect conferred by current treatments. By designing nano-sized targeted drug delivery systems, the strategy is to release the active molecule locally on the inflamed area, sharply reducing adverse effects. Nanocomposites (NCs) are innovative delivery systems, made of a nano-scaled phase, loaded into a micro- or macro-polymeric matrix, that can therefore offer interesting characterics (3, 4). The aim of the present project is to develop an original NC for oral administration combining mucoadhesive and mucopenetrating properties to target intestinal inflammation. More precisely, a nano-in-micro system made of nanoemulsions (NEs) containing a JAK-STAT inhibitor and loaded into a polymeric hydrogel was designed. A stable and monodisperse O/W NE was successfully formulated using the microfluidic technique, with satisfying drug loading and encapsulation efficiency. Thereafter, the drug-loaded NEs were encapsulated into spherical alginate microbeads (MBs), following to the ionotropic gelation technique. The stability and changing of size of the MBs and the drug-loaded NEs' release depending on time were reviewed in simulated gastrointestinal fluids. In vitro experiments confirmed the safety of the NC on a co-culture model, and cellular internalization and anti- inflammatory capacities were explored. Finally, mucoadhesion studies on ex vivo murine colons were sucessfully conducted.

Keywords: Nanocomposite, inflammatory bowel disease, oral administration, tofacitinib, nanoemulsion, hydrogel

A5. Prednisolone Prodrugs Lipid Nanoparticles as an alternative management of inflammatory diseases

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In this study we designed aliphatic prodrugs of prednisolone. The prodrug 1 and Prodrug 2 were then characterized by mass spectrometry, nuclear magnetic resonance spectroscopy and Fourier transform infrared spectroscopy. Structural elucidation was followed by nanoformula- tion of designed prodrugs (1,2) into nanoscale prodrugs by using ethanol injection method. The excipients used for nanoformulation were hydrogenated soya phosphatidylcholine (HSPC) as lipid and tween 80 as surfactant for stabilizing the nanoformulation. The designed nanopar- ticles were characterized by using dynamic light scattering (DLS) Malvern NanoZetasizer. The Hydrodynamic diameter (dH) of nanoparticle were in range of 110 to 180 nm with measure of polydispersity index varying from 0.242-0.275. The surface charge of the designed nanoparticle ranges from -22 to -29 mV. Designed nanoparticle possessed percent encapsulation efficiency of ≥ 90% for each prodrug. The spherical type morphology of the designed nanoparticles was confirmed by using scanning electron microscopy (SEM) and atomic force microscopy (AFM). The results of AFM and SEM showed spherical type nanoparticle having homogeneity in size distribution. Prodrug (1,2) Nanoparticles were tested for in vitro cytotoxicity against THP-1 cell line up to dosage of 100 µg/mL. The designed higher dose showed minor toxicity compared to standard drug prednisolone. Following toxicity assay the designed nanoparticle were tested for in vitro inhibition of THP-1 cells stimulated with lipopolysaccharide (LPS). The designed nanoparticles (1,2) possessed significant inhibition of inflammatory cytokine such as TNFα, IL-6, MCP-1 and IL-1β. In conclusion, NPs 1-2 are potential anti-inflammatory agents for the treatment of inflammatory diseases and may be potential drug candidate for treatment of inflammatory diseases which would need further insightful studies of the designed prodrug nanoparticles.

Keywords: Prednisolone, Glucocorticoids, Prodrug, Nanoparticle, Inflammation

A6. Investigation of a Hydrophobic Lipid-Based Solid Formulation Technology for Biomolecule Protection in Enhancing Exocrine Pancreatic Function

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Context. Exocrine Pancreatic Insufficiency (EPI) induced by conditions like cystic fibrosis, chronic pancreatitis, and Crohn's disease, is a frequently overlooked and underdiagnosed gastrointestinal ailment. It causes inadequate intestinal digestion due to insufficient pancreatic juice secretion, leading to discomfort, pain, and, ultimately, severe malnutrition.
Objectives. To address such insufficiency, several treatment turned out inefficient for the last three decades. In this project, we propose a new formulation to restore the digestive function by targeting the amphiphilic nature of bile salts.
Methods. This lipid technology exploits the surfactant properties of bile salts by incorporating the active ingredient within a hydrophobic lipid matrix. This matrix consists of a blend of long-chain fatty acids, including palmitic and stearic acid (C16/C18), together with fatty acid esters. The synthesis process entails the dry incorporation of the active ingredient (alongside excipients) into the hydrophobic lipid matrix through thermal co-crystallization and cryo-milling. The resultant particles, sized between 500 to 900 µm, are subsequently encapsulated within oral administration capsules. The formulation is subsequently characterized in terms of morphology, loading rate, enzymatic activities, release kinetics, and enteric resistance. Moreover, this char- acterization has been carried out over time to ascertain storage conditions and their influence on the treatment stability. Lastly, preclinical trials have been conducted for the purpose of establishing an in vitro-vivo correlation.
Results. The results demonstrated that this formulation effectively shields an acid-sensitive active ingredient during enteral passage (SGF: Simulated Gastric Fluid) while enabling its rapid release upon entering an artificial duodenal environment (SIF: Simulated Intestinal Fluid). Furthermore, the preservation of a protein-based active ingredient pertains not only to its primary or secondary protein structure but also to its function, such as enzymatic function.
Perspectives. In summary, this technology provides an innovative solution for treating EPI by overcoming the challenges related to gastric pH. It enables the efficient release of the medi- cation in the duodenum, where nutrient absorption is vital, offering hope for patients suffering from this complex gastrointestinal condition. This approach could enhance treatment efficacy, increase patient comfort through reduced dosing, and minimize side effects. Comprehensive clin- ical studies are essential to evaluate the effectiveness and safety of this technology in patients with EPI.

Keywords: Drug Delivery, Digestion, Nanoparticles, Acute Pancreatitis.

A7. Ferrocifen loaded Lipid Nanocapsules: Targeting MDR tumors via the Thioredoxin Reductase Pathway

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Regardless of the leaps being achieved in the field of cancer treatment, 90% of the chemother- apy failures arise from the multi drug resistance aspect gained by these tumors (1). Aiming to overcome these limitations, ferrocifens, a family of molecules obtained by combination of a fer- rocene with hydroxytamoxifen derivatives (2), encapsulated in lipid nanocapsules (LNCs), could offer an innovative approach to combat this resistance. Moreover, the functionalization of the obtained nanoparticles with polyethylene glycol would imbue them with stealth properties (3), increasing their half-life and circulation time, while utilizing the enhanced permeability and re- tention effect (EPR) (4).
Ferrocifen has been hypothesized to act via targeting the thioredoxin reductase (TrxR), a system responsible for thiol redox homeostasis and overexpressed in cancer cells leading to increased resistance. This action might stem from TrxR, similar to another anti-ROS mechanism, the Glutathione Peroxidase 4 (GPX4) system, were both display a selenocysteine residue, which can be targeted by metal complexes.

To establish the mode of action of ferrocifens, we used A549, a lung cancer cell line, with high TrxR and a low GPX4 expressions, and compared it to HepG2, a hepatocellular carcinoma cell line with low TrxR and high GPX4 expressions. Using Erastin/Ferrostatin based viability tests, we were able to establish that blank LNCs were able to induce cell death via ferroptosis, with a more prominent effect on HepG2 cells. Additionally, several assays including Annexin V/ Propidium Iodide, TrxR enzymatic assays and Western blots showed that ferrocifen loaded LNC was able to act on both the TrxR and GPX4, inducing apoptosis through the TRxR pathway and ferroptosis via the GPX4 pathway following their inhibition, leading to the accumulation of ROS, causing cellular damage.
This project, along-side producing and characterizing ferrocifen stealth nanoparticles, was able to verify and establish the mode of action of ferrocifens, having an important anti-cancer effect on cell lines expressing low TrxR and high GPX4, proving that this ferrocifen can target both the thioredoxin reductase and glutathione peroxidase anti-ROS systems. This original effect can further synergize with the ferroptotic ability of the LNCs, thus causing an accumulation of intracellular ROS cumulating to cellular death by both the apoptotic and ferroptotic pathways.

Keywords: Ferrocifen, TrxR, GPX4, Stealth LNC

A8. Enhancement of Phosphate Removal in peritoneal dialysis using designed magnetic Iron oxide Nanostructures

Sylvie Begin [1,2]

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Chronic kidney disease (CKD) results in the progressive loss of the kidney's purification func- tions and leads to complications linked to the accumulation of toxins in the body. Phosphates are molecules whose presence in excess in the blood is particularly dangerous, as hyperphos- phatemia can lead to vascular calcification, cardiac arrest, etc. In the absence of a transplant, the treatment of CKD involves dialysis techniques. Peritoneal dialysis (PD) is best suited for infants and children but its blood purification efficiency is always too low.

In order to improve the phosphates capture during PD, a promising approach consists in working on the formulation of the dialysate by incorporating iron oxide nanoparticles whose affinity with phosphates has already been demonstrated. Various types of nanostructures (IONs) satisfying precise specifications (biocompatibility, size, colloidal stability, etc.) and coated with differ- ent type of biocompatible surfactants were synthesized by testing different synthesis methods. These different methods were optimized to obtain suspensions of IONs coated with surfactants colloidally stable in physiological media and displaying a mean hydrodynamic size in the range 50-300 nm. Then, their capacity in phosphate removal was evaluated as function of the IONs size, the surface specific area (SSA) and the surfactant. Analytical protocols have been de- veloped to quantify phosphates and toxins using chemical approaches as well as via a hospital apparatus. Kinetic and adsorption experiments for phosphates and other toxins were performed on these designed IONPs in different media and pH. The kinetics was found very favorable to reduce the PD duration and experiments conducted in dialysate at pH 7 has shown a phosphate adsorption similar to that in water. Beside the expected effect of the SSA, we demonstrate further an effect of the nature of the surfactant on phosphate removal and some surfactants were shown very efficient to remove toxins.

Acknowledgment: This research project was funded by French national agency for research (ANR) under the project PHODIA 2021.

Keywords: iron oxide nanostructures, peritoneal dialysis, phosphate removal, surfactants, colloidal stability

A9. Novel lipophenol quercetin derivative formulations for preclinical pharmacological evaluation to prevent macular degeneration

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Dry age-related macular degeneration (Dry-AMD) is a chronic and disabling disease that progressively evolves from the age of 60 years (1). It is one of the main causes of poor vision in adults. Stargardt disease (STGD1) is an autosomal recessive inherited retinal disease (≈ 1:10000), which appears in children (7 to 12 yearsold), caused by variants in the ABCA4 gene. Carbonyl and oxidative stresses (COS) are aggravating factors for both diseases as they lead to the formation of toxic entities, such as bis-retinoid N-retinyl-N-retinylidene ethanolamine (A2E). Photo-oxidation of A2E is toxic to photoreceptor and retinal pigment epithelium cells (RPE) leading to central vision loss that evolves to legal blindness (2). Currently, there are no curative or preventive treatments on the market for these diseases. One of the potential ways to prevent the progression of these diseases involves using molecules with capacities against oxidative and carbonyl stresses (3). In this context, drugs with proper- ties against oxidative and carbonyl stresses are potential candidates to prevent dry age-related macular degeneration (Dry-AMD) and inherited Stargardt disease (STGD1). Previous studies developed in collaboration between INM and IBMM have demonstrated the capacity of a new lipophenol drug: 3-O -DHA-7-O -isopropyl-quercetin (Q-IP-DHA) to protect ARPE19 and pri- mary rat RPE cells respectively from A2E toxicity and under oxidative and carbonyl stress con- ditions. As other alkyl-lipophenolic compounds studied (4), they are classified as BCS class IV, Q-IP-DHA are poorly water-soluble molecule. Different approaches have been used to improve their solubility for IV and oral administration in collaboration with ICGM during LiPoPheRet project (ANR-18-CE18-0017). An intravenous formulation with micelles and an oral formula- tion using lipid nanocapsules (LNC) were developed. Micelles were formed with Kolliphor® HS 15 and saline solution 0.9 % (mean size 16 nm, drug loading 95 %). The oral formulation was optimized and successfully allowed the formation of LNC (25 nm, 96 %). The evaluation of the therapeutic potency of Q-IP-DHA was performed after IV administration of micelles loaded with Q-IP-DHA (M-Q-IP-DHA) at 30mg/kg and after oral administration of LNC loaded with Q-IP-DHA (LNC-Q-IP-DHA) at 100mg/kg in mice. Q-IP-DHA formulated in micelles and ad- ministered IV at 30 mg/kg efficiently protected the function and integrity of photoreceptors in ABCA4 -/- mice (model of STGD1) after induction of photoreceptor degeneration. LNC-Q-IP-DHA administered orally in mice demonstrated also its protective effect on the function and integrity of photoreceptors after light-induction degeneration and is more adapted for chronic ad- ministration. LNC-Q-IP-DHA were able to fully protect photoreceptors in BALB/c mice against light induced photoreceptors damages at a dose of 100 mg/kg both to oily solubilization and to permeability enhancement. Protection effect was dose dependent and reached already 87 % of protection using only 75 mg/kg of Q-IP-DHA. The LNC formulation significantly improved photoreceptor protection, compared to Labrafac® solution. Altogether, these interesting in vivo results highlight lipophenolic quercetin derivatives as potential candidates to reduce the progression of macular degeneration in retinal dystrophies. Chronic administrations should be evaluated in due course in order to reduce efficient doses and investigate their potential side ef- fects. Despite numerous results describing in vitro potency of quercetin in the field of oxidative stress, few studies were able to present in vivo efficiently of quercetin or quercetin derivatives. The present study focused on the importance of determining the appropriate formulation and dose to enhance the bioavailability of polyphenolic compounds and demonstrated the photore- ceptor protection after induction of retinal degeneration by acute light stress making Q-IP-DHA a promising preventive candidate against dry-AMD and STGD1.

Acknowledgment: This research project was funded by French national agency for research (ANR) under the project PHODIA 2021.

Keywords micelles, lipid nanocapsules, lipophenol, intravenous and oral routes, antioxidant, age, related macular degeneration AMD

A10. Design of a multifunctional nanoplatform for photothermal therapy and temperature sensing

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Cancer was responsible for the death of almost 10 million individuals in 2020, consolidating its status as one of the principal causes of mortality nowadays. Many therapies are used for its treatment, including ablation for non-vital affected organs (breast, prostate.), chemotherapy, or radiotherapy. However, these therapies are particularly heavy for patients with numerous side effects (nausea, asthenia, hair loss, cognitive disorder), requiring new innovative treatments.
Recently, photothermal therapy (PT), consisting of a temperature elevation pro- duced by given nanoparticles submitted to light irradiation, has attracted great interest in cancer treatment as it permits local heat release. Among all the panels of nano-objects suitable for PT, Prussian blue NPs demonstrate outstanding po- tential as a photothermal therapy agent in biomedical research and clinical settings thanks to their photothermal properties, biocompatibility, photostability, and ab- sorption range in the near-infrared (NIR).

However, controlling and sensing the temperature in real-time during PT remains a significant challenge. For this purpose, the project aims to develop new intelligent nano-systems acting as a unique tool for photothermal therapy action and simulta- neous measure of a local temperature elevation. To do so, nanoplatforms comprising a Prussian blue core as a nanoheater with lanthanide complexes grafted on the sur- face as thermometers through a silica shell surrounding the core are developed. Lanthanide complexes have been chosen because of their temperature-dependent luminescence, allowing their use as emissive self-referenced nanothermometers.
The interest of a PB@SiO2-Ln-complex nanoplatform will be presented to address the limitation mentioned above as well as the first preliminary results obtained.

Keywords: Prussian blue nanoparticles, temperature sensing, photothermal therapy

A11. Amphiphilic PCL-g-Dex nanoparticles: Towards hybrid hydrogel/nanoparticles dual-cancer drug delivery system

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Triple-negative breast cancer (TNBC) is a prevalent and challenging subtype of cancer due to the absence of targeted therapy options, its aggressive nature, and its high recurrence risk.(1) Re- searchers are exploring combination treatments using multiple modalities to enhance anticancer therapies and developing innovative dual-action drug delivery systems to improve efficacy, reduce side effects, and overcome drug resistance. This project focuses on designing a novel biodegrad- able polymeric hydrogel/nanoparticle hybrid system as a dual-drug delivery system of anti- cancer drugs and antibodies for enhanced cancer treatment. Hydrogel and nanoparticles (NPs) are based on an amphiphilic graft copolymer of the same nature, poly(caprolactone)-g -dextran (PCL-g -Dex), with different hydrophilic-lipophilic balance to ensure a more homogenous system and a more controlled release. In this contribution, we focus on the design of paclitaxel (PTX)- loaded PCL-g-Dex NPs. PCL-g -Dex copolymers are first synthesized by azide-alkyne Huisgen 1,3-dipolar cycloaddition between propargylated PCL (PCL-yne, Mn =16.000 and 35.000 g.mol- 1; Substitution degree= 12%),(2) and an azido-dextran (Dex-N3) (Mn = 3.000 g.mol-1 Substi- tution degree= 100%) synthesized via reductive amination of the chain ends.(3) PCL-g -Dex with DEX/CL ratios of 2 and 1.5 were obtained for PCL16k-g -Dex3k and PCL35k-g -Dex3k, re- spectively. The copolymers were used to prepare PTX-loaded NPs through a nanoprecipitation process. PCL-g -Dex NPs sizes were characterized by DLS showing diameters of 190 and 230 nm, for PCL16k-g -Dex3k and PCL35k-g -Dex3k, respectively. For the PCL16k-g -Dex3k copolymer, an encapsulation efficiency of 40%, and drug loading of 10% were determined by HPLC. PTX release kinetics and in vitro biological evaluations of the loaded PCL-g -Dex NPs are currently under investigation.

Keywords: PCL-g-Dex, nanoparticles, amphililic, cancer therapy

A12. Evaluation of functionalized liposomes' placenta targeting efficiency by assessing in vitro uptake and in vivo biodistribution

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Our team focus on delivering active substance to treat the placenta during pregnancy. Preg- nant women are a particular population with specific therapeutic needs. The active substance and its carrier should not be able to cross the placenta, but the delivery should be as specific as possible to avoid off-target effect for the safety of the mother. To this extent, active targeting strategies seem promising when combined with a carrier not able to cross the placenta.

A specific ligand to target the placenta has been identified by our laboratory. It relies on the high affinity of VAR2CSA, a protein found on the surface of erythrocytes infected by the plasmodium falciparum, for the CSA (chondroitin sulfate A), a receptor overexpressed by the placenta. A peptide of 16 amino acid sequence length derived from VAR2CSA sequence was identified thanks to the PEPScan technique.

Liposomes were functionalized with the peptide by optimizing the thiol/maleimide coupling for this specific application. Size and potential zeta were assessed by DLS. After a purification step, the coupling success was monitored by a LC/MS method developed to check the presence of the peptide on the liposomes. Moreover, an indirect quantitative technique by the reaction of fluorescamine and peptides from supernatants enable to determine the coupling efficiency. In vitro internalization and toxicity were evaluated on the BeWo cell line. An improvement of the internalization of the functionalized formulation by a factor 3.5 was found in vitro compared to the control formulation.

After assessing the improvement of the internalization of the functionalized formulation in vitro, the development of methods to evaluate the biodistribution in vivo are needed. Two methods are currently being developed using the fluorescent lipid DOPE-Cy5 in the liposome formulation to assess the biodistribution. Preliminary results were obtained by the acquisition of images by IVIS on ex vivo organs of pregnant mice. The placenta was found to be the second organ with the most signals, right after the liver. A second method using UPLC setting with 2 fluorescent channels is currently being developed, to confirm the results (1).

(1) Pauline Chavrier et al, Tools to quantify liposomal uptake in vitro and biodistribution in vivo, SFNanoJR 1stseminar on Nanoparticle applications in nucleic acid delivery and imaging (09-06-2023)

Keywords: Targeting, Placenta, Liposomes, VAR2CSA

A13. Nanovectors for drug delivery activated by temperature and/or light and monitoring by MRI.

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Since the 1980's, nanoscience and nanotechnologies have experienced a real boom and have opened promising prospects in nanomedecine with the design of more specific and efficient ther- apeutic agents. These new methods are actually being used to develop new anti-cancer drugs to fight one of the deadliest diseases of our century. Some of the keys for the development of efficient drugs is the improvement of their bioavailability and the possibility to control spatially and temporally their release by an external stimulus. Drug delivery triggered by endogenous acidification of tumour microenvironment or localized temperature jump would allow delivery of anticancer drug at the tumour site while reducing rapid clearance and side effects. This approach would enable to achieve better cure rates of patients by reducing relapse related to surgery margins. The work of this PhD topic, funded by the Impulsion Research Network on biomedical imaging (IMPACT), aims to encapsulate, then release under light irradiation, suf- ficiently concentrated anti-cancer drugs in the target area from vesicles called thermosensitive polymersomes. The goal is to convert deep red-near Near InfraRed (NIR) light source (600 to 900 nm) into thermal energy using small organic nanoparticles 'hyper-functionalised' by dyes which are able to absorb the NIR light and generate localized heating by relaxation. Thermal elevation will be monitored and controlled by a high-performance MRI temperature imaging technique recently developed by one of the IMPACT Network team, opening an innovative ther- agnostic approach. This is a complex and highly innovative approach that uses different types of nanoobjects and involves collaboration between synergistic disciplines (chemistry, physics, biology) and laboratories. This study received financial support from the French government in the framework of the University of Bordeaux's France 2030 program / RRI "IMPACT ".

Keywords: Nanoparticles, FONs, MRI, drug delivery, polymersomes

A14. Docetaxel-loaded nanodroplets as therapeutic agents against Glioblastoma

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Glioblastoma is one of the most aggressive cancer to treat with a very poor prognosis. This outcome is mainly due to the difficulty of drugs to cross the blood-brain barrier.(1) One of the strategies developed to improve the drug delivery was to encapsulate the drug in a nanocarrier concomitantly with the application of focused ultrasound to disrupt the blood-brain barrier us- ing commercially available microbubbles.(2)
In this optic we have developed a dry formulation of perfluorocarbon nanodroplets stabilized with an in-house surfactant. The core of these nanodroplets was constituted of a mixture of perfluorooctyl bromide and a biocompatible oil, where this latter served to encapsulate a hy- drophobic drug or dye.(3)

First, we have characterized the resulting nanodroplets and demonstrated that they were safe and able to reach the brain after blood-brain barrier disruption. Secondly the biodistribution and pharmacokinetics of these nanocarriers were studied in mice. Finally, Docetaxel, a chemothera- peutic drug, was encapsulated and survival experiment in mice bearing orthotopic glioblastoma were conducted.

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Keywords: Nanodroplets, Cancer, Blood, brain barrier, Ultrasound

A15. Super-resolution imaging of antibody-conjugated biodegradable periodic mesoporous organosilica nanoparticles for doxorubicin delivery in prostate cancer

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Biodegradable periodic mesoporous organosilica nanoparticles (nanoPMOs) have been syn- thesized through the sol-gel procedure and then we analyzed their properties. Next, nanoPMOs have been functionalized with an anti-mannose-6-phosphate receptor (M6PR) antibody in dif- ferent ways in order to target prostate cancer cells as M6PR is overexpressed in these cells. We have applied direct stochastic optical reconstruction microscopy (dSTORM), a single-molecule super-resolution microscopy technique, to quantify the degradation of nanoPMOs and compare it with TEM. Furthermore, the multivalency of antibody-conjugated nanoPMOs is evaluated with dSTORM analysis. Subsequently, cancer cell targeting, drug loading and release properties, and anticancer activity are also studied. It has been found that the orientation and multivalency of antibodies on the surface of nanoPMOs are crucial for efficient endocytosis of the nanoPMOs in prostate cancer cells and efficient delivery of doxorubicin in these cells.

Keywords: PMO, Antibody, dSTORM, Prostate

A16. Nano-designed carbon monoxide donor SMA/CORM2 exhibits protective effect against acetaminophen induced liver injury through modulating macrophage reprograming

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Acetaminophen (APAP) induced liver injury is the most common drug-induced liver injury, accounting for the top cause of acute liver failure in the United State, however the therapeutic options for it is very limited. Excess generation of reactive oxygen species (ROS) and the subse- quent inflammatory responses are the major factors of the liver injury. Carbon monoxide (CO) is an important gaseous molecule with versatile functions including anti- oxidation and anti- inflammation, and we previous reported the therapeutic potential of a nano-designed CO donor SMA/CORM2 in a dextran sulphate sodium (DSS) induced mouse colitis model. In this con- text, we investigated the effect of SMA/CORM2 in an APAP-induced mouse acute liver injury model and tackled the mechanisms involved. We found upregulation of heme oxygenase-1 (HO- 1, endogenous CO generating enzyme) and the dynamic changes of macrophage polarization (pro-inflammatory M1/anti-inflammatory M2), which played important roles in the process of live injury. SMA/CORM2 treatment remarkably increased the CO levels in the liver and circu- lation, by which oxidative stresses in the liver were significantly reduced, and more importantly, it remarkably suppressed the expression of M1 macrophages but alternatively increased M2 po- larization. Consequently the liver injury was significantly ameliorated, and the proliferation and regeneration were greatly promoted through the Pi3k/Akt/mTOR signaling pathway. The shift of macrophage polarization accompanied with the downregulated hypoxia-inducible factor-1α (HIF-1α) level. These findings suggested CO released from SMA/CORM2 manipulated the macrophage reprogramming toward M2 phenotype by inhibiting HIF-1α, which subsequently protected liver against inflammatory injury and benefited tissue repair. Moreover, compared to native CORM2, SMA/CORM2 exhibited superior bioavailability and protective effect. We thus anticipate the application of SMA/CORM2 as a therapeutic regimen for APAP induced liver injury as well as other inflammatory diseases and disorders.

Keywords: Acetaminophen Drug, induced liver injury, Carbon monoxide, Styrene maleic acid copolymer, Micellle, Macrophage reprogramming, Hypoxia, inducible factor, 1α

A17. Ultrasound responsive polymer microbubbles for the targeted treatment of stroke

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Systemic injection of thrombolytic drugs is the gold standard treatment for non-invasive blood clot resolution. The most serious risks associated with the intravenous injection of tissue plasminogen activator-like proteins are the bleeding complication and the dose-related neuro- toxicity. Indeed, the drug has to be injected in high concentrations due to its short half-life, the presence of its natural blood inhibitor (PAI-1) and the fast hepatic clearance. Overall, there is a serious need for a dose-reduced targeted treatment to overcome these issues. We present in this study a patented acoustic cavitation-based method for polymer MBs synthesis, three times faster than the current hydrodynamic-cavitation method. The generated echogenic MBs are stable, biocompatible and enable US visualization of the mouse brain vasculature. Their func- tionalization enabled the efficient and targeted treatment of stroke, without side effects. The stabilizing shell of the MBs is composed of Poly-Isobutyl Cyanoacrylate (PIBCA), copolymer- ized with fucoidan. Widely studied for its targeting properties, fucoidan exhibits a nanomolar affinity for activated endothelium and activated platelets (P-selectins). Secondly, the throm- bolytic agent (rtPA) was loaded onto microbubbles (MBs) with a simple adsorption protocol. We validated the in vivo efficiency of rtPA-loaded Fuco MBs to be over 50% more efficient than regular free rtPA injection for ischemic stroke resolution. In addition, the relative injected rtPA grafted onto targeting MBs was 1/10th of the standard effective dose (1 mg/kg in mice). As a result, no hemorrhagic event, no BBB leakage nor unexpected tissue distribution were observed. The echogenic targeting-MBs produced in this work now bring promising perspectives for the development of US molecular imaging of thrombotic diseases and the US assisted clot resolution: sonothrombolysis.

Keywords: Microbubbles, Fucoidan, Fibrinolytic, Ultrasound, Targeted thrombolysis, Stroke

A18. Optimization of Lorecivivint-loaded cationic liposomes for osteoarthritis therapy

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Osteoarthritis (OA) represents a widespread degenerative joint disease, causing significant morbidity and impairing the quality of life for millions of individuals worldwide. Lorecivivint, a promising therapeutic agent, has shown potential in slowing OA progression and promoting car- tilage regeneration. However, its clinical application is hindered by fast clearance from the syn- ovial fluid, limited cartilage targeting, and solubility. Our research study aims to develop a novel cartilage-targeting drug delivery system for Lorecivivint, employing cationic liposomes prepared through a microfluidic system. The cationic charge will allow it to interact electrostatically with the anionic glycosaminoglycans (GAGs) of cartilage. The use of microfluidic technology enables precise control over the particle size and homogeneity, leading to improved stability and enhanced drug encapsulation efficiency. The project started by formulating blank cationic liposomes including N-(1-(2,3-Dioleoyloxy) propyl)-N,N,N-trimethylammonium)(DOTAP) as cationic phos- pholipids and characterizing their size and zeta potential by dynamic light scattering (DLS). Then, the microfluidic system was used to produce Lorecivivint-loaded cationic liposomes, and various process parameters such as the total flow rate (TFR) and the flow rate (FFR) were var- ied to optimize the physicochemical properties of the liposomal formulation. The encapsulation efficiency (EE) of the optimized formulation measured by UPLC was 41%. The size and the zeta potential measured by DLS were respectively 137.7 d.nm and 53.2 mV, which is favorable to cartilage penetration. Finally, an in vitro release assay was realized in PBS. The controlled drug release profile is critical for achieving sustained drug levels and maximizing therapeutic efficacy while minimizing adverse effects.

Keywords: Osteoarthritis, cartilage, cationic liposomes, microfluidics

A19. Through the respiration to the general circulation, a promising path to reach the target?

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Administration of drugs intravenously or orally is a common practice, but it presents many obstacles that are difficult to overcome. These obstacles range from the highly acidic conditions of the stomach and the difficulty of passing the intestinal membrane for the oral route to the immune system triggered at the injection site for the intravenous administration, reducing or preventing the amount of drug in the blood. For some organs, such as the heart, another route of administration could be more direct and more efficient: the pulmonary route which has been increasingly studied in recent years. This route of administration allows nanomedicines to be aerosolized or nebulized to reach the deep lung, where the epithelium is no thicker than 0.2 µm. This delivery strategy could be used to counteract the side effects of doxorubicin on the heart. This widely administered anti-cancer agent is toxic to the heart, causing the basification of cardiomyocyte lysosomes and preventing them from their natural property to degrade. To restore healthy lysosomes, one strategy is to deliver PLGA which, once uptaken by the organelles, could acidify them, thereby restoring their natural capacity.

To this end, we synthesized silica nanoparticles (SiO2-NPs) with a fluorescein isothiocyanate (FITC) core, making them fluorescent. Those SiO2-NPs where then grafted with PLGA via an EDC/NHS coupling reaction. The SiO2-PLGA-NPs obtained where then fully characterized by transmission electron microscopy (TEM), dynamic light scattering and fluorescent spectroscopy. We obtained tiny, spherical, negatively charged nanoparticles with a zeta potential of -31 mV and a hydrodynamic diameter of 25 nm, enabling them to be administered by nebulization. In vitro and in vivo tests were carried out by Dr. Mialet-Perez's team and showed promising results for the restoration of cardiomyocytes lysosomes activity.

Keywords: pulmonary route, nanomedicine, drug delivery, silica nanoparticles

A20. Diclofenac prodrugs nanoparticles: an alternative and efficient treatment for rheumatoid arthritis?

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We have synthesized lipidic prodrugs of diclofenac by grafting aliphatic chains (C10, C12, C16 and C18) to diclofenac through an ester bond. Their molecular formulas were confirmed through HR-MS and the formation of ester bond by FTIR and NMR spectroscopy. Nanoparti- cles of the different prodrugs were successfully formulated using emulsion evaporation method and DSPE-PEG2000 as the only excipient. All nanoparticles were spherical and had a size between 110 and 150 nm, PdI ≤ 0.2 and negative Zeta potential values from -30 to -50 mV. In addition, they were stable upon storage at 4◦C up to 30-35 days. The encapsulation efficiency of the prodrug was above 90% independently of the aliphatic chain length grafted. Nanopar- ticles did not induce any toxicity on LPS-activated THP1 cells up to a concentration of 100 µg/mL (equivalent diclofenac) whereas diclofenac sodium salt IC50 was around 20 µg/mL. Following incubation of nanoparticles with LPS-activated THP1 cells, a dose dependent inhibition of TNF-α was observed comparable to standard diclofenac sodium. Based on in vitro studies representative nanoparticles, Prodrug 3 NPs (C16 aliphatic chain) were selected for further in vitro and in vivo studies. Upon incubation in murine plasma, Prodrug 3 NPs underwent an enzymatic cleavage and almost 70 % of diclofenac was released from nanoparticles in 8 hours. In vivo studies on a collagen induced arthritis murine model showed contrasted results: on one hand Prodrug 3 NPs led to a significant decrease of arthritis score and of paw volume compared to PBS after the second injection, on the other hand the third injection induced an important hepatic toxicity with the death of half of the mice from the NP group. To promote the reduction of inflammation while avoiding hepatic toxicity using NPs would require to precisely study the No Observable Adverse Effect Level and the schedule of administration in the future.

Keywords: Diclofenac, Prodrug, Nanoparticle, Inflammation, Rheumatoid arthritis

A21. Nanolipid-based strategies for improving the pulmonary delivery of non-small cell lung cancer treatment

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Non-small-cell lung cancer (NSCLC) is the most common form of lung cancer, and a lead- ing cause of cancer deaths worldwide. NSCLC intrinsic mechanisms of resistance are a major cause for therapeutic failure, resulting in tumor recurrence and disease progression. Within this context, the aldehyde dehydrogenases (ALDHs) family of detoxifying enzymes are receiving increasing attention, since often upregulated in NSCLC and associated with chemo- and radio- therapy resistance. Owing to their therapeutic potential, Advanced BioDesign develops ALDH inhibitors (ALDHin) with high target specificity and potency as drug candidates for NSCLC. Yet, the use of these ALDHinin vivo is challenged by their low aqueous solubility and short half-life. Here, we address these challenges by proposing a lipidic nanoformulation approach aimed to promote lung-specific delivery and potentiate the therapeutic activity of the ALDHin, ABD-0171. To increase the tropism of lipid nanoemulsions (LNE) to the lungs, we exploited an endogenous targeting mechanism based on the protein corona. Recent work has demon- strated that the addition of selective organ targeting (SORT) molecule allows to control the global/apparent pKa and serum protein interactions at the surface of lipid nanoparticles, which then determine the particle biodistribution leading to lung accumulation (1). On this basis, we prepared reverse micelles of SORT molecule and incorporated it into the oily core of a LNE. This reverse-micelle incorporation was compared to a ”one-pot” fabrication, for which all lipids were mixed before the LNE preparation (2,3). An initial screening that involved physico-chemical characterizations (size and surface charge) allowed for the preselection of 3 LNE candidates. Using cellular uptake assays, we observed a more efficient internalization in LNE formulations containing SORT molecule. Consistently, these LNE forms of ABD-0171 improved the inhibitor cytotoxic activity towards NSCLC cells. No toxicity was observed for empty LNE. Finally, a first in vivo evaluation of the most promising ABD0171 LNE forms was performed to assess their toxicological profiles and set the experimental conditions for in vivo efficacy and biodistribu- tion studies. Our preliminary results already suggest that the incorporation of SORT molecule within LNE constitutes a promising research axis for the targeted delivery of ALDHin to the lung tissue.

Keywords: Nanoemulsions, lung cancer, NSCLC, endogenous targeting, aldehydes deshydrogenase inhibitors

A22. A new analytical method to study the interactions of proteins and innovative nanoparticles: toward the treatment of Type-2 Diabetes

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According to the WHO, the worldwide prevalence of Diabetes in adults will reach 5.4% by 2025(1). Most diabetics have Type-2 Diabetes, and to date, no curative treatment is available on the market. The misfolding and oligomerization of an amyloid peptide hIAPP (human islet amyloid polypeptide) is an established etiology of Type-2 Diabetes(2). Janus nanorods (JNR), two-faced nonsymmetrical nanoparticles, could form the basis of an innovative therapeutic strat- egy to prevent the pathological process of this disease(3). One side is dedicated to capturing hIAPP to inhibit their oligomerization or fibril formation. The other side binds to human serum albumin (HSA) to increase the circulation time in the bloodstream(4).

This work aims to investigate JNR dual interactions with HSA and hIAPP. Initial research focused on characterizing the size and shape of the nanoparticles by individual nanoparticle tracking analysis (NTA) and multi-angle dynamic light scattering (MADLS). From these meth- ods the length and width of JNR were estimated. Furthermore, the surface charge was also determined (zeta potential around -12.5 mV). After determining the nanoparticle's size, shape, and charge, the interaction between JNR-HSA was developed using a new capillary electrophore- sis method. As the JNR has low electrophoretic mobility, we opted for a method able to dose HSA remaining free after incubation with JNR. Due to HSA being a protein that adsorbs to the silica capillary wall and experiments needing to be conducted under physiological conditions (pH 7.4), we extensively optimized the analytical parameters. Currently, this fast and reproducible method allows the construction of adsorption isotherms in 30 min. Thus, from the quantifica- tion of free HSA, we successfully determined interaction parameters between protein and JNR, such as binding constant and stoichiometry. Finally, different nanoparticles were tested, such as PLGA and PLGA-PEG nanoparticles. Similar electropherogram profiles were obtained, sug- gesting that this method can be generic for nanoparticle-HSA interaction studies.

This project is part of the ANR consortium https://anr.fr/Project-ANR-21-CE06-0017

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Keywords: Long circulating nanorods, Type 2 Diabetes, capillary electrophoresis, human serum albumin, interactions

A23. Interaction of nanoparticles with key corona proteins impacting their circulation time ‚Äì an in vitro-in vivo correlation study

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Upon contacting biological fluids, nanoparticles (NPs) interact with proteins, lipids, and sug- ars, creating the biomolecular corona thereby conferring, a new biological identity and physic- ochemical properties to the NP. Protein corona composition depends, besides other factors, on NP surface composition, size, surface charge, and hydrophobicity.
In a previous study, superparamagnetic iron oxide nanoparticles (SPIONs) coated with Polyvinyl alcohol (PVAL) with neutral (OH), positive (NH2), and negative (COOH) surface charges were injected into the tail vein of the rat, and the protein corona compositions were tracked over time. NPs showed different retention times in blood circulation depending on their surface chemistry/charge and resulting corona composition. SPIONs-PVAL positively charged showed the shortest circulation time, which was attributed to high hemoglobin adsorption. Moreover, neutral charged NPs had the longest circulation time, which could be related to several proteins, such as alpha-2H-glycoprotein (fetuin).
This work explores the interaction between the previously identified key proteins and the PVAL- coated SPIONs, by studying binding affinity and protein structural changes upon binding to NPs. Besides hemoglobin and fetuin, albumin and transferrin interaction with NPs is also studied, since they are two of the most abundant proteins in the plasma. To do so, fluorescence quench- ing and quartz crystal microbalance determined binding affinity, while the potential secondary structure changes were characterized by circular dichroism. The results are compared with the data obtained from the in vivo experiment, to draw conclusions on how simple protein-NP experiments can be correlated with the in vivo complexity of the protein corona.

Keywords: nanoparticle, protein corona, protein structure, binding affinity, conformational changes

A24. Size Distribution by Taylor Dispersion Analysis (SD-TDA): An innovation for resolutive size distribution measurement of lipid nanoparticles and monoclonal antibodies

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The assessment of the absolute size of mixture of nano-objects is a challenge in biophysical characterization. There are a limited number of affordable technologies that provide accurate particles size distribution down to 0.5nm. Current technics such as DLS, A4F/SEC-MALS, NTA are often not suitable to work in native form. Indeed, denaturation occur during sample preparation or analysis: filtration, dilution or shear stress.
Nanoscale Metrix developed a new methodology to characterize the size distribution of nanoob- jects, the Size Distribution - Taylor Dispersion Analysis. With SD-TDA, only 10 nL of sample are mobilized under low pressure differential in a capillary. Under Taylor dispersion conditions, the hydrodynamic radius can be calculated from the detected signal (U.V, L.I.F) through Stokes- Einstein equation.

In this work, SD-TDA was first used as a new alternative method to determine the size and size distribution of nanovectors: LNPs encapsulating mRNA, coated metallic nanoparticles.

The size of LNP is known to affect intracellular delivery and therefore vaccine efficacy and SD-TDA allow a very accurate size measurement. Thus, several LNP formulations were success- fully analyzed and obtained results were compared to those obtained with DLS or FFF-MALS. Second part of this work was dedicated to the thermal stress of human IgG. At high temper- ature, a kinetic study of the IgG degradation was carried out. A modification in the IgG size is measured and degradation products were also detected. The size of obtained IgG fragments is compared to the size of purified fragments directly characterized by SD-TDA (Fc, Fab, and F(ab')2 fragments)

Keywords: LNP, Size characterization, Size Distribution, Taylor Dispersion Analysis, Antibody,

A25. Cytotoxicity and Antibacterial Effects of Peppermint Synthesized Zinc Oxide Nanoparticles

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This study evaluates the cytotoxicity and antibacterial activities of zinc oxide nanoparticles (ZnO NPs), synthesized by a biogenic route using peppermint extract. The obtained NPs have a hydrodynamic size of 171.0 ± 5.0 nm, with polydispersity index of 0.22 ± 0.01, and zeta potential value of -18.6 ± 0.2 mV. Fourier-transform infrared spectroscopy demonstrated the presence of phytochemicals derived from the plant extract as capping agents on the surface of NPs, while X-ray diffraction confirmed the formation of ZnO NPs. Transmission electron microscopy revealed the presence of organic molecules derived from peppermint extract on the surface of well dispersed ZnO NPs.

The cytotoxicity of ZnO NPs was evaluated against prostate cancer cells (PC3) and compared with human fibroblast cells (FN1) (non-cancer cell line). The results demonstrated a concen- tration dependent cytotoxicity against both cell lines. Specifically, for the PC3 cells, a 75% reduction in cell viability was observed at a concentration of 50 µg/mL of ZnO NPs. In con- trast, the FN1 showed a 25% reduction in cell viability at the same NP concentration. This indicates a selective cytotoxic effect of ZnO NPs, with a higher cytotoxicity towards the cancer cells compared to the non-cancer cells. Complementary, the antibacterial assays demonstrated that ZnO NPs completely reduced the cell viability of Escherichia coli at a concentration of 15.6 µg/mL. The observed antibacterial activity could be attributed to the ability of the NPs to penetrate the bacterial membrane and generate reactive oxygen species. Moreover, the antioxidant activities of peppermint synthesized ZnO NPs, commercial ZnO NPs and the peppermint extract were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. This assay is based on the measurement of the scavenging capacity of antioxidants towards it. The results showed that the scavenging capacity is dependent on the concentration of plant extract. At a concentration of 3.1 mg/mL of peppermint synthesized ZnO NPs, scavenging capacity was approximately 80%, compared to control. Furthermore, the antioxidant activity of the ZnO NPs synthesized with peppermint extract was found to be 33% higher than commercial ZnO NPs, at a concentration of 15.63 µg/mL.

Thus, our results demonstrate that peppermint extract can be successfully used in the green synthesis of ZnO NPs, which demonstrated toxicity against PC3 cells, antibacterial activity, and antioxidant properties, highlighting their potential use in biomedical applications.

Keywords: zinc oxide, green synthesis, biomedical applications

A26. Advanced porous hybrid hydrogels for sustained drug delivery

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Injectable hydrogels have received increasing attention in biomedical areas due to their po- tential to serve as drug reservoirs for localized drug administration.(1)Injected Intraperitoneally, they could find application in the treatment of peritoneal carcinomatosis as they can ensure (i) efficient retention and controlled release of drugs and (ii) high drug concentrations locally for prolonged periods. However, the benefits achieved so far are insufficient. The main issues af- fecting hydrogels arise from their poor stability, the abrupt release of physically encapsulated drugs and the potential toxicity of crosslinking reagents. It is therefore necessary to develop a new strategy to ensure controlled and sustained delivery of active molecules in the peritoneal cavity.
To reach this goal we have developed an injectable hybrid system by combining an adhesive hydrogel matrix and drug nanocarriers. Hyaluronic acid has been chosen as main component of the gel matrix and opportunely functionalized with cyclooctynes and catechol-containing moi- eties. Drug-loaded nanocarriers decorated with azide functions have been covalently attached to the hydrogel matrix by biorthogonal copper-free click chemistry and serve as crosslinking points. The presence of catechol confers adhesive properties to the material, enhancing in situ residence.

The characteristics of the hybrid system can be modulated by adjusting the properties of the individual components, and we have rationally studied how the combination of building elements will enable us to meet the required specifications.

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Keywords: hybrid hydrogel, nanoparticles, click chemistry, cancer therapy

A27. A study of drug release kinetics with advanced analytical methods

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Polymer nanocarriers designed for controlled drug release represent an advanced therapeu- tic approach, offering improved therapeutic efficacy while minimizing potential side effects. The accurate assessment of drug release kinetics plays a main role in the development of safe and effective nanomedicines. In this study, we introduce a novel application of analytical techniques, specifically surface plasmon resonance biosensor technology, capillary electrophoresis, and 1H and diffusion-ordered nuclear magnetic resonance, for the precise determination of drug release profiles.
These methods were innovatively employed to quantitatively determinate the pH-triggered re- lease of three structurally distinct drugs: dexamethasone, docetaxel, and hexyl ester of aminole- vulinic acid, from polymer-based carriers. Various parameters, such as their adaptability to diverse sample types, biological relevance within the experimental setup, method complexity, and analysis outcomes, were thoroughly evaluated and analyzed. Furthermore, the performance of these techniques was compared with the conventional ”gold standard” high-performance liq- uid chromatography method.

This research highlights the potential of emerging analytical approaches in enhancing our under- standing of drug release from nanocarriers and their applicability in the development of advanced therapeutic solutions.

Acknowledgement
This work was supported by the Ministry of the Health of the Czech Republic (NU20-08-00255), the Czech Science Foundation (grant 19-00956Y), and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) funded by the European Union, Next Generation EU, and Charles University (project SVV260690).

Keywords: Polymer nanocarriers, drug release kinetics, surface plasmon resonance biosensor technology, capillary electrophoresis, 1H NMR, DOSY NMR

A28. Dexamethasone-loaded DSPE-PEG(2000) micelles as a drug delivery system to treat dysregulated inflammatory response

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Sepsis arises from a systemic infection that triggers an uncontrolled inflammatory response which may worsen into multi-organ failure and death1. Currently, other than antibiotic reg- imens in combination with cardiovascular/respiratory supports, there is no established cures for sepsis. Among the therapeutic approaches, glucocorticoids, notably dexamethasone (DXM), have been widely used to ameliorate the excessive inflammation associated with sepsis. This work presents the formulation and efficacy assessment of DXM-micelles, a drug delivery system based on DSPE-PEG(2000) micelles encapsulating DXM.

First, we comprehensively investigate the physicochemical properties, stability, in vitro release kinetics, and cytotoxicity of DXM-micelles on both human and murine monocytes. Additionally, we investigate the in vivo therapeutic efficacy of DXM-micelles using two mouse models, namely the endotoxemia model and the caecal ligation and puncture (CLP) model for sepsis. Further- more, the targeted delivery and preferential accumulation of DXM-micelles within immune cells were explored, shedding light on the potential immunomodulatory benefits of this innovative drug delivery approach. The results indicate that DXM-micelles have the potential to revolu- tionize anti-inflammatory therapy, offering a safer and more effective alternative to conventional treatment.

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Keywords: Sepsis, dexamethasone, micelles, immunomodulation

A29. Anisotropic phospholipid-containing nanoscale dispersions: self-assembly mechanism and manufacturing

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There is a growing interest in asymmetry in the field of nanotechnologies for drug delivery. Anisotropy makes it possible to give original properties of mobility, encapsulation or interaction with biological media to the nanoparticles considered.(1)
The present project focuses on asymmetric particles in their composition in order to improve the co-encapsulation of compounds for biomedical uses. Over the last few years, our group has been interested in the development of multicompartmental (Janus) supramolecular self-organization from amphiphilic derivatives.(2) The general objective of this project is to understand the impact of formulation and preparation process on the properties of anisotropic nanoparticles obtained from different amphiphilic materials (short-PEG-based surfactants or amphiphilic cy- clodextrins). Key-parameters will be investigated mainly at three levels: formation mechanism, stability and encapsulation efficiency.
Finally, to anticipate further use as multidrug delivery systems, the formulations showing suit- able structure stability will be evaluated as delivery systems through intravenous administration by performing in-vitro assays to ascertain their compatibility with this route of administration.

Keywords: multidrug delivery, anisotropic nanoparticles, phospholipid, self assembly

A30. Liposomal formulation of docetaxel: biodistribution and immunomodulatory benefit in HER2 breast cancer treatment

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Despite the success and use of docetaxel in combination with trastuzumab in HER2+ breast cancer, there are still serious limitations to docetaxel, including poor water solubility and the systemic toxicity of taxanes. To overcome these side effects of taxanes we have first, developed an Immunoliposome loaded with docetaxel and grafted with trastuzumab, using a microfluidic method. We will then test these nanoparticles in the blood and analyse their effect on immune cells in immunocompetent mice bearing murine breast cancer. We have chosen to analyse stealth liposomes (Lipo) or stealth immunoliposomes grafted with trastuzumab (ANC) versus control. To analyse the biodistribution of ANC, plasma was collected 1h, 2h, 3h, 6h, 8h and 24h after their injection (docetaxel 1,9 mg/kg and trastuzumab 160 ng/kg) or a mixture of free drugs at the same dose, into healthy C57BL/6 mice by intraperitoneal injection. Frozen plasma was analysed by HPLC-UV. We injected lipo or ANC repeatedly (ie MD) or singly (ie SD) into Balbc mice carrying KT1 breast cancer. We then collected blood, spleen and tumor over time (Day 1 to 18) and used flow cytometry to characterise immune cells (i.e. B cells, T cells (CD4 and CD8), Treg, MDSC (Gr MDSC and Mo MDSC)) over time. The ANC were characterised by a mean diameter of 140 nm, a docetaxel encapsulation rate of 73% and a trastuzumab uptake rate of 400 units of trastuzumab by nanoparticle. This liposomal formulation showed a marked change in pharmacokinetics and an advantage in terme of plasma exposure time. Preliminary results show a superior half-life for liposomal docetaxel of 40.76h compared to free docetaxel of approximately 4.33h. The concentration of docetaxel appeared to stabilise over time, up to 24 hours (52.72± 9.45 ng/ml) when the animals were treated with ANC compared to free docetaxel which is undetectable at T24h. Liposomal docetaxel showed higher AUC0-t and AUC0-∞ values compared to non-liposomal docetaxel due to the liposomal encapsulation. The PK results obtained showed a 3.75-fold increase in AUC0-t and a 5.9-fold increase in AUC0-∞ when compared to free docetaxel. Regarding the effect of liposomes or ANC on immune cells, no clear effect of the nanoparticles was observed from D-1 to D-18. Compared to the control, treatment of animals with MD-ANC resulted in a higher CD8/CD45 ratio in blood, spleen and tumours at D18. Similarly, at D18, all treatments appeared to induce a decrease in Tregs in blood and tumors. Conversely, a greater number of Tregs were found in the spleen after treatment with MD ANC, suggesting that they were sequestered. MD ANC showed a better CD8/Treg ratio in the tumor. The increase in CD8+ cells in blood and tumors, combined with the decrease in Tregs in tumors, suggests that multiple dose of liposomal nanoparticles could help turn cold tumors into hot ones. In conclusion, these preliminary results suggest that we have developed a promising immunoliposome for systemic delivery of docetaxel in HER2+ breast cancer with optimal pharmacokinetics and promising harnessing of tumor immunity. This study demonstrates that the immune response induced by liposomal nanoparticles is significant and sustained over time. Sequencing treatments with immunotherapy (i.e., 2 weeks after nanoparticles) may be the best strategy to achieve synergism.

Keywords: nanoparticles, liposomes, mice model, bio distribution, immunotherapy

A31. Development of a personalized 3D printed form loaded with MCM-41 silica for chronic disease

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Chronic diseases require frequent administration per day leading to non-observance of the treatment. Furthermore, these treatments may concern patients (such as newborn or children) or active pharmaceutical ingredients (API) with a narrow therapeutic window needing personalized treatment with adapted dose (per kg weight). In these specific cases, personalized extended- release forms need to be developed to reduce the frequency of administration and adapt API dose.

Among innovation research on extended release form, ordered mesoporous silica (MCM-41) is a particularly appealing material due to its high pore volume, enabling high drug loading. It also features an organized pore network with a narrow pore size distribution, which can be adjusted to control drug release. Commercial silica are available but direct loading method allows simultaneous the mesostructuration of the silica and drug incorporation, which is time-saving (1), (2). Concerning the development of personalized form, pharmaceutical 3D printing has emerged as a potential solution. Among the various 3D printing techniques, Fused Deposition Modeling (FDM) stands out due to its low cost and accessibility as evidenced by the increasing number of research articles published in the last decade (4-fold between 2012 and 2022) (3).

Thus, our work aims to develop a personalized FDM 3D printed form loaded with MCM-41 silica to control drug release in order to improve treatment compliance and safety. MCM-41 silica was synthesized and loaded in a one-pot step using Poly(poly(ethylene glycol)-methyl ether acrylate)-b poly(sodium 4-vinylbenzenesulfonate) (PEO-PSS) copolymer in the presence of silica precursors (TEOS) and neomycin as a model drug. The resulting MCM-41 was characterized using BET, TEM, and the drug load was quantified. Subsequently, PEG filaments loaded with MCM-41 silica were prepared using hot melt extrusion and printed using FDM (1). A drug release study was then conducted on the loaded hybrid materials and material formulation will be adapted to obtain required form.

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Keywords: 3D printing, Mesoporous silica, Custom, made, Compliance

A32. Cartilage targeting nanosized delivery system for osteoarthritis therapy

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Osteoarthritis (OA) is the most common degenerative joint disease worldwide. Disease evolution in knees and hips is associated with cartilage degradation. Treatment options are only symptomatic, and no disease-modifying therapy able to stabilize or revert OA progression has passed clinical trials due to systemic toxicity and lack of cartilage targeting. In this context, the aim of our research is to design an avidin drug delivery system containing kartogenin (KGN) as a disease-modifying OA drug (DMOAD) for intra-articular (IA) administration. The drug should penetrate the cartilage's full depth to reach the chondrocytes and stimulate chondroge- nesis. Cartilage has a negative fixed charge density that can be used to overcome the cartilage barrier by making drugs positively charged. We selected avidin, a positively charged protein as the delivery system, to target chondrocytes in the cartilage's deep zone, the nanosized allowing penetration through the cartilage's porous matrix. The KGN was biotinylated in 2 steps and purified by preparative HPLC with a global yield of 27%. The biotin-PEG2-KGN was characterized by Electrospray ionization mass spectrometry (ESI-MS), Matrix-Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF), and Nuclear Magnetic Resonance (NMR). The HABA (4'-hydroxyazobenzene-2-carboxylic acid) assay was used to estimate the molar ratio of biotinylated conjugates to avidin. The assay proved a preserved biotin-avidin affinity after KGN coupling. 4 molar ratios of biotin-PEG2-KGN were necessary to displace the HABA and generate the avidin conjugate. The size and zeta potential measured by dynamic light scattering (Nano ZS) were 8.4 ± 3.6 nm and 18,3 ± 5,15 mV, respectively.An in vitro drug release study over 7 days was performed in the presence of 1U and 100 U of porcine esterase. After 24 h, 40 - 45 % of KGN was released from the avidin nanocarrier. The enzymatic kinetic was faster with 100 U however a plateau was reached after 1 week. The fluorescence was used finally to show the avidin-biotin-5-fluorescein cartilage uptake in an ex-vivo bovine explant model, compared to neutravidin-biotin-5-fluorescein and free biotin-5-fluorescein used as controls. The avidin- biotin-5-fluorescein positively charged showed higher cartilage uptake compared to neutravidin. This supports the electrostatic interaction-driven delivery of the DMOAD within the negatively charged cartilage matrix.

Keywords: nanosized delivery system, cartilage, osteoarthritis therapy

A33. Controlling PEG-b-PTMC Polymersome Size through Microfluidic-Assisted Self-Assembly: Advancing the Understanding of Polymersomes Self-Assembly

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Polymersomes are typically more stable than liposomes in biological environments because of their thicker membrane and their ability to adjust membrane properties(1). The copolymers self-assembly is thermodynamically and kinetically controlled and thus depends on several crit- ical parameters, including the physicochemical properties of the copolymer(2), the appropriate selection of organic and aqueous solvent and the choice of the formulation technique(3,4).
Using microfluidic systems, solvent displacement method was used to assess the self-assembly of poly(ethylene glycol)-block -poly(trimethylene carbonate) PEG-b-PTMC copolymers into vesicles. The effect of process parameters on vesicle generation was assessed using different microfluidic chips with various flow regimes and with various aqueous and organic solvents.
We demonstrated the choice of microfluidic systems and organic solvent had minimal impact on vesicle formation while copolymer concentration and total flow rates are the main parameters that influence vesicles size. Overall, microfluidic-assisted self-assembly offers a highly reproducible method to produce monodisperse polymersomes with adjustable sizes. Nanoparticles formulated with microfluidic were confirmed to be vesicles through the use of multi-angle light scattering in combination with cryo-TEM characterization (5).
Following these results, the focus was on investigating the self-assembly mechanism of PEG-b-PTMC. Intermediate morphologies were examined using two different organic solvents by directly observing them under confocal microscopy during the addition of water. All tested organic solvents exhibited similar behavior: an initial liquid-liquid phase separation occurred, concentrating the copolymer in coacervate droplets. These droplets then coalesced up to a certain water content, and budding was observed on the surface of the droplets. It appeared that when the solvent is removed, vesicles were formed and spontaneously detached from the coacervate surface. Through this study, we aim to demonstrate that understanding the vesicle self-assembly mechanism and using microfluidic system to precisely control their morphology and size will enable better prediction of drug encapsulation inside polymer vesicles.

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Keywords: Microfluidic, polymersomes, vesicles, nanoparticles, formulation, nanoprecipitation

A34. Vectorization of M2e peptide by nanostructured lipid carriers for universal flu vaccine

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Objective: The current Influenza vaccines are considered the most effective ways to con- trol influenza infections. However, they are based on hemagglutinin, which is highly changeable, thus resulting in a varying protection. The M2e peptide, a surface antigen highly conserved among Influenza A viruses, is a key cross-protective antigen. However, its poor immunogenicity hampers its use in a universal vaccine (1).
Nanostructured lipid carriers (NLCs) are nano-emulsions highly stable over time. Their small size (50-90 nm) improves their interactions with antigen-presenting cells and their lymphatic drainage. In previous studies, NLCs have been used to deliver both antigen (HIV-p24) and adjuvant (CpG) leading to strong antibody and cellular responses in mice and macaques (2).
The aim of this study is to vectorize M2e by NLC to improve its immunogenicity and demon- strate its potential as a universal flu vaccine.
Method: M2e was vectorized by two different NLCs: neutral NLC (NLC) and positively charged NLC (NCL(+)). M2e was either conjugated to NLCs surface through click chemistry (NLC-M2e) or was complexed to NLC(+) surface by electrostatic interactions (NLC(+)/M2e). CpG ODN1826 was used as adjuvant and also complexed to NLC(+) (NLC(+)/CpG). These nanoparticles were injected in BALB/c mice of 8-9 weeks. The sera were sampled to determine the anti-M2e antibody response (ELISA) and the spleens were collected to determine the cellular response (ELISA, Flow Cytometry).
Results: M2e was successfully vectorized by both NLC and NLC(+). High density of ˜315 M2e/NLC could be reached, i.e. ˜20 times the density of M2e on Influenza A virion. Multimeric presentation and efficient carrying by NLCs in combination with NLC(+)/CpG strongly improve M2e immunogenicity compared to its free form. Both vectorization methods led to the threshold of response expected for a protection (> 50µg/mL anti-M2e IgG) (3) and the orientation of the immune response (LT CD4+ Th1 bias) complied with the M2e mechanism of protection against flu (1). NLC(+)/M2e triggered a quantitatively higher humoral response than NLC-M2e. How- ever, evidences suggested that the specificity of the elicited antibodies was different depending on the vectorization context and support NLC improved native epitopes presentation of M2e.
Conclusion: NLC-M2e and/or NLC(+)/M2e have the potential to be included in a univer- sal flu vaccine. A challenge in mice with different subtypes of influenza A viruses is planned to assess this potential.

Keywords: : Influenza – Vaccine, Lipid nanoparticles

A35. Biodegradable polymersomes as controlled drug delivery system for the treatment of neurodegenerative diseases

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Significant breakthroughs in the field of drug delivery system are conditioned by the design of polymer nanoparticles, which can control the drug release in time and location. (1) The project aims to develop nanoscale vesicles called ”polymersomes” loaded with specific drugs and composed of biodegradable polymers in order to obtain a dual action of restoring lysosomal activity in dopaminergic neurons, one of the issues leading to the development of Parkinson's disease. (2) In this project, PEG-b-PLA and PEG-b-PLGA copolymers were studied for self-assembly, nano-vesicles formulation, and bioactivity.

PEG-b-PLA and PEG-b-PLGA were chosen as amphiphilic copolymers for polymersomes formulation because it offers a tunable degradability and a good biocompatibility. Furthermore, the degradation of the lactide-based blocks generates an acidity that regulates lysosomal activity in deficient neurons. Such polymers were synthetized via ring-opening polymerization of lactide and glycolide using PEG2000 and PEG5000 as macroinitiator and DBU as organocatalyst. A precise molar mass was targeted to obtain a specific hydrophilic fraction. (3)

Polymer vesicles were formulated by nanoprecipitation using a solvent displacement method. Monodispersed and nanosized vesicles of 150 nm with a membrane thickness of 15 nm were formed and characterized by light scattering and transmission electron microscopy. It has been demonstrated that particular attention must be paid to the hydrophilic fraction but also to the formulation conditions and techniques to achieve a homogeneous population of vesicles. (4) Encapsulation of drugs was performed during the self-assembly process.

Cell viability and polymersomes internalization were study by encapsulating a fluorescent dye in their membrane. No toxicity was observed for PEG-b-PLA vesicles. Confocal microscopy demonstrated that nanoparticles were colocalized in lysosome of neurons.

Next, release study of the drug encapsulated will be investigated under physiological conditions mimicking intracellular neuronal environment. Meanwhile, the degradation of the vesicle membrane and the generated acidity will be studied. The last step of the project will be to functionalize the nanovesicle surface with ligands that will enable the vesicle to cross the blood-brain barrier and be specifically internalized by dopaminergic cells.

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Keywords: : Polymersome, Nanovesicle, Nanoprecipitation, Parkinson Disease

A36. Optimizing ASO-based therapeutics formulation and administration to treat idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic disease consisting to a loss of pulmonary functions due to a collagen overproduction by lung fibroblasts (1) (2). Actually, no efficient treatment exists but one could be RNA-targeted therapies using modified oligonucleotide (ASO) which have already shown promising outcomes (3). ASO delivery in vivo for IPF remains to be optimized and in particular we need to define adapted formulations to administer the ASOs : 1) lung myo-fibroblasts responsible of the formation of fibrosis and are known to be difficult to access due to pulmonary tissue complexity; 2) an appropriate administration route; and finally 3) without inducing an inflammatory response. We are thus developing different type of ASO-containing nanoparticles such as self-assembled Gold-nanoclusters, liposomes or lipid nanoparticles adapted to an efficient encapsulation and protection of the ASOs and capable of efficiently silence different genes or lncRNA in fibroblasts in vitro. Finally, the intravenous and intratracheal administration routes will be studied in a mouse model of bleomycin-induced pulmonary fibrosis.

Keywords: : Fibrosis, oligonucletide, nanoparticles

A37. Interfering with the YAP1/TAZ pathway in chemotherapy-resistant gastrointestinal stromal tumors to develop new pharmacological molecules

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Gastrointestinal stromal tumors (GIST) are the most common sarcoma of the gastrointesti- nal tract. These tumors mostly arise from oncogenic gain of function mutations in receptor tyrosine kinase genes, mainly KIT. Current treatments are based on tyrosine kinase inhibitors (TKI), such as imatinib mesylate (IM). However, a substantial proportion of patients develop over time resistance to such therapy and rapidly cancer relapse rapidly occurs (1). One cause of IM resistance is the activation of signaling pathways unsensitive to IM among which the YAP1/TAZ pathway is a promising candidate (2,3). The Hippo pathway effectors YAP1 and TAZ are master regulators for multiple cellular processes and cancer development. Dissecting YAP1/TAZ pathways and elucidating down-stream effects could help to better understand GIST resistance to conventional chemotherapy and to develop novel therapeutics.

Thus, my thesis work aims to propose a new therapeutic approach targeting YAP1/TAZ in GIST. The objective is to manipulate YAP1/TAZ expression using specific siRNAs in IM sensi- tive/resistant GIST cells. For this purpose, the team designed WRAP (W- and R-rich Amphi- pathic Peptide) able to transfect siRNA in vitro and in vivo (4).

We designed and validated siRNAs efficient to specifically target YAP1 or TAZ. First results evidenced that WRAP-based nanoparticles encapsulating siYAP1 or siTAZ could specifically reduce YAP1 or TAZ levels up to 70% in GIST cells. More importantly, we determined the effi- cient ratio to obtain nanoparticles encapsulating simultaneously both siRNAs (siYAP1/siTAZ) to silence both proteins. Cellular consequences of such YAP1/TAZ inhibition will be further investigated regarding the activity of KIT, the YAP1/TAZ downstream pathways, the viability, proliferation and apoptosis of IM sensitive/resistant GIST cells.

In conclusion, reducing YAP1/TAZ activity in GIST should allow to reduce IM doses and/or to re- sensitize cancer cells to IM treatment.

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Keywords: : YAP1/TAZ, gastrointestinal stromal tumors, peptide, based, nanoparticles, siRNAs

A38. Fate of silicon and Zinc oxide nanoparticles in liver cancer cells

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In the last decades, nanotechnology-related research has exponentially grown in the biomedical field. Among diverse properties and applications, nanoparticulated formulations enable a designed treatment, targeted applications, and reduced side effects. Conventional cancer treatments still face difficulties related to poor targetability, non-specificity, and increasing tumor resistance. Thus, nanoparticles (NPs) may be a promising solution for the difficulties encountered. Among several metal oxide NPs, zinc and silicon oxides (ZnO and SiO2) consist in a potential strategy for an efficient drug delivery. Although, the fate of these commonly used NPs is still not fully described. Herein, we aimed to identify molecular mechanisms of ZnO and SiO2 NPs in liver cancer cells: (i) differentiated hepatoblastoma cells (HepG2) and (ii) mesenchymal liver cancer cell (SNU449). The NPs were synthesized and characterized by several techniques, confirming their physicochemical properties. Both NPs were evaluated in doses from 5 to 50 µg/mL, in which we had observed significant cytotoxicity for ZnO NPs in HepG2 and SNU449 cells (24 h), but no significant cytotoxicity for SiO2 NPs. We have determined NPs intracellular localization using transmission electron microscopy (TEM). ZnO NPs demonstrated a higher accumulation in mitochondrial fraction in HepG2, and a non-specific localization in SNU449 cells, while SiO2 NPs tended to be localized in lysosomes in both cell lines. Interestingly, when evaluating cell respiration (MitroStress, Seahorse, Agilent), ZnO NPs decreased oxygen consumption rate in both cell lines, while, as expected, SiO2 NPs did not impact cells respiration, confirming the mitochondrial target only observed for ZnO NPs. Overall, SiO2 demonstrated no cytotoxic profile against HCC cells, promoting no significant alterations in the parameters evaluated. By contrast, ZnO NPs demonstrated a high cytotoxic potential against both HCC cells, directly impacting in cells respiration, which might be related directly to ZnO NPs accumulation in mitochondria. Moreover, our analysis suggests a different death pathway promoted by ZnO NPs in HepG2 and SNU449 cells, indicating the presence of necroapoptotic pathways only in SNU449 cells.

Keywords: : zinc oxide nanoparticles, silicon nanoparticles, liver cancer, death pathways

A39. Synthesis and evaluation of self-assembling properties of fluorinated lipid-like (3,5-bisalkyl-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides

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The application of nanotechnology to healthcare has led to the development of a variety of novel innovative nanoproducts. Fluorinated phospholipids possessed different properties com- paring with analogue CH2 group containing ones.(1)
This study is aimed to elaborate to new kinds of nanoparticles formed by cationic lipids with fluorine containing substituents; evaluation, characterisation of compounds and their self-assembling properties.
A synthesis of 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin- 1-ium iodides 4 with was performed according to Scheme 1. Treatment of the corresponding 4-pyridyl-1,4-DHPs 1 with Selectfluor® (2) followed by treatment with methyl iodide of pyri- dine moiety in the obtained 1,5-diketones 3 gave the desired pyridinium iodides 4.
This type of compound would be useful as synthetic lipids for further development of the delivery systems. The estimation of self-assembling properties and characterization of the nanoparticles obtained by ethanol solution injection in an aqueous media were performed by dynamic light scattering (DLS) measurements. DLS measurement data showed that compound 4b with nonyl moiety formed liposomes with the average diameter of 300–400 nm and polydispersity index (PDI) value around 0.30–0.40, while compound 4a with ethyl moiety formed a heterogeneous sample with PDI value 1, which indicated the heterogeneity of the sample. For more details see (3).
Acknowledgements: Funded by the EuroNanoMed3 Project TENTACLES.

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Keywords: : synthetic lipids, selfassembling properties, nanoparticles, DLS, pyridinium

A40. Squalene-based nanomedicines combining enkephalins and enkephalinase inhibitors: a synergistic approach for chronic pain treatment

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Endogenous neuropeptides such as Enkephalins represent a promising way to alleviate the pain sensation by using natural analgesics devoid of opioids side effects. However, their fast metabolisation in blood stream restrict their clinical use. To circumvent this limitation, our lab developed a novel nanomedicine approach (1), using Leu-enkephalin-squalene (LENK-SQ) prodrugs (2), which allows the specific delivery of the neuropeptide into inflamed tissues for efficient pain control, by targeting peripheral opioid receptors (and not central). In order to potentiate the analgesic effect of these enkephalin-based nanoparticles (NPs) for the management of intense pain, we now intend to develop a multidrug approach based on the combination of Leu-enkephalin (LENK) with the opiorphin (OPN), an enkephalinase inhibitor, or STR324, its stable analog. Indeed, these endogenous peptides have already shown a significant pain-relieving effect, with no morphine-like side effects, by inhibiting the degradation of enkephalins (3). It is expected that the nanoformulations combining LENK-SQ and OPN-SQ (or STR-SQ) will enable the simultaneous release of LENK and OPN (or STR) from NPs in the peripheral injured tissues thus allowing an local increase of enkephalin concentrations. To provide a proof of concept of a possible synergistic effect, a library of different enkephalinase inhibitor bioconjugates using squalene has been developed. SQ-STR nanoformulations have been evaluated in vivo and exhibited a significant analgesic effect but with a high mortality rate. Further investigations are in progress in order to understand whether it is a dose-dependent toxicity or a problem with the formulation per se. SQ-OPN NPs, on the other hand, look promising, as preliminary tests have shown no mortality.

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Keywords: : Pain control, enkephalin, enkephalinase inhibitors, squalene based nanoparticles

A41. In vitro assessment of the photoprotective efficacy of a linseed oil-based nanoemulsion loaded with avobenzone and tris-biphenyl triazine UV filters

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Nanoemulsions offer a promising approach in the development of sunscreens due to their stability over time, capacity to carry both hydrophilic and lipophilic ultraviolet (UV) filters, and uniform application on the skin. Moreover, natural oils, such as linseed oil, serve as alternatives to mineral oils in these products, potentially adding multiple beneficial properties to the for- mulations. The objective of this study was to evaluate the in vitro sun protection effectiveness of a nanoemulsion containing linseed oil and loaded with avobenzone and tris-biphenyl triazine (TBPT) as UV filters, referred to as NEUVMIX. The formulations consisted of 10% linseed oil for NEUVMIX and 10% Miglyol® 812N for a control version (NEMIGUV). Surfactants (Kolliphor® RH 40 and Span® 80) were added to attain the necessary Hydrophilic-Lipophilic Balance for each oil, along with 1% vitamin E, 0.2% potassium sorbate, 0.55% xanthan gum, 1% avobenzone, 8% TBPT, and 69.25% purified water. These formulations were prepared at 50 ºC using a phase inversion method with magnetic stirring (950 rpm) and were characterized based on their hydrodynamic diameter, polydispersity index (PdI), and zeta potential. The results showed that NEUVMIX had a hydrodynamic diameter of 132.5 ± 8.3 nm, a PdI of 0.23 ± 0.03, and a zeta potential of -23.33 ± 0.7 mV, while NEMIGUV exhibited a hydrodynamic diameter of 123.0 ± 1.9 nm, a PdI of 0.21 ± 0.01, and a zeta potential of -25.09 ± 0.4 mV. In terms of Sun Protection Factor (SPF), both NEUVMIX and NEMIGUV showed similar results, with SPF values of 21.7 ± 6.7 and 21.7 ± 3.3, respectively, indicating effective protection against UVB radiation for all skin phototypes, since formulations with SPF higher than 15 can protect against 93 % of UVB radiation. However, the presence of linseed oil did not contribute to SPF enhancement of the formulation. For UVA protection, NEUVMIX demonstrated a UVA/UVB ratio of 0.88 ± 0.01 and a critical wavelength (λc) of 381.67 ± 0.43 nm, while NEMIGUV ex- hibited values of 0.78 ± 0.01 and λc of 378.33 ± 0.63 nm. According to the Boot's Star Rating system, NEUVMIX achieved a UVA protection rating of four stars, categorized as "Super," whereas NEMIGUV attained three stars, categorized as "Good." Although linseed oil did not significantly affect SPF, these results suggest its potential to enhance UVA protection. In con- clusion, this study demonstrated that linseed oil enhances the UVA photoprotective efficacy of nanoemulsion formulations compared to inert oil. This makes the produced nanoemulsion a valuable option for new sunscreen products.

Keywords: : Photoprotection, UV filters, nanoemulsions, linseed oil

A42. Synthesis of Gold Nanorods for targeted phototherapy of cancer cells

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Although much progress has been made in radiotherapy, chemotherapy, immunotherapy and surgery, cancer remains a worldwide public health issue. A solution of interest is minimally invasive thermal ablation, where tumor temperature is locally increased in order to induce tumor cell injuries such as apoptosis and coagulative necrosis. Photothermal therapy can amplify the effectivness of this therapeutic procedure, especially by using wavelengths between 800 and 1200 nm, in the so-called ”biological window” where vascularized tissue have decreased absorption coefficient (1). Yet, clinical practice is today limited by the insufficient procedure selectivity. Plasmonic nanostructures can improve the procedure by enhancing light-absorbance capability on the specific area where they are positioned, the tumor. Among the plasmonic nanostructures, gold nanorods (GNRs) are of particular interest due to their tunable aspect ratios and resulting localized surface plasmon resonance (LSPR) (2). This project aims at developing an antitumoral treatment method combining targeting of cancer cells by GNRs labelled with antibodies and improved phototherapy.
GNRs are synthetized and functionalized according to the biomarkers of interest. A first linker grafting step is carried out, leading to stable suspensions. The next step of covalent conjugation of the recognition biomolecules is carried out through different strategies: peptidic bonding and click chemistry. Different antibodies or proteins from tumor necrosis factor (TNF) family are used for cancer cells targeting. Various characterizations highlight the grafting of additional lay- ers and maintained stability at each step of the synthesis (DLS, UV-visible spectroscopy, XPS and TEM). Then, preliminary tests were carried out by applying GNRs and thermal treatments to different carcinoma cell lines (MDA-MB-231, HCT116...) to evaluate their antitumoral po- tential. Results obtained are very encouraging on the efficiency of combined treatments in vitro. Work is in progress to confirm these results in vivo and to extend the portfolio to other relevant antibodies.

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Keywords: : gold nanorods, immunotherapy, photothermal therapy, cancer

A43. Combination therapy of terbinafine nanocrystals-loaded thermosensitive gels and fractional ablative laser to improve ungual drug delivery

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Limited drug permeation and poor diffusion are major factors responsible for the failure of topical treatments. The low permeability of the nail plate combined with poor drug solubility of drugs leads to insufficient drug delivery to this tissue.

The goal of the present project is to combine formulation and delivery strategies to provide local drug depots for sustained topical drug delivery to treat nail disorders. Previous studies showed that fractional laser ablation could enable drug delivery in the nail by removing the nail plate's barrier. A sustained-release formulation with high drug-loading that could fill the pores created by the laser and release the drug for an extended period of time would reduce the frequency of applications, thus improving compliance and treatment outcome. The first part of this work focused on developing a formulation for the sustained delivery to the nail of the antifungal agent terbinafine (TBF).

A thermoresponsive poloxamer 407 (P407) gel was formulated. The rheological and thermo-gelation properties of the gels were determined. A thermosensitive formulation could easily fill and gelify in the micropores at the temperature of the nail (32◦C) to increase the residence time and decrease treatment frequency. In parallel, a size reduction of TBF raw material was performed by wet milling to obtain nanocrystals to be incorporated into the gel. Previous work showed that a high drug loading and an extended release could be achieved by using nanocrystals. The amount of drug in the pores created by laser ablation could be increased by using nanosized drug particles, and the active surface area in contact with the nail would also be increased. A TBF nanocrystal-loaded P407 gel containing 10 % (w/w) of TBF nanocrystals with a state transition between 25◦C and 32◦C was successfully formulated with 16 to 17% (w/w) of P407.

Porcine hooves were selected as an ex vivo model for ungual drug delivery. Hoof pieces were microporated using a CO2 fractional ablative laser at different fluences. Micropores dimensions were assessed by Scanning Electron Microscopy and X-ray Computed Tomography. Preliminary results showed that micropores depth increased with fluence, up to 1200 µm with a fluence of 150 mJ, whereas diameter remained fixed at 200 µm.

The perspectives are to assess drug distribution from the formulations in microporated hooves with optimized laser settings.

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Keywords: : Topical drug delivery, sustained release formulation, nanocrystals, fractional laser ablation, nail

A44. Liposomal co-loading of two hydrophobic drugs for cancer treatment: overview, workflow and preliminary data

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Liposomal formulation drug delivery has evolved significantly over the years, with the aim of enhancing therapeutic efficacy while minimizing adverse effects. One promising approach involves co-loading multiple anticancer drugs within the same delivery system and offers the potential for a ratio-dependent synergistic effect.
We have previously optimized multidrug combinations, based on drug-drug interaction, with a high efficacy and lack of toxicity (1). The optimization was performed in human renal cell carcinoma models and showed activity also in melanoma and colon carcinoma. Within this optimized drug cocktail, 2 drugs, i.e. tubacin, and Erlotinib.HCl exhibit a high degree of synergism. However, the two APIs have disparate physico-chemical properties. Hence, achieving and maintaining optimal drug retention levels within liposomal formulations is a critical challenge. Through ethanol injection and remote loading processes, we explored the effects of cholesterol content and differences in internal pH on drug retention.

The result of our experiment revealed a clear trend: as the cholesterol content increased, there was a corresponding rise in Erlotinib.HCl encapsulation. As for internal pH, the same trend was observed towards acidic pH. Furthermore, our experimental findings demonstrate that the degree of encapsulation remains consistent whether drugs are loaded individually or co-loaded within liposomes. These results indicate that there is no discernible impact of one drug on the encapsulation of the other.

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Spindle Pole Clustering. Cancers (Internet) 2019;11(10):1612.

Keywords: : liposome, co, loading, cancer treatment

A45. Enhancing Wound Healing with Apple Plant Stem Cell-Loaded PAA/Alginate Nano- Hydrogel

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This study aimed to investigate the cultivation and extraction of apple plant stem cells and their potential application in wound healing using a Polyacrylic acid (PAA)/Alginate (Al) nano-hydrogel. Incorporating apple stem cells into the PAA/Al scaffold was found to enhance its regenerative properties and improve wound healing efficiency. The hydrogel was subjected to various morphological, physical, and cellular assessment tests.

The nano-hydrogel, with a porous structure and a size of 65 nanometers, closely resembles the extracellular matrix. The composite scaffold of PAA-alginate/apple stem cells offers numerous advantages for tissue engineering applications. The study also investigated the impact of the hydrogel on skin fibroblast cells and observed that the presence of flavonoid and polyphenol groups in the hydrogel had positive effects on antioxidant, anti-inflammatory, and regenerative properties. The uniform structure of the hydrogel, its increased transparency, and its hydrophilic nature create an optimal environment for wound healing. These characteristics facilitate the diffusion of oxygen and nutrients to the wound site while maintaining a moist environment, promoting effective wound healing.

Additionally, the nano-hydrogel demonstrated efficient absorption of the apple stem cell extract, enabling the delivery of beneficial properties such as antioxidants and anti-inflammatory effects to the wound site. These properties contribute to tissue regeneration and reduce inflammation, both crucial for successful wound healing. The addition of apple stem cell extract increased the surface tension of the hydrogel, making it relatively hydrophilic. Importantly, the study confirmed the non-toxicity and good biocompatibility of the hydrogel, making it a promising candidate for wound healing applications. Furthermore, the study highlighted the potential of apple plant stem cells in promoting cell regeneration and repair, thereby improving the overall wound-healing process.

In conclusion, the findings of this study suggest that the PAA/Al nano-hydrogel containing apple plant stem cells has the potential to be an effective and safe treatment for wound healing. Further research and development in this field could lead to the practical application of this hydrogel in clinical settings.

Keywords: : wound healing, plant stem cell

A46. Polyoxazolines with various lipid anchors for effective intracellular delivery

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Polymeric micelles have been extensively used as nanocarrier for cellular delivery of active molecules such as for cancer therapy. Indeed, they are able to enhance drug retention in tissues, cellular uptake and protection from enzymatic degradation (1). Among the polymers used, poly(ethylene glycol) (PEG) is the most well-known hydrophilic polymer for micellar drug delivery. However, its systematic use has led to serious clinical concerns such as anti-PEG antibodies, bioaccumulation in some organs and hypersensitivity (2). In addition, PEGylation can lead to reduced cellular internalization and endosomal escape of polymeric micelles due to the steric hindrance of PEG corona, known as PEG dilemma (3). Therefore, there is an urgent need to find alternatives to PEG overuse and among the potential candidates, poly(oxazolines) (POx) have sparked great interest (4). Indeed, POx gathers interesting biological properties such as cyto- and hemo-compatibility, degradability, immunomodulation, and stealth-behavior (5).
To work with amphiphilic POx, the hydrophilic 2-methyl-2-oxazoline was combined with a hydrophobic lipid chain to obtain a lipopolyoxazolines (LipoPOx) (6). LipoPOx were synthesized by cationic ring opening polymerization (CROP) with various possible nature of lipid anchors such as linear (saturated C16 and unsaturated C18:2), grafted (C18(SC12)2 and C18(SC8)2)
(7) or cholesterol group (8). The nature of theses linear and grafted lipid anchors was shown to have no impact on the resulting physicochemical properties but strongly on the interactions with cells looking at cell viability and internalization. Indeed, the lipid anchor interact and insert in lipid membrane such as the one of giant unilamellar vesicles (GUV), cell membranes, and skin with different kinetics upon lipid nature (9).
Regarding the cholesterol anchor, the LipoPOx was able to spontaneously form vesicles then able to rapidly deliver the loading molecule in cells within 15 minutes thanks to membrane fusion. Therefore, thanks to their lipid anchor the LipoPOx hold great promises for effective intracellular delivery of active molecules.

Keywords: : Micellar delivery, PEG alternatives, lipopolyoxazolines

A47. A study on chitosan-coated liposomes as potential delivery systems

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Encapsulation of drug into liposomes may reduce a number of drawbacks, such as rapid clearance, poor biodistribution, low intracellular absorption, and toxicity that may limit their therapeutic efficacy.(1) Decoration of liposomes with chitosan is one of the methods for improving their physical-chemical and biochemical characteristics.(2) Chitosan's coating on liposomes is primarily based on electrostatic forces.(3) Previously we established that multifunctional pyridinium derivatives on the 1,4-dihydropyridine (1,4-DHP) core formed liposomes and were found to be active as gene delivery agents (4). Additionally, the effects of lipid head-groups, linker structure, and cationic moieties' shift from 1,4-DHP core on transfection activity were also studied (5).

In this study, liposome compositions were formed from 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,4-DHP amphiphile at various ratios of the components (19:1; 10:1; 2:1). Chi- tosan derivatives for coating of nanoparticles were synthesized employing different surface mod- ifications, such as formation of carboxymethyl (6) or N,N,N - trimethylated chitosans (7). The obtained chitosan derivatives were applied for the coating of liposomes formed by DOPE and
1,4-DHP.

Liposomes at various lipids ratios and chitosan-to-lipid weight ratios (1% - 50%) were stud- ied by DLS and ITC methods. It was assessed how the change in composition affected the stability and size distribution of the formed nanoaggregates. According to ITC, chitosan can be adsorbed on liposomes as much as 1:5 lipid-to-chitosan weight ratio. The increase of the ratio of chitosan-to-total lipids (1:1 w/w) led to the rise of the particle average size compared to the uncoated ones; stability studies were also carried out.

Acknowledgements
Funded by the EuroNanoMed3 Project TENTACLES.

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Keywords: : liposome, amphiphile, modified chitosan, coating, delivery

A48. Formulation of polymer sonosensitive agents for ultrasound brain therapy

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The blood-brain barrier (BBB), poses a formidable challenge for delivering therapeutics exceeding 450 Daltons in size, (1) limiting their clinical utility in the treatment of brain patholo- gies. A promising way to overcome this barrier has emerged through the use of microbubbles in conjunction with focused ultrasound, enabling localized, non-invasive and reversible permeabilization of the BBB to facilitate drug delivery into the brain.(2) However, commercially available ultrasound contrast agents and agents designed for therapeutic purposes suffer from a short in vivo half-life (only few minutes).(3) This project aims to formulate new polymer microbubbles optimized for BBB opening which will be stable, sonosensitive and non-toxic for prolonged treatment.
Successfully, three degradable polymers were synthesized. The structure and synthesis of these polymers are not presented due to patenting. Their physicochemical properties at the air/water interface were assessed at different concentrations using a tensiometer. All three polymers stabilized the interface. Polymeric microbubbles were then formulated with two perfluorocarbon gases (C3F8 and C4F10) at a polymer concentration of 1 mg/mL. Microbubbles were observed by optical microscopy to determine their size and concentration. Microbubbles formulated with C4F10 gas were more stable than with C3F8 gas. The acoustic signature was characterized by exciting microbubbles at f0=1 MHz for a range of peak negative pressure between 0 and 400 kPa using an unfocused transducer and recording their signal with a 2.25 MHz piezoelectric transducer. All the formulations respond to ultrasound and the appearance of ultra-harmonic components (destabilization) occurs at a lower pressure than SonoVue, a commercial agent clinically approved. Future work involves in vivo validation of these novel sonosensitive polymer microbubbles.

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Keywords: : Therapeutic ultrasound, Sonosensitive agents, Polymer

A49. Design and development of innovative formulations by nanoencapsulation of antidotes, against intoxications by chemical warfare agent

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Organophosphorus compounds (OPs) such as sarin, Novichok or VX are notorious for being extremely hazardous and lethal chemical warfare agents. Used as pesticides, OPs are also responsible for the acute poisoning of millions of people. All these OPs have a common mechanism of action. They act by inhibiting acetylcholinesterase in the central nervous system (CNS) (1). Given the limitations of current antidotes, new molecules have been developed by Dr Rachid BAATI and his team (2). Among these synthetic compounds, the patented molecule JDS364
(2) stood out in in vitro tests. However, solubility problems in the blood prevent the molecule from reaching the CNS.
Our work consisted first in synthetizing JDS364 at the gram scale through a 9-step synthesis. Several options were then considered to improve the solubility and stability of JDS364, including the use of co-solvents, surfactants, di- and triglycerides and cyclodextrins. Among them, the use of polyethylene glycol 400 was effective. However, the best stability was obtained by forming a complex between JDS364 and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). No crystallization or degradation of the formulated antidote was observed after a month of storage at room temperature. Furthermore, a 1:1 stoichiometry of the complex was confirmed with Job's plot method and a 2D ROESY spectrum was used to show the interaction between the molecules.
These results are promising but further studies are still required, particularly with regard to pharmacokinetics, biodistribution and efficacy in a poisoned murine model. If the formulated antidotes prove to be effective, they could be industrialized on a semi-industrial scale at the Pharmacie Centrale des Arm´ees (Chanteau, France).

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Keywords: : Organophosphorus compounds, Antidote, Acetylcholinesterase, Reactivator, JDS364, Inclusion complex

A50. Nanoformulation of a promising molecule targeting an innovative bacterial target to overcome antibioresistance

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According to the WHO report, antibiotic resistance has emerged as one of the most signif- icant risks to global public health, leading to higher healthcare costs and increased mortality.1 This antibiotic resistance crisis calls for an urgent need to identify innovative molecules and novel modes of action for the treatment of infectious diseases. Recently, the PIMs team of the MICALIS Institute (INRAE) has recently discovered a promising target (Mfd - Mutation Frequency Decline Protein), implicated in the bacteria resistance to the human immune system response, together with a "first-in-class " molecule specifically targeting the active site of the bacterial Mfd protein (called NM102).2,3

Despite its high potential, NM102 poor water solubility and restricted solubility in organic solvents (e.g., DMSO) provide a significant obstacle to its clinical application. To address this issue, we designed a DMSO-free drug-loaded nanoformulation that enables the delivery of this new compound. The sonication assisted-nanoprecipitation method was used to formulate the nanoparticles with the NM102 molecule.4 The resulting drug nanoformulation exhibited an intensity-averaged diameter Dz = 180 nm, with narrow particle size distribution (polydispersity index = 0.2), a neutral surface charge (ζ potential = -2 mV) and a drug content of 0.6 mg.mL-1. In-vivo results provided the first proof of evidence for NM102 efficacy in combating antimicrobial resistance.

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Keywords: : nanoformulation, antibiotic resistance, nanoparticles, PLGA

A51. Anti-fibrotic potential of liposomes encapsulating inhibitors of the C-terminal domain of HSP90

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Pulmonary fibrosis is a fatal chronic disease, and with only two treatments of moderate efficacy on the market, a large therapeutic space is still to be covered. HSP90 is a protein overexpressed in idiopathic pulmonary fibrosis, involved in synthesis of extracellular matrix, cells migration and proliferation. Its inhibition has been shown to decrease the progression of pulmonary fibro- sis and to attenuate differentiation of fibroblasts into myofibroblasts (1).

This work aims to address the need for new therapies by encapsulating in liposomes an inhibitor of the C-terminal domain of TRAP1, a mitochondrial isoform of HSP90. TRAP1 participates in mitochondrial metabolism and cell proliferation (2). To allow a better targeting of the myofibroblasts, these liposomes are decorated with hyaluronic acid, a natural ligand of the CD44 receptor. Our goal is to decrease myofibroblasts proliferation and extracellular matrix production, combined with a metabolic reprogramming of the cells. To support this work, a solid cellular model is needed. Therefore, part of this project consists in the establishment of a co-culture model combining myofibroblasts, with two other actors of pulmonary fibrosis, M2 macrophages and alveolar epithelial cells.

Up to now, we have set up a cellular model of differentiated myofibroblasts, by treating IMR90 cells with TGF-β1 for 48 to 72 hours. Following the same protocol, an alveolar epithelial model undergoing epithelial mesenchymal transition has also been established with the A549 cell line. Stable formulations decorated or not have been produced and evaluated on differentiated IMR90, showing a decrease of mitochondrial activity and a slowdown of cells proliferation.

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Keywords: : Liposomes, Pulmonary Fibrosis, TRAP1

A52. Enhancing chemotherapy of triple negative breast cancer: TROP-2 targeting immunoliposomes to co-deliver metformin and doxorubicin

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Triple negative breast cancer (TNBC) is commonly treated using chemotherapy, as hormonal and targeted therapies are highly limited. However, in many cases, TNBC develops high drug resistance leading to poor prognosis. Thus, there is a dire need of new treatment approaches, such introducing an adjuvant to chemotherapy. Metabolic modulator metformin hydrochloride (Met) has recently been associated with reduced cancer risk prompting in an increase of clinical trials of using it as an adjuvant to conventional cancer therapies (De, A., Current Problems in Cancer, 2020). Met can reduce adenose triphosphate (ATP) production, oxygen, and glucose consumption by inhibiting mitochondrial complex I, and activating AMP-activated kinase (AMPK) leading to the suppression of tumour growth regulators (Saini, N., Acta Biochim Biophys Sin, 2018). As a result, Met reduces drug resistance for chemotherapeutics, such as doxorubicin (Dox), reduces cell proliferation, and improves immune system response. Moreover, targeted therapy of trophoblast cell-surface antigen 2 (TROP-2), which recently was shown to be a promising TNBC marker, could further improve the effects of chemotherapy (Aslan, M., NPJ Breast Cancer, 2021). Thus, the aim of this project is to achieve the co-delivery of Dox and Met using immunoliposomes decorated with anti-TROP 2 single-chain variable fragments (scFvs).

Formulation of Met-Dox-liposomes was done using thin film-hydration method, encapsulating hydrophobic Dox in the lipid bilayer (by prior desalting Dox), and highly hydrophilic Met in the aqueous compartment of liposomes. The formulation resulted in liposomes of 150 nm, with low polydispersity index (< 0.1), and slightly negative surface charge (- 18 mV). The drug loading of Met and Dox was 150 mg/g and up to 550 µg/g, respectively. Imaging inside TNBC MDA-MB-468 cells showed co-localisation of liposomes inside lysosomes, indicating favourable physical properties for endocytosis based cellular uptake. Met is able to reduce cell prolifera- tion, while disintegrated cell nucleus indicated successful Dox effect to induce cell apoptosis. For combined treatment assessment different Met and Dox ratios inside liposomes are being tested to find the optimal conditions for enhanced TNBC treatment. Moreover, immunoliposomes were formulated by performing scFv conjugation through PEG2000- maleimide-cysteine binding (Michael addition). Surface functionalisation did not affect scFv functionality, nor the physical properties of liposomes, indicating the advantage of using scFv instead of a whole antibody. The link between maleimide and cysteine remained stable when exposed to foetal calf serum after 24 h. The assessment on MDA-MB-468 by flow cytometry with the optimised immunoliposomes showed a 5-fold increase in cellular uptake compared to unconjugated liposomes.

The formulated liposomes show a promising potential to enhance the chemotherapy based treatment of TNBC. The next steps in this study are to optimise Met and Dox ratio inside liposomes, and combine it with TROP-2 conjugation for 2D and 3D assessment on TNBC cells.

Keywords: : triple negative breast cancer, TROP 2, scFv, doxorubicin, metformin, immunoliposomes

SESSION B – Installation Tuesday 7th at 13:45 pm

Removal Wednesday 8th at 16:00 pm

Inorganic and hybrid Nanomaterials

B1. New methodologies for Taylor Dispersion Analysis – Theory, Proof of Concept and Application to gold nanoparticle – protein interaction

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Since proteins can act as ligand targeting a specific receptor, and nanoparticles (NP) can be used as probe, the knowledge of the interactions between both agents is important for the design of an innovative sensor (1). The change in hydrodynamic radius (rH) induced by the formation of the nanoprobe can be followed using several technics, such as dynamic light scattering or Taylor dispersion analysis (TDA). TDA is a technique dedicated to the determination of the molecular diffusion coefficient (D) of species using band broadening of an analyte in a laminar flow. The advantage of TDA towards other methodologies is the possibility to measure molecules with lower sized (down to 0.2 nm), with the possibility to analyze multimodal samples. Two modes are commonly used: Pulse and Frontal modes. In each case, a fitting of the signal (denoted as Taylorgram) is required and limitations are occurring (2).

To extend the use of TDA beyond the classical requirements, we propose here two innovative approaches:

- Firstly, a third mode denoted as *Cross-frontal mode* (3) is proposed, combining two crossed sample fronts without modification of a classical capillary electrophoresis device for the rapid and accurate determination of D of caffeine, reduced glutathione, insulin from bovine pancreas, bovine serum albumin and citrate-capped gold NP (AuNP).
- Secondly, a *new mathematical description* of the Taylorgram obtained using Pulse mode is proposed, allowing for increased injection volume (up to 15 % of the volume of the capillary inlet, against 1%), which allows for higher sensitivity and a better signal fitting.

Theoretical aspects and methodology of classical and new TDA methodologies will be described, showing a good correlation between experimental results and measurements from DLS and TaylorSizer® device. Finally, the use of this new approach allows for the study of the interaction between AuNP and Concanavalin A (ConA), a lectin known for its interaction with sugar moieties like D-Mannose (4). Using TDA, formation of an AuNP-ConA nanoprobe is demonstrated with a D changing from 5.5 to 2.0 .10-11 m2.s-1 between the native protein and the nanoprobe. These methodologies are promising for metallic NP-proteins interaction characterization and study, while extending the potential use of TDA as a conventional and reliable method for NP size determination.

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Keywords: Taylor Dispersion Analysis, Nanoparticle, methodology

B2. Fusogenic liposomes as nanocarriers for the intracellular delivery of magnetic nanoparticles

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Magnetic nanoparticles (MNPs), as any other type of nanoparticles, are internalized by cells through endocytosis, and thus are trapped in intracellular vesicles called endosomes (1). But for a number of bio-applications, such as cellular engineering or magnetic hyperthermia treatments, it can be of great interest to have MNPs able to reach the cell cytoplasm. This would allow to have less dipolar interactions between the MNPs, and hence increase their intracellular heating properties (2). We have recently demonstrated that the functionalization of core-shell magnetic nanoparticles with cationic peptides poly-His promotes their access to the cytosol (3). Here, we propose a novel strategy based on the use of magnetic fusogenic liposomes. Fusogenic liposomes are able to fuse with plasma membrane and as a consequence to deliver their content into the cytosol. We propose to study the fusion of liposomes on a pure electrostatic model al- ready described in the literature (4). Cationic Large Unilamellar Vesicles (LUV, lipid molar ratio DOTAP/DOPE/PEGPE/Topfluor, 1/1/0.1/0.1, 200 nm in diameter) were prepared by extrusion as well as anionic LUV (POPC/POPG, 50:50). Anionic LUV will play the role of acceptor liposomes that mimic the cell membrane. The evolution of the diameter and the zeta potential of the acceptor system is analyzed by DLS, the heat release during the fusion is measured by isothermal titration calorimetry (ITC). This allows the determination of two key parameters that trigger the fusion i.e the best lipid composition and charge ratio (anionic/cationic). Magnetic fusogenic liposomes were then prepared by reverse phase evaporation, the protocol has been adapted from a previous study to avoid the precipitation of anionic MNP in contact with cationic lipids (DOTAP)(5).Their fusion with cell model will be presented.

Keywords: liposomes, magnetic nanoparticles, fusion, hyperthermia

B3. Biocompatible polymer conjugates bearing octahedral molybdenum clusters: potential nanomaterials for photodynamic therapy

Robert Pola*[1], Michal Pechar[1], Marina Tavares[1], Kaplan Kirakci[1], Tomas Pribyl[1], Jaroslav Zelenka[1], Tomàš Ruml[1], Kamil Lang[1], Tomàš Etrych[1]

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Octahedral molybdenum clusters (Mo6) have shown promise as photo/radiosensitizers for photodynamic therapy applications (1,2). Mo6 have red luminescence, which is quenched by oxygen to produce singlet oxygen in high quantum yields. However, most of these compounds reported so far showed a limited solubility in water and underwent hydrolysis connected with aggregation, sedimentation, and diminishing luminescence and singlet oxygen-sensitizing properties. Also, their direct administration and delivery to the desired target can be limited by their poor solubility and low stability in physiological conditions, as well as unsuitable biodistribution leading to little or no therapeutic effect and so increasing the side effects of the therapy. The solution could be coating the Mo6 clusters with polymer carrier to overcome these problems and improve their bioavailability. Here, we have conjugated biocompatible hydrophilic *N* -(2- hydroxypropyl)methacrylamide (HPMA) copolymers with a Mo6 bearing azido ligands via a copper-free click reaction. Prepared Mo6 polymer conjugates exhibited long-term colloidal stability in phosphate-buffered saline. Luminescence properties and the quantum yields of singlet oxygen of these nanoparticles remained unchanged. The strong phototoxic effect was observed by illumination of HeLa cells after 24 h of incubation with polymers bearing Mo6. Attaching Mo6 to the HPMA-based copolymer slightly increased its phototoxicity but it significantly lowered its dark toxicity in comparison with Mo6 alone which is phototoxic even in dark. The polymer conjugates can suppress the side-effects of low molecular Mo6 and can be used as a suitable nanocarriers for molybdenum cluster-based photosensitizers intended to use for photodynamic therapy.

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Keywords: polymer carrier, photodynamic therapy, octahedral molybdenum clusters

B4. Size Distribution by Taylor Dispersion Analysis (SD-TDA): An innovation for resolutive size distribution measurement of lipid nanoparticles and monoclonal antibodies

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The assessment of the absolute size of mixture of nano-objects is a challenge in biophysical characterization. There are a limited number of affordable technologies that provide accurate particles size distribution down to 0.5nm. Current technics such as DLS, A4F/SEC-MALS, NTA are often not suitable to work in native form. Indeed, denaturation occur during sample preparation or analysis: filtration, dilution or shear stress.
Nanoscale Metrix developed a new methodology to characterize the size distribution of nanoobjects, the Size Distribution - Taylor Dispersion Analysis. With SD-TDA, only 10 nL of sample are mobilized under low pressure differential in a capillary. Under Taylor dispersion conditions, the hydrodynamic radius can be calculated from the detected signal (U.V, L.I.F) through Stokes- Einstein equation.

In this work, SD-TDA was first used as a new alternative method to determine the size and size distribution of nanovectors: LNPs encapsulating mRNA, coated metallic nanoparticles.

The size of LNP is known to affect intracellular delivery and therefore vaccine efficacy and SD-TDA allow a very accurate size measurement. Thus, several LNP formulations were success- fully analyzed and obtained results were compared to those obtained with DLS or FFF-MALS. Second part of this work was dedicated to the thermal stress of human IgG. At high temperature, a kinetic study of the IgG degradation was carried out. A modification in the IgG size is measured and degradation products were also detected. The size of obtained IgG fragments is compared to the size of purified fragments directly characterized by SD-TDA (Fc, Fab, and F(ab')2 fragments).

Keywords: LNP, Size characterization, Size Distribution, Taylor Dispersion Analysis, Antibody, IgG

B5. Functionalized 2 nm gold nanoparticles for motion in living cell and in situ immunolabeling at cryo-Electron Microscopy resolution

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To identify molecules of interest in situ without sample denaturation, we aimed at revisiting the gold immunolabeling methodology. Toward this goal, 2 nm gold nanoparticles (AuNPs) are synthesized and functionalized with bioactive molecules to allow their delivery into living human cells by electroporation and to facilitate motion and binding to targets inside living cells. As a proof of concept, AuNPs of optimized composition are functionalized on their surfaces with 2 kDa polyethyleneglycols (PEG) and SV40 Nuclear Localization Signals. Our data demonstrate that PEG is necessary to shield the particles from unspecific interaction with cytoplasmic components and to permit the AuNPs to enter into the nucleus via the NLS-mediated import. Moreover, we will present data showing that 2 nm AuNPs are clearly depicted within frozen hydrated sections containing the cell nucleus. We are currently synthesizing AuNP-nanobody conjugates and will present first data on their behavior inside living cells Altogether, we demonstrated for the first time that this identified 2 nm size range and surface coating functionalization offer a good compromise between the electron contrast using cryo-EM and the free diffusion of probes within living cells. The gold surface functionalization with mixed bioactive thiolates opens up opportunities to generate specific gold immunolabelling tools and decipher the distribution of molecular components that compose the cell.

Keywords: gold nanoparticle, cryo, Electron microscopy, intracellular delivery, antibody, gold immunolabeling

B6. Assessing the particle concentration of mRNA-LNP using Videodrop :A new metric for LNP manufacturing, optimization & standardization

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LNP (Lipid Nanoparticles) are a leading class of RNA delivery systems, used in the field of vaccines and gene therapy. They are typically composed of a lipid formulation that encapsulates the desired nucleic acid (RNA). A common method for LNP production involves mixing the lipid composition (in the organic phase) with the nucleic acid (in the aqueous phase) using a microfluidic device.
The analytical characterization of these nanoparticles is crucial for ensuring product quality and understanding their *in vitro* and *in vivo* activities. The analytical strategies employed for LNPs include the characterization of the RNA (as the active pharmaceutical ingredient), the lipid composition, and the (mRNA-LNP) nanoparticles.

The current analytical strategies employed for mRNA-LNP characterization lie primarily on size, polydispersity, and zeta-potential. However, particle concentration, evaluated through a single particle measure- ment technique, is an important parameter to monitor for several reasons:

- Quality control tests
- Stability assessment
- Standardization for comparative studies

When developing new formulations, manufacturing processes, or different storage conditions, quantifying the number of particles enables efficient comparison studies. By comparing particle numbers under different conditions, researchers can assess the impact of the process or composition on transfection activity.
Here, we propose to use Videodrop for the analysis on mRNA-LNP and highlight the interest of measuring the particle concentration of mRNA-LNP.

Keywords: LNP, particle concentration, interferometry, lipid nanoparticle, particle counter

B7. Cell penetrating foldamers as nanovectors for synergic drug delivery and gene silencing

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Small interfering RNAs (siRNAs) are promising molecules for developing new therapies based on gene silencing. However, their delivery into cells remains an issue. This weakness has led several groups to develop pseudopeptide and peptidomimetic sequences, including foldamers. In this study, we took advantage of foldamers, which provide not only proteolytic resistance, but also increased cell-permeability(1). For this purpose, we designed *α*-amino *γ*-lactam foldamers which adopt a stable ribbon structure. These foldamers are non-toxic vectors that were able to efficiently encapsulate siRNA, transport them into the cell and induce gene silencing. The grafted foldamers with a pro-apoptotic peptide (KLAKLAK)2 are able to induce a synergic effect : gene silencing and drug delivery.

Keywords: Foldamers, siRNA, nanovectors, drug delivery, gene silencing.

B8. Lipid-based nanoparticles as game-changers in mRNA-mediated B Cell engineering

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Delivery of mRNA to immune cells has emerged as a promising strategy for engineering and harnessing the therapeutic power of immune cells, especially T, natural killer and B cells. However, current methods for gene delivery, such as viral vectors or electroporation, have limitations in terms of safety, efficiency and scalability. Lipid-based nanoparticles have emerged as an interesting alternative, offering a versatile platform for efficient and targeted mRNA de- livery to these cells. Nevertheless, there is still a necessity for improving the performance and to understand the underlying mechanisms of efficacy. Currently, there is a growing industrial and clinical interest in B cell engineering and subsequent adoptive B cell transfer, which already displayed good preclinical data. Effective, simple & scalable solutions to engineer B cells still remain to be uncovered and are a significant research priority.

Thus, we aim to develop and optimize lipid-based nanoparticles, lipid nanoparticles (LNPs) & lipoplexes (LXs), for improved delivery of mRNA to B lymphocytes, to uncover the efficacy drivers, and achieve therapeutic potency through CRSIPR-Cas engineering of B cells. To achieve this, we designed and synthesized a series of lipid-based nanoparticles. After physico-chemical characterization, we evaluated their efficiency for eGFP mRNA delivery into B cells and plasma cells. Impressively, our LNPs achieved 100% transfection efficiency in B cell lines and primary B cells. To improve overall performance of our LNPs, their optimization was preformed through Design of experiment - Response Surface methodology by fine-tuning production parameters: Flow Rate Ratio (FRR), Total Flow Rate (TFR), and lipid concentration on the Nanoassemblr® Ignite microfluidic device. These parameters significantly affect LNP properties and transfection potency in BL41 lymphoma cells. The study demonstrated the robustness of our LNP formulation, enabling enhanced B cell transfection through fine production adjustments. Ultimately, we focused on the use of CRISPR-Cas system for B cell engineering with our model featuring GFP-expressing BL41 cells. Cas9 mRNA and sgRNA were loaded into LNPs used in transfection, eventually resulting in more than 70% of GFP knocked-down B cells. Ongoing investigations comprise a comparison of our technology with electroporation, essential to highlight its strengths, and embracing therapeutically relevant CRISPR-Cas engineering, as it stands as a key pathway to fully harness the potential of B cells.

Overall, our developed LNPs offer a scalable platform for highly efficient transfection of B cells, enabling the versatile utilization of the CRISPR-Cas system for cellular engineering.

Keywords: mRNA, Lipid nanoparticles, CRISPRCas, B cells.

B9. Treating myocardial infarction using pH-sensitive nanoparticles for FADD silencing

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Cardiovascular diseases are the number one cause of death representing 31% of all deaths worldwide. Infarct size is a major determinant of myocardial functional recovery and mortality after an acute myocardial infarction (AMI) (Burns RJ et al., 2002). Limitation of infarct size is required to prevent post-ischemic heart failure (HF). The recommended intervention for AMI patients is reperfusion; however, this treatment can induce detrimental side effects, called lethal reperfusion injury culminating in apoptotic cell death within the cardiac tissue.
In this context, the crucial role of the extrinsic apoptotic pathway mediated by the FAS receptor and its associated protein FADD was demonstrated causing detrimental apoptosis when activated (Roubille F. et al., 2007). Our research hypothesis is to propose an innovative therapeutic strategy for AMI patients with a focus on the FADD pathway using specific siRNA encapsulated in peptide-based nanoparticles (PBNs).

For this purpose, our team designed WRAP (W- and R-rich Amphipathic Peptide) able to form PBNs with siRNA and to induce significant gene silencing *in vitro* and *in vivo* (Konate K. et al., 2019, Ferreiro I et al. 2021). These PBNs will be further optimized using targeting peptides and polyethylene glycol (PEG) in order to develop multifunctional nanoparticles. Furthermore, since cardiac ischemia triggers an immediate extracellular pH drop (Fliegel L., 2008), we propose to connect the different entities to the PBNs through acid-sensitive linkages.

First, the different WRAP-derivates bearing pH-sensitive linkages with the targeting sequence or PEG were synthesized and characterized for their ability to form PBNs in the presence of siRNA. Then, our first results *in vitro* evidenced that WRAP-based nanoparticles encapsulating siFADD reduce specifically FADD expression. Now FADD downregulation using PBNs will be further evaluated and characterized in different pH conditions.
In conclusion, WRAP-based pH-sensitive nanoparticles could represent an efficient heart-targeting drug delivery system for AMI treatment.

Keywords: Myocardial infarction, pH sensitive, peptide based nanoparticles, FADD.

B10. Are Argonaute 2 protein loaded nanoparticles an efficient siRNA delivery system in glioblastoma?

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Glioblastomas (GB) are the most frequent and aggressive primary tumors of the human central nervous system. Despite of the combined approach of surgery, chemotherapy and radiotherapy, the median survival is 15 months. Indeed, recurrences systematically occur after surgical resection.
The development of innovative local treatments represents a major technological challenge today. RNA interference (RNAi) is a potent biological mechanism which down-regulates the expression of single or multiple genes (1,2). This makes the RNAi molecules (microRNA and small-interfering RNA) an ideal therapeutic strategy to treat the diseases that are characterized by genomic instability such as GB. Argonaute (AGO) proteins are the core of RNAi machinery; they are involved in the biogenesis of microRNA and functional gene-silencing activity3,4; although it's structural and functional properties have been extensively studied5, its role as extrinsic vectors remains to be explored.

In this context, we are exploring clinically relevant strategies to use human AGO protein as a safe and efficient functional delivery system for RNAi molecules. By nanoprecipitation process, neutral polymeric siRNA/ AGO 2 loaded nanoparticles were formulated with about 300 nm size. *In vitro* biological activity by Luciferase knockdown assay shows a statistically significant reduction in relative luciferase expression for siRNA-Luciferase/ AGO 2 complexes with respect to siRNA-control/AGO 2 complexes. *In vitro* cytotoxicity by resazurin assay demonstrated no toxicity effect of siRNA/AGO 2 herein developed system.

Those results demonstrate the great potential of siRNA vectorization system for future therapeutic applications in GB.

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Keywords: nanoparticles, interfering RNA, glioblastoma, argonaute protein

B11. Development of an ovarian selective miRNA delivery system based on gold nanoparticles to reduce side effects of chemotherapy

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Cancer treatments are known to cause several side effects, like follicle apoptosis, and thus increase the risk of infertility. A previous study has identified the miRNA let-7a to be strongly downregulated in mouse ovaries following chemotherapy, and this latter seems to play a key role towards apoptosis.(1) Our objective is to develop an ovarian selective Delivery System based on gold nanoparticles (AuNPs) for this miRNA with the aim to restore a normal activity level of let-7a. Nucleic acids (NA) are usually attached to AuNPs using thiol chemistry (AuNPs-S-NA); however, this strategy presents some limitations such as the robustness of the organic layer and the lack of control over the NA loading. We worked on the development of AuNPs functionalized with calixarenes, macrocyclic molecules that allows the formation of a robust layer on the NPs through multiple carbon-gold bonds, and different possibilities of subsequent bioconjugation.(2,3) To date, we have developed AuNPs functionalized with calixarenes bearing PEG chains to promote biocompatibility, but also functional groups allowing the covalent attachment of DNA-RNA strands with a high density. Using mixtures of calixarenes bearing different functional groups, orthogonal bioconjugation of various biomolecules can also be achieved, opening the possibility to add a targeting ligand.(4) Very promising results were already obtained with this new bioconjugation nanoplatform. Indeed, the resulting AuNPs-calixarenes-NA allow a very high NA loading through post-hybridization and showed remarkable stability over time compared with AuNPs-S-RNA that aggregate after few weeks of storage. Moreover, RNA expression was still visible after months highlighting the strong stabilization effect of AuNPs-calixarenes-NA as vector. Several biological studies were also performed with these NPs that showed high cellular uptake, no significant cytotoxicity and also efficiency as miRNA delivery system regarding the increasing of let-7a expression observed. Finally, let-7a target genes expression was significantly modulated using AuNPs as carriers, even in context of chemotherapy.

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Keywords: Gold nanoparticles Nucleic acids delivery Bioconjugation Cancer therapy

B12. Cationic Nanostructured Lipid Carriers for mRNA delivery promote potent anti-tumoral T cell immunity

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Objective: We tested the capacity of Nanostructured lipid carriers (NLCs) for the delivery of mRNA encoding antigen and for eliciting potent antitumor immune responses. NLC were of small size (50-90 nm) and have been previously used to deliver proteins as antigen leading to strong antibody and cellular responses in mice and macaques (1,2).

Methods: Cationic NLC were used for the delivery to antigen presenting cells of mRNA encoding Ovalbumin (OVA) as target or an irrelevant protein as control. The vaccine was administered 3 X 3 μg of vectorized mRNA in mice bearing subcutaneous tumors expressing OVA (B16-OVA or E.G7-OVA). Intratumoral immune responses were analyzed by flow cytometry and ELISA and histology. Experiments blocking effectors cells (CD8+ T cells, NK cells) and the PD-1/PD-L1 axis were carried out by injecting intraperitoneally respective blocking monoclonal antibodies.

Results: Vaccination slows B16OVA and EG.7-OVA tumor growth drastically for short-term experiments (17 days), increases intratumoral CD8+ T cells compared with control mice, with higher frequencies of activated CD8+ T cells expressing PD-1, CD69, and coproducing IFNγ and TNFα or IFNγ and Gzb. Blocking CD8+ T cells, but NK cells, prevents the antitumor effect. Combining the vaccination with anti-PD-1 improves the survival of mice bearing tumors. This combinatory treatment leads to a long lasting antitumoral immune response by completely preventing the growth of a secondary tumor engraftment.

Conclusion: Collectively, these data demonstrate that Cationic NLC are able to vectorize mRNA promoting antitumoral immunity by eliciting CD8+ T cell-dependent antitumor immune response. The association with immunotherapy blocking the PD-1/PD-L1 axis improves long-term memory immune responses and thus prevent tumor recurrence.

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Keywords: Nucleic Acid delivery

B13. In vitro delivery of pDNA by β-sitosterol-based cationic lipids with different polar headgroups

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Cystic Fibrosis (CF) is an autosomal recessive genetic disorder affecting the gene encoding the Cystic Fibrosis Transmembrane conductance Regulator (CFTR). This alteration induces a dysfunction of ions transport and dehydration of mucus in the lung. Viscous mucosal accumulation leads to bacterial colonization, chronical inflammation and pulmonary fibrosis responsible for lung failure. Trikafta® is an association of three therapeutic molecules which positively affects lung function. However, this treatment is mutations dependent. Gene therapy is another solution to restore the CFTR protein and consists of introducing a transgene encoding cftr directly towards the airway epithelium. Aerosol delivery represents the most suitable route to achieve this purpose due to its safety. Strategies using non-viral vectors, like cationic lipids (CLs), have been demonstrated to resists to the shear forces during nebulization processes and to be clinically well tolerated (1,2). This approach remains to be improved in order to restore CFTR function in the lung. For instance, β-sitosterol, the most abundant sterol presents in the membrane of plant cells, has recently been used as colipid to deliver nucleic acids (NA) in lipid-based nanoparticles (LNP) formulation (3). Results highlighted the high potency of β-sitosterol to enhance NAs delivery when used as a helper lipid. This phytosterol has also important biological interests such as anticancer or antimicrobial activities which can have interest in CF context. In the present study, three new β-sitosterol-based CLs were designed, synthesized and formulated as cationic liposomes in combination with DOPE (as co-lipid) and compared to cholesterol-based CLs such as DC-Chol. Liposomes and lipoplexes (pDNA/CLs mixture) were characterized regarding their size and zeta potential at various charge ratios. Thereafter, lipoplexes were investigated for their gene delivery properties using four cell lines (A549, 16HBE14o-, CFBE41o- and Calu-3). Results, using gene reporter technique (luciferase and eGFP), showed that β-sitosterol-based CLs can efficiently deliver gene toward the cells. Further investigations are ongoing on these new CLs to optimized the formulation for aerosol delivery.

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Keywords: Gene delivery, Cystic Fibrosis, Cationic lipids

B14. Addressing Replication Challenges in Nanoscience Research: The Curious Case of Nanoparticles Endosomal Escape

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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.

Acknowledgement
This work is part of NanoBubbles, a Synergy grant from the European Re- search Council (ERC), within the European Union’s Horizon 2020 programme, grant agreement no. 951393.

Keywords: Replication, Endosomal Escape, Intracellular Sensing

B15. Reprogramming tumor-associated macrophages using siRNA-LNPs

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Cancer is one of the most prominent causes of mortality worldwide, responsible for approximately 10 million deaths in 2020. Macrophages are innate immune cells abundant in the tumor microenvironment, which can change their activation states depending on environmental cues. In response to tumor-derived factors, tumor-associated macrophages (TAMs) tend to exhibit an immunosuppressive phenotype (M2) that promotes angiogenesis as well as tumor initiation and progression. As such, high TAMs infiltration has been strongly correlated with poor clinical outcomes in many solid tumors. In the last few years, the reprogramming of TAMs from an M2 phenotype into an immunostimulatory phenotype (M1) has emerged as a promising therapeutic strategy to enhance the treatment of solid tumors.
Recently, J.L. Perfettini's group discovered a novel immune checkpoint that modulates macrophage polarization. Based on this finding, we aim to develop siRNA-loaded lipid nanoparticles (LNPs) to block the P2Y2-dependent signaling pathway and reprogram TAMs into M1-like. To date, we have successfully synthesized siRNA-LNP systems with uniform size distribution and high encapsulation efficiencies via a microfluidic method. These siRNA-LNPs can be efficiently internalized by bone marrow-derived macrophages (BMDMs). We are currently studying gene expression kinetics in the BMDM model to confirm the proinflammatory reprogramming of macrophages after treatment with P2Y2 siRNA-loaded LNPs.

Keywords: Lipid nanoparticles, siRNA delivery, gene silencing, tumor associated macrophages, cancer immunotherapy

B16. Scale up strategy for the encapsulation process of LNP-mRNA

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Thanks to the COVID-19 crisis, the first mRNA vaccine was marketed in 2019. The speed and ease with which it is adaptable from one application to another makes it a major asset for the pharmaceutical industries. In addition, the process (rapid mixing of an aqueous phase with an organic phase) allows the optimal control of production conditions. However, the processes currently used are mainly micromixers which are parallelized to achieve an industrial production volume.
The objective of this poster is to describe the strategy defined by Sanofi to develop a scale-up of the encapsulation process to have a large-scale process, usable for industrial scale. This strategy focuses on three axes:

- The modeling of the fluids flows in the mixer to understand the fluids mixing.
- The definition of the key parameters to achieve a scale up.
- The development of a digital twin capable of predicting the physicochemical and biological properties of LNPs according to the process parameters applied.

The modelling of fluids flow is done by computational fluid dynamic (CFD) and validated by experimental assays. Model results will then be related to physicochemical and biological properties of LNPs to better understand the LNP formation and find the key parameters to achieve a scale up. These results will also be used to develop the digital twin.

Keywords: mRNA, mixing, scale up, modelling, process, LNPs

B17. Study of therapeutic effects of two-photon controlled gene delivery with nanoparticles in uveal melanoma using organ-on-chip model

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The ocular melanoma is an intraocular malignant tumor, usually affecting the adult eye. It develops melanocytes located in the highly pigmented uveal tract, which is also the main oxygen and nutrient supplier to the retina. This cancer is caused by mutations, some of which are well known, such as the Q209 and R183 mutations in the GNAQ and GNA11 genes, which theoretically cover 80-90% of the most common uveal melanomas. It can affect the choroid, iris or ciliary body. This cancer affects 5 out of 1 million people, and current treatments such as radiotherapy, chemotherapy or ablation can only slow the disease down in the best cases. Moreover, the prognosis and long-term survival of patients remains limited, as this cancer has a strong tendency to metastasize and induce high mortality rates. Our project focuses on the treatment of ocular melanoma, using porous silicon nanoparticles (pSiNPs). pSiNPs offer very interesting optical and surface properties, such as intrinsic photoluminescence and high porosity, enabling them to be loaded with large molecules, drugs or nucleic acids. For this purpose, a system composed of size-controlled pSiNPs functionalized with cationic porphyrins is proposed. The aim is to efficiently complex negatively charged siRNA, with the porphyrin, internalize it into the endosome of melanoma cancer cells, then trigger endosomal escape of the siRNA by PCI mechanism under biphotonic photoactivation. The use of pSiNPs offers a number of advantages, since they are bioresorbable and biocompatible. Cytotoxicity and tumor cell internalization carried out on MM28 and 92.1 melanoma cell lines will be presented.

Keywords: nanoparticles, pSiNPs, siRNA, uveal melanoma

B18. Engineering lipid-based micro-RNA therapeutics for treatment of intervertebral disc degeneration

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Low back pain (LBP) stands as a global health burden, afflicting almost 80% of adults a some point in their lives, with substantial disability and socioeconomic consequences. Remarkably, around 40% of all LBP cases are attributable to intervertebral disc degeneration (IVDD), a condition that unfortunately lacks a definitive cure. Hence, there is a dire need for innovative therapeutics that not only offer symptomatic relief but also have the capacity to stop or even reverse the IVDD process.

Given the accumulating evidence that multiple micro-RNAs (miRs) are dysregulated during disc degeneration, they could have a huge potential as biomarkers, targets, or active principles against this debilitating condition. Our miR of choice, miR-155, is downregulated in IVDD and is known to be involved in protecting the IVD from apoptosis, decreasing degradation, and controlling inflammation. However, miRNA delivery encounters extracellular and intracellular barriers. Extracellularly, they would be rapidly degraded by endogenous nucleases, and intracellularly, their uptake will be hindered by their relatively high molecular weight and negative charge. A promising technique to address this challenge is the vectorization of miRNAs within lipid nanocapsules (LNCs), providing both protection from degradation and improving their uptake within the scarce target cells, called nucleus pulposus (NP), of the degenerated IVD.

Henceforth, in this study, we developed miR-155-loaded LNCs to be safely injected for the treatment of IVDD. These LNCs were formulated using the phase inversion process by adding cooled miR-155-lipoplexes in the phase inversion zone (PIZ) of a mixture of caprylic-capric triglycerides and hydroxy-stearate-PEG. The LNCs were fully characterized to be 78 nm in hydrodynamic diameter, 0.08 of polydispersity index, and +11 mV in zeta potential. Finally, surface modification of the LNCs with targeting peptides was proposed. Nucleus pulposus (NP) cell affinity peptides such as short Link-N (s-Link-N) peptide have a direct anabolic effect on the NP cells and could be utilized in LNCs surface decoration either covalently or by adsorption techniques. Loading the LNCs with both miR-155 and s-Link-N could offer a huge potential in terms of synergetic pro-regenerative effects with enhanced ability to target the NP cells *in situ* and *in vivo*.

Keywords: Intervertebral disc degeneration (IVDD), Lipid nanocapsules (LNCs), microRNA (miR), RNA interference (RNAi)

B19. Robust Calixarene-Coated Gold Nanorods for Photothermal Therapy and Nucleic Acid Delivery

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Gold nanorods (AuNRs) have emerged as highly promising materials for advancing biomedical *in vivo* applications. They exhibit two distinct Localized Surface Plasmon Resonance (LSPR) bands, namely transverse and longitudinal. The maximum absorption wavelength of the latter can be precisely tuned by adjusting the aspect ratio of the rods. For high aspect ratios, the absorption wavelength falls within the near-infrared (NIR) region, where minimal absorption of light by endogenous molecules enables excellent penetration into tissues.

Photothermal therapy (PTT) is a promising non invasive cancer treatment strategy exploiting the conversion of light to heat by photothermal agents for thermal ablation of cancer cells. PTT has attracted much attention due to its simple operation, short treatment duration, and minimal intrusion. AuNRs possess several qualities that make them excellent photothermal agents: high biocompatibility, long circulation times, ease in functionalization, and intense optical extinction coefficients in the NIR region. However, one limitation is their anisotropic structure that is susceptible to deformation upon laser irradiation leading to a rapid decrease of their NIR extinction and photothermal efficiency. To address this challenge, we have employed an effective approach to stabilize the rod shape, involving the use of calix(4)arene-tetradiazonium salts, which can be irreversibly and strongly grafted onto surfaces through the reduction of their diazonium groups. We have adapted this calixarene-based coating technology to fabricate ultrastable AuNRs which exhibit superior stability compared to conventional AuNRs, notably under laser irradiation.

The photothermal effect can also be exploited for light-triggered drug delivery, enabling opportunities for combination therapies that can significantly enhance treatment outcomes. Notably, light-triggered nucleic acid delivery has been shown to be a promising strategy for gene and antisense therapies in the context of cancer treatment. A commonly employed system involves AuNRs functionalized with thiolated nucleic acids. Nevertheless, such systems suffer from several limitations including Au-S bond thermolysis, resulting in AuNR destabilization and release of free thiols which can have detrimental effects in cells. Additionally, premature release can be induced by exchange with endogenous thiols, even before reaching the targeted cells. To address these limitations, we exploited the multiple anchoring points and strong Au-C bonds of the calixarene layer. We used double stranded oligonucleotides in which only one of the strands is conjugated to the calixarene coating: irradiation of these systems raises the local temperature causing the unpairing and release of the guide strand. Enhancing the stability and efficiency of AuNRs in the context of PTT and nucleic acid delivery has the potential to drive significant advancements in the field.

Keywords: gold nanorods, calixarene, photothermal therapy, nucleic acid delivery

B20. Efficient lipid nanoparticle-based mRNA delivery system

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The recent success of mRNA-based COVID-19 vaccines has impressively highlighted the therapeutic potential of nucleic acid-based therapeutics. However, delivery of the nucleic acid payload to the target cells is still a major challenge within the development of mRNA-based therapeutics. Over the last years, various delivery vectors have been extensively investigated for nucleic acid delivery. Among them, lipid nanoparticles (LNPs) have gained great interest from groundbreaking research to cutting-edge applications with the commercially approved On-pattro® and mRNA-vaccines. The purpose of the present study was to develop mRNA-lipid nanoparticles for modulating protein expression in skeletal muscle cells as proof-of-concept for vaccine applications. In fact, transfecting muscle cells is a coveted milestone to ensure the pro- duction of the viral antigens and trigger the specific immune response. As proof-of-concept to modulate protein expression in skeletal muscle cells, eGFP fluorescent protein was selected as reporter gene. The present investigations demonstrated the high efficiency of our recently patented LNP (FR2112931) to associate mRNA. All complexes were monodispersed and characterized by a hydrodynamic diameter around 200 nm. The most suitable nanosystems were then tested in vitro on C2C12 murine skeletal muscle myoblasts and myotubes. Data demonstrated a high transfection efficiency with up to 90 % of myoblasts and 80 % of myotubes expressing eGFP without any overt signs of toxicity on both cell types. Finally, nanosystems were tested ex vivo on muscle fibers explanted from healthy mice. Results demonstrated that nanosystems were accumulated at the membrane level and a high eGFP expression was observed following 24 hours, highlighting the high efficiency of our nanosystems to transfect skeletal muscle cells. Overall, the present study demonstrates the suitability of our LNP to deliver mRNA in skeletal muscle cells, opening the range of possibilities for vaccine applications. Further investigations will be devoted to study antigens expression in vivo.

Keywords: mRNA, lipid nanoparticles, muscle, vaccine

B21. Enhanced Stability of Lipid Nanoparticle-Based ANRIL Delivery System: A Promising Approach for Colon and Pancreatic Cancer Treatment

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Antisense Non-Coding RNA in the INK4 Locus (ANRIL) is involved in the regulation of gene expression and cellular processes. It is associated with gene expression control and cell cycle regulation. ANRIL's involvement has been prominently documented in various pathological conditions, spanning cancer, cardiovascular disorders, diabetes, and neurodegenerative ailments. Dysregulation of ANRIL significantly contributes to the etiology and progression of these pathologies. Despite ANRIL shows promise as an effective and safe target-specific therapy, there are notable barriers that must be addressed for their successful delivery. One significant challenge is the instability of ANRIL in biological fluids, which limits their availability in their intact form at the target site. To surmount this challenge, we have developed an advanced ANRIL delivery system, centered on lipid nanoparticles. Our lipid nanoparticle-based delivery system leverages the cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) to promote electrostatic interactions with ANRIL. Several formulations were investigated, encompassing DOTAP combined with auxiliary lipids such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and cholesterol, denoted as DOTAP/DOPE, DOTAP/Cholesterol, and DOTAP/DOPE/Cholesterol. Lipid nanoparticles were obtained by solvent emulsification-evaporation method and subsequently complexed with ANRIL. Lipid Nanoparticle-ANRIL (Lip-ANRIL) complex, obtained by varying the nitrogen/phosphate (N/P) ratio from 2 to 10, was characterized using a Zetasizer Nano 90. The binding efficacy of lipid nanoparticles to ANRIL was ascertained through agarose gel electrophoresis and quantification of ANRIL with a Qubit 4 Fluorometer. Stable lipid nanoparticles were achieved with an average size of approximately 100 nm and a zeta potential ranging from +38 to +66 mV. However, Lip-ANRIL complexes formed with DOTAP alone exhibited a rapid loss of stability within 3 days. The inclusion of auxiliary lipids (DOPE and/or cholesterol), remarkably extended the stability of the formulations. Stable Lip-ANRIL complexes (N/P ratio of 10) generated with DOPE exhibited an average size of approximately 180 nm, a zeta potential ranging from +50 to +60 mV, achieved ANRIL complexation levels exceeding 98%, and 30-days stability. In conclusion, the incorporation of auxiliary lipids, particularly DOPE, has significantly enhanced the stability of Lip-ANRIL complexes, thus presenting a promising avenue for the treatment of colon and pancreatic cancer.

Keywords: Lipid nanoparticles, ANRIL, cancer

B22. Gene-Activated Injectable Cell carrier for Cartilage Repair: Controlling MSC Differentiation by Tuning siRNA Nanovectors Characteristics.

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The combination of mesenchymal stromal cells (MSCs) with active injectable carriers brings about innovative solutions to current issues in the field of tissue engineering. In particular, repair of adult articular cartilage lesions, especially those affecting the knee, remains a clinical challenge because of the limited cartilage self-healing capacity. A previous work of our team demonstrated that the open porosity of homemade collagen microspheres allowed for the entrapment and progressive release of TGF-β3, which efficiently triggered the chondrogenic differentiation of MSCs *in vitro* and *in vivo*, and the production of neo-cartilage tissue. However, one major hurdle in MSC-based therapies for cartilage repair is their late hypertrophic differentiation and subsequent tissue calcification. In this context, we identified Runx2 protein, which plays a central role in chondrocyte hypertrophy, as the main molecular target to be repressed. We developed a new siRNA nanovector that showed interesting properties, such as tunable size, stability in cell culture conditions and high efficiency to transfect primary human MSCs. After entrapment of nanovectors in porous collagen microspheres, we showed their size influenced both loading capacity and release kinetics. These different release profiles led to differences in the transfection kinetics of human MSCs. This original and unique type of gene activated matrix, with adaptable release kinetics, is one step forward in the control of MSC differentiation, and must allow future advances in the treatment of osteoarthritis and other cartilages lesions.

Keywords: Tissue Engineering, Cartilage repair, Lipid nanovector, siRNA, Biomaterial

B23. WRAP-based nanoparticles for mRNA delivery.

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After the COVID pandemic, mRNA-based therapy has great potential for therapeutic applications for an immense number of indications. However, mRNA delivery is still challenging and requires a specific delivery system. In this context, beside other non-viral vectors, cell-penetrating peptides (CPPs) gain more and more interest as delivery systems by forming a variety of nanocomplexes depending on the formulation conditions and the properties of the used CPP/mRNA.
Recently, our team developed cell-penetrating peptides called WRAP able to vectorize efficiently into cells siRNA *in vitro* and *in vivo*. We thus studied the ability of these peptides to pro- mote the cellular delivery of different mRNAs.

As a first step, we characterized WRAP:mRNA nanoparticles formation by Dynamic Light Scattering depending on the charge ratio between the peptide and the mRNA and on the final mRNA concentration. First results evidenced that nanoparticles of ˜60 nm were obtained at a charge ratio of 5. Upon a simple mix of peptide and mRNA solutions, these nanoparticles internalize human heterologous HeLa cells as shown by confocal microscopy and GFP mRNA is translated as exemplified by the GFP protein expression recorded by Western blots after incubation of cells with mRNA.

In a second step, we demonstrated that WRAP:mRNA nanoparticles are stable and remains fully efficient over an extended period (up to 3 months) upon a simple storage at 4◦C. Moreover, we showed that this delivery system could be used for the transfection of mRNAs of different lengths (900-4800 bases). We are now investigating the *in vivo* delivery of these nanoparticles in different animal models.
In conclusion, peptide-based delivery of mRNAs could promote new formulations strategies to make easier in a near future the access to mRNA-based immunotherapeutic strategies.

Keywords: Cell penetrating peptides, Nanoparticles, mRNA delivery, vectorization

B24. mRNA loaded Lipid Nanocapsules: a promising therapeutic strategy to treat Pseudoxanthoma elasticum?

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Pseudoxanthoma elasticum (PXE) is a rare genetic disease, characterized by a slow and progressive ectopic calcification due to mutations in the ABCC6 gene, affecting the elastic fibers in the skin, the Bruch's membrane of the retina and the cardiovascular system. Unfortunately, no treatment exists. To replace the deficiency of the protein expressed in the hepatocytes, the use of the corresponding mRNA seems to be a promising therapeutic strategy, but requires a nanocarrier to protect it, to cross the cell membrane and finally to reach its target.

During Covid-19 pandemic, lipid nanoparticles highlighted the huge potential of nanomedicines and have successfully demonstrated to be a relevant vector to protect and carry a mRNA. Among the lipid nanoparticles, lipid nanocapsules (LNC) were designed and patented by MINT laboratory in 2014 (patent FR 3026009 (A1)), with the aim to protect genetic material such as DNA, siRNA and miRNA.

The objective of this project consists in formulating and characterizing mRNA loaded lipid nanocapsules (LNC) and in evaluating the *in vitro* toxicity on hepatocytes cell line HepG2. As a model to validate the system, the mRNA translating the Enhanced Green Fluorescent Protein (EGFP) was first chosen and in parallel a total amount of 948.2 µg *ABCC6* mRNA of was pro- duced in Prof. Pichon's laboratory. Afterwards *ABCC6* and *EGFP* mRNA loaded LNC were fully characterized. A dose-dependent effect was observed by MTT and LDH assays on HepG2 cells with a IC50 of 1 mg/mL. Moreover, cell internalization was followed by flow cytometry after 30 min, 1h, 4h, 8h, 24h, 48h and 72h of treatment by DID loaded *EGFP* mRNA LNC: an efficient cell internalization of the LNC with in parallel a protein expression was observed after 8h, demonstrating the potential of this strategy.

Keywords: ABCC6, mRNA, LNC, genetic disease, PXE, mRNA loaded LNC, ABCC6 mRNA loaded LNC

B25. Synthesis and characterization of a new peptide-based self-assembled theranostic nanostructure

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Peptide-based self-assembled nanostructures are attractive materials with tunable morpholo- gies and properties, allowing to build from the same original building block adaptable nanoplat- forms for targeted applications such as drug delivery and *in vivo* imaging. Peptides as building blocks are extremely versatile, due to their straightforward synthesis and modification, leading to a great diversity in sequences that can be used to design well controlled self-assembled nanos- tructures. Self-assembly property is particularly interesting as it is often stimuli-responsive, making nanostructure reconfigurable and very well suited for drug loading and release steps. Eventually, accessible surface functional groups allow for easy grafting of targeting or labeling agents toward multimodal platform development. (1,2)

In this work, a short amphiphilic peptide sequence (3) was obtained by solid-phase peptide syn- thesis under continuous flow process, reducing the time of one amino acid cycle from one day to one hour. To help in the optimization of the synthesis, we developed an electrokinetic method based on CE-DAD-ESI-MS to characterize crude peptide samples. This method allowed for an easy and quickly identification and quantification of predominant amino acid sequences with- out the need of their prior purification as direct sample quality control. Peptide self-assembly following two different processes, either spontaneous by pH adjustment or dialysis assisted in presence of a model hydrophobic drug, yielded robust nanostructures of 50 or 170 nm in equiv- alent spherical diameter, respectively. Besides, the peptide was functionalized with a DOTAGA ligand (80% reaction yield) for imaging properties and in view of further co-assembly.

Our combined results show that the described amphiphilic peptide-based self-assembled nanos- tructures have great potential as theranostic agents for the development of drug delivery systems with controlled release and post-injection monitoring.

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Keywords: Peptide self assembly, pH sensitive, theranostics, organic nanomaterials

B26. Nanoclusters for Preparing Irinotecan Hydrochloride Trihydrate Parenteral Injection Concentrates

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Irinotecan hydrochloride trihydrate is an antitumor and antileukemic water-soluble prodrug. The commercial active pharmaceutical ingredient has been identified as the thermodynamically stable Form b of the compound. The commercial injection concentrate of 1 mL contains 20 mg of the salt, 45 mg of D-sorbitol powder, and 0.9 mg of L-lactic acid dissolved in water by heating. However, the solubility of Form b crystals in water at 25 ◦C was reported to be only about 10 mg mL^{-1}. Despite the apparent supersaturation, this concentrated solution of 20 mg mL^{-1} is surprisingly stable for long-term storage.
The unusual stability behavior observed was assessed by our group through different approaches. Multiple techniques were employed to characterize the solid state of the compound (i.e., FT-IR, NMR, PXRD, HT-PXRD, DSC, TGA, and DVS). Water-equilibrium solubility was evaluated by the van't Hoff and Hildebrand plot, which revealed a non-linear behavior. Furthermore, the stability of various frustration levels was assessed until the supersaturated solution crystallized. We noted that the formation of irinotecan hydrochloride nanosized clusters of 150 nm confers the enhanced stability of the preparation. The formation pathway involves three distinct stages: (1) densification of monomers and dimers in the solution stage, (2) pre-nucleation of dimers liquid-like clusters of 1.5 nm with a local energy minimum, and (3) molecular ordering of dimers in the crystal nucleation stage.
Indeed, the frustration in the second stage explains the long-term stability of the commercially formulated injection concentrates. Irinotecan hydrochloride trihydrate's concentration above the equilibrium solubility does not affect the cluster size distribution. The latter also implies no complexation between the excipients and the drug was occurring. Consequently, at concentrations higher than the equilibrium solubility value, the apparent solubility of the drug is increased, allowing the formulation of nanomedicines with improved physicochemical and biopharmaceutical properties.

Keywords: liquid, like cluster, nanocluster, non stoichiometric hydrate, dimer

B27. Synthesis of lipopolypeptides towards controlled membrane destabilization

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Delivering a drug at the right time and right place is still a major challenge for nanomedicine. Stimuli-sensitive lipids or polymers have been explored to modulate membrane permeability and trigger the delivery of drugs from nanovesicles in the presence of ions, light, temperature, or redox conditions. Here, we intend to incorporate such switchable lipids, based on molecular tweezers into a polymer-based biomimetic scaffold to enhance and control the membrane destabilization effect. We report the first step of this research work: first, the multi-step synthesis of pH- and Zn^{2+}-responsive lipids, via different coupling reactions such as the Suzuki and Sonogashira reactions, and the study of their conformation change by NMR and XRD upon pH changes or exposure to metallic cations. Then, we also report the synthesis of the polymeric backbone: these molecular tweezers were conjugated to a polypeptide polymer via the ring-opening polymerization of N-carboxyanhydrides. Interestingly, such switching elements are promising blocks which could be used in the building of biomimetic synthetic cells, which also need the controlled transport of nutrients through their membrane.

Keywords: Molecular tweezers, Switchable lipids, Lipopolypeptides, Membrane destabilization

B28. Sizing up the nanomedicines: a thorough examination of techniques and data interpretation

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Size is a universal and first in mind property relevant for nanomedicine/nanomaterial characterization, representing the most suitable measurand. However, due to the acknowledged lack of standardized protocols that are able to provide reliable results, especially in the biorelevant context, researchers often engage with the procedures that are successful in acquiring data, but not necessarily the correct ones. In line with the current initiatives of the regulatory authorities, and aiming to address this issue, in the presented research, a set of orthogonal sizing techniques was conducted (dynamic light scattering – DLS, analytical ultracentrifugation – AUC, asymmetric field flow fractionation (AF4), atomic force microscopy (AFM) and scanning electron microscopy (SEM). Insights into the different ”types of size” generated through these experiments were pro- vided, each method critically examined to highlight crucial aspects for result interpretation and data reporting. For this purpose, three lipid-based nanosystems (nanoemulsions) intended for i.v. application were selected: one non-PEGylated (NPEG) and two corresponding PEGylated formulations, containing either PEG2000-DSPE (P21) or PEG5000-DPPE (P51).
Notably, the method of sample preparation prior to the measurement - such as dilution ratio and media composition (water vs. PBS; media containing human serum albumin - HSA), yielded significantly different results. While batch mode DLS confirmed monomodal size distribution for NPEG, P21, and P51 (with Z-ave values of 91.70±0.59 nm in water and 101.67±1.19 nm in PBS; 89.70±0.59 nm in water and 92.71±0.41 nm in PBS; 96.00±0.52 nm in water and 102.56±0.77 nm in PBS, respectively), AUC revealed the existence of numerous smaller nanodroplets (which was also indicated after calculating number-based size distribution through the DLS measure- ments). Similarly, AF4, as another separation technique, discriminated between two distinct particle populations. On the other side, microscopy techniques provided insights to the shape and morphology of the droplets (indicating the difference with respect to the presence of the PEG coating), but faced limitations in evaluating interactions with proteins.

DLS characterisation of this kind of samples clearly suffers from the drawback of the light scattering based method: overestimation of the size due to the strong scattering effect of the bigger particles, which might hide the smaller ones. Therefore, for reliable sizing experiments at least two additional orthogonal techniques ought to be performed, one of which enables particle visualization.
Recognizing that size measurements are incomplete without contextual details about the methodology, a comprehensive measurement report was also proposed, emphasizing the need for a systematic approach to data analysis and interpretation.

Keywords: nanomedicines, sizing techniques, PEGylation, nanomedicine CQAs

B29. Optonutrics: a new technology at chemistry-biology interface for photo-controlled release of bioactive molecules in the brain

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In Europe, 180 millions of people suffer from a neurological or neuropsychiatric disease, but treatments remain ineffective for several brain disorders. Indeed, sustainable and safe delivery of therapeutic molecules across the blood brain barrier (BBB) is a major hurdle for successful brain therapies. To improve the efficiency and selectivity of therapeutic molecules, their loading in synthetic polymer vesicles represents a promising strategy. Polymersomes are ideal candidates due to their high stability, versatility and ability to transport drugs with high efficiency. However, the use of these vesicles does not allow the control of their release in time and space in the body, limiting certain therapeutic applications. In parallel, neuroscience research has shown an impressive development with the emergence of light-controlled technologies (e.g., optogenetics, fiber photometry, etc.), which allows control of brain circuits by optical fibers. However, these developments do not allow the release of bioactive molecules. Here, the Optonutrics technology combines the principles of encapsulation and photostimulation, in order to release molecules into the brain with high spatial and temporal control, as well as regulation of concentration. Following the work done by the project partners (1), we formulated by emulsion-centrifugation several combinations of polymersomes loaded with new hydrophilic photocleavable dyes. Characterization of polymersomes with confocal microscopy showed that the mean diameter of the vesicles is around 8 μm. The time of irradiation for the rupture of polymersomes at a specific wavelength was quantified in solution and in a gel environment mimicking brain tissue. Furthermore, biosafety of polymersomes was assessed *in vitro* and *in vivo* for up to three weeks. Altogether, this work established a solid proof-of-concept demonstrating the unique properties of biocompatible photoactivable polymersomes for the delivery of bioactive molecules to the brain *in vivo*.

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Keywords: Brain, Polymersome, Encapsulation, Photostimulation, Confocal microscopy

B30. Self-Assembled Quantum Gold Clusters for Biophotonic Applications

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Gold Quantum Clusters (GQCs) are a class of ultra-small particles presenting tunable photoluminescence (PL) in the first (NIR-I) and second (NIR-II/SWIR) near-infrared windows, making them appealing as tracers for high-resolution imaging of blood vasculature in mice. Interestingly, we also remarkable change in their optical properties with a boost of their absorbance and PL in the NIRs windows when GQCs self-assemble. The main objectives of the SEQUOIA project are now to make the proof of concept that such self-assembly can be obtained and controlled by *in vivo* bio-orthogonal click chemistry after successive intravenous administrations of biocompatible polymer chains and GQCs bearing complementary functions such as strained cyclooctynes (DBCO) and azides. After *in situ* generation, the nano-assemblies will present enhanced biophotonic properties. Thanks to the presence of cRGD-peptide targeting ligands on the polymer, non-invasive diagnostic applications will lead to the sensitive detection of angiogenesis and of a specific biomarker expressed on activated neo-endothelial cells (integrin $\alpha v\beta 3$). Controlled formation and the properties of the nano-assemblies will be studied in increasingly complex environments: serum-containing solutions, microfluidic devices, 2D/3D cell culture and then in mice carrying proangiogenic cellulose sponges implanted subcutaneously. All methods are currently available in our laboratories. The versatility and new optical properties of this platform could be exploited for the definition of the next generation of theranostic agents, but also eventually biophotonic sensors and optoelectronic devices.

Keywords: nanoclusters, biophotonics, self assembly

B31. Impact of the presence of a PEG linker between targeting ligands and the surface of functionalized nanoparticles on their cell internalization efficiency

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Among females, breast cancer is the most diagnosed cancer and the leading cause of cancer death. There is a strong need of new treatments without side effects. In nanomedicine, the goal is to develop multimodal nanoparticles (NPs) to speed up targeted diagnosis, to increase its sensitivity, reliability and specificity for a better management of the disease. The selective accumulation of NPs in desired organs to enable precise diagnosis and targeted therapy remains an important issue. Most of developed NPs accumulate, after intravenous injection, in eliminatory organs and only low amounts are seen accumulating in tumors. For a precise treatment, active targeting with affinity ligands to achieve tumor specificity is crucial. Among NPs developed for nanomedicine, superparamagnetic iron oxide nanoparticles (IONPs) are promising as they may be designed to display multimodal therapy. Indeed, besides being excellent T2 contrast agents for MRI, IONPs are promising as therapeutic agents by hyperthermia when suitably designed. However, the targeting efficiency is often reported lower than expected and one issue would be that the targeting ligand is ”hidden” in the organic coating and is thus not available for inter- acting with targeted cell receptors. In that context, we developed IONPs coated with a dendron molecule (DIONPs), which have been demonstrated in several *in vitro* and *in vivo* studies to display antifouling properties. We have studied the targeting of breast cancer cells by coupling on the nanoparticles’ surface a selected targeting ligand (TL) bearing or not a PEG linker. We have chosen peptides with high affinity for specific membrane receptors overexpressed in these cancer cell lines: MDA-MB-231 (triple negative) and MCF-7 (ER+). We succeeded in establishing a reproducible method for the coupling of the TL and for the quantification of their amount at the surface of DIONPs. The impact of the PEG linker on the DIONPs internalization in breast cancer cells has been studied by combining different characterization techniques and will be discussed.
This project received funding from ANR (EURONANOMED2020-121 - THERAGET) under the umbrella of the ERA-NET EuroNanoMed (GA N◦723770 of the EU Horizon 2020 Research and Innovation and from Alsace contre le Cancer.

Keywords: Iron oxide nanoparticles, dendron coating, targeting, Breast cancer, PEG linker

B32. Functionalized lipid nanoemulsions loaded with near-infrared fluorescent dyes for targeted bioimaging

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Lipid nanoemulsions (LNEs) are biomimetic self-assembled nanostructures with oil core and surfactant shell. The use of LNEs for bioimaging-based diagnosis has gained attention due to their low toxicity, and high biocompatibility compared to inorganic and other organic nanoparticles (1). Near-infrarred fluorescent dyes are ideal for bioimaging purposes because the optical interferences from the tissue are minimal (2). The challenges here are to design LNEs encapsulating these dyes, and further functionalize their surface with antibodies for targeting specific cells in vitro and in vivo. To this end, highly apolar Cy5.5 dye with bulky counterion were loaded into the oil core LNEs. To functionalize the LNEs with antibodies, we devised a novel method for nanoemulsion surface functionalization with biotin, which can be readily applied for antibody conjugation via avidin-biotin interactions. The developed nanoemulsions were success- fully applied for targeted imaging of senescent and cancer cells.

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Keywords: lipid nanoemulsion, near, infrared dyes, fluorescence, targeted imaging

B33. Polymeric nanoparticles as a platform for nose-to-brain imaging and drug delivery

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Central nervous system (CNS) diseases such as neurodegenerative conditions, brain cancers, epilepsy, stroke, and even mental health disorders, represent today a huge challenge for medicine (1,2). Drugs administered via oral route, or intravenous route reach the systemic circulation before being able to enter the brain. Therefore, once in the blood, therapeutic molecules need to cross the blood-brain barrier (BBB) to attain the brain. This physiological barrier is a challenging obstacle for the delivery of molecules as less than 5% of small molecules can cross it (3). Intranasal delivery is being investigated to deliver drugs to the CNS by bypassing the BBB. This avoidance of the BBB is possible thanks to the olfactory mucosa at the top of the nasal cavity that allows a direct connection to the brain (4). Even if this route of administration is very advantageous, effective drug delivery systems are necessary to protect the therapeutic molecules against both chemical and biological degradation as well as prevent efflux transport.

We aim to develop a delivery platform based on nanoparticles (NP) composed of polylactic acid and a poloxamer that can cross the olfactory mucosa and reach the brain. These NP will be functionalized with contrast agents to image *in vitro* as well as *in vivo* their potential crossing of the olfactory mucosa. Moreover, the NP will be used to deliver therapeutic drugs to the brain, either by adsorbing them onto the NP or by encapsulating them.

This development will involve the optimization of the nanoprecipitation technique, and the characterization of the formulation, to obtain a formulation that will meet the expectations of the European Pharmacopeia. Part of this work will be to evaluate the impact of those formulations on different cell types *in vitro* and to further explain how and by which means the NP cross the olfactory mucosa *in vivo*.

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Keywords: nasal delivery, polymeric nanoparticles, nose, to, brain delivery, drug delivery system

B34. Modulation of the nanoparticles ”Protein Corona” by tailoring their surface chemistry

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It is clearly established that the protein corona (PC) formed by interaction with plasma proteins on injected nanoparticles (NP) determines their biological identity, dictating the NP cell uptake properties and their fate *in vivo (*1).The objective of this project is to develop nanoprobes capable of targeting resident foamy macrophages within vulnerable atherosclerotic plaques. Therefore, the PC composition related to the synthetic identity of the NP needs to be adapted for better pharmacokinetic and targeting efficiency. To achieve this objective, chemical moieties will be grafted on the surface of PEGylated NP to promote interactions with plasma proteins of interest such as clusterin (ApoJ) known to act as dysopsonin against macrophages of the reticuloendothelial system (2). Within the different surface identities, the best proteomic profiles in terms of ApoJ abundance, among others, will be considered for further *in vitro* and *in vivo* tests. The chemical surface modification will be applied on two types of NPs of the same size (20 nm): (i) fluorescent silica NP that incorporates two fluorophores in visible and near infrared (NIR) range for *in vitro* test, cytometry and fluorescence imaging experiments; (ii) maghemite nanoflowers used for their remarkable magnetic relaxivities useful for MRI. The affinity of selected plasma proteins with the functionalized internal surface of the PEGylated NPs will be the subject of a specific physicochemical study using fluorescence quenching (3) and their conformational properties measured by circular dichroism. Comparative *in vitro* studies of interactions with macrophages will be conducted using the different synthetic identities generated by incubation of NP in serum from healthy mice. Moreover additional *in vitro* tests will involve blood cells in order to predict the elimination of NP towards the liver, experimental models mimicking the macrophages of the plaque as well as macrophages present *in situ* will be implemented to assess the interactions with the generated bioidentities.

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Keywords: PEGylated nanoparticles, Protein Corona, Surface chemistry, Bioimaging

B35. Silica nanoparticles as an EPR effect guided nanoplatform for nuclear imaging

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Silica nanoparticles (NPs) designed for medical use are attractive as a platform because of their biocompatibility, their ease of functionalization and their large surface/volume ratio allowing multifunctionalization. Moreover, their size potentially induces their accumulation in solid tumours via the enhanced permeability and retention effect (EPR effect), making them suitable vectors to bring diagnostic or therapeutic tools specifically to these tumours.
With this in mind, we modified silica NPs with a dipicolylamine clamp (DPA) allowing radiolabeling with technetium-^{99}m, the most used radionuclide for Single Photon Emission Computed Tomography (SPECT) imaging.

The aim of the project would therefore be (i) to prepare and characterize new technetium-99m radiolabelled NPs and (ii) to study their pharmacocinetics and also their passive targeting on different murine cancer models via the EPR effect.

Our results will be divided in three parts, which are the three stages of the design of this object: (i) we will review the synthesis of the NPs which allows to modulate the morphology of the final object and the functionalization by polyethylene glycol (PEG) chains to improve their biocompatibility. The obtained nanomaterials were characterized by a set of complementary techniques (DLS, TEM, AFM/Raman, MS, RMN); (ii). the choice of the best linker (carbamate, urea or thiourea function) between the DPA clamp and the NPs will be discussed based on a study of their stability in the grafting reaction conditions, and (iii) the results of complexation with ($Re(CO)_5Cl$) or ($Re(CO)_3(H_2O)_3$) precursors as "cold" models of ^{99}mTc-tricarbonyl cores) and ^{99}mTc-radiolabelling study will be presented.

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Keywords: Silica nanoparticles, EPR effect, SPECT imaging, technetium ^{99}m

B36. Chemical Insights into the Preparation of Multifunctional Metal-Oxide Nanoparticles for Enhanced Imaging and Theragnostic

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Non-centrosymmetric metal oxide nanoparticles exhibit a wide range of functional properties similar to their bulk counterpart. Among them, lithium niobate nanocrystals (LiNbO3) have received great attention due to their high nonlinear optical coefficients making them useful in biomedical imaging, nonlinear optics, and sensing applications (1). In the nanomedicine field, several proof-of-concept studies have also been evidenced in terms of potential applications after surface functionalization with Gd^{3+} chelates allowing an MRI (Magnetic Resonance Imaging) contrast for deep imaging and for the local on-demand release of chemotherapeutics through harmonic generation (2-3). Their extremely rich non-linear optical properties, long-term photostability, and excitation-wavelength tunability for deep optical imaging (4) indeed make them excellent candidates to go beyond the usual tissue transparency window (NIR I, 650-950 nm), in the newly addressed NIR II (1100-1350 nm) and NIR III (1600-1870 nm) regions.
To go further, our recent work focuses on developing multimodal agents capable of generating several contrasts through multiple physical mechanisms including MRI, computed tomography (CT), luminescence, and nonlinear processes in a single product. In this presentation, the reaction pathways and formation mechanisms recently demonstrated for LiNbO3 nanoparticles will be discussed (5). This synthesis route was extended to other types of metals, such as lithium tantalate ($LiTaO_3$). Substituting Nb for Ta allows improvement of the nanoprobe by adding a new feature. A higher CT contrast is indeed expected since tantalum has a K-edge at 67.4 keV which is well-suited for most current CT scanners. The resulting NPs were characterized *via* X-ray diffraction (XRD), dynamic light scattering (DLS), and transmission electron microscopy (TEM). Their Second Harmonic properties under femtosecond excitation were assessed from colloidal suspensions and compared to the well-known ones of $LiNbO_3$.

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Keywords: Metal oxide nanoparticles, LiNbO3, LiTaO3, nonlinear optics, SHG

B37. Innovative peptide-based nanomaterial for the surface modification of screen-printed carbon electrodes on paper using a plasma assisted vapor deposition strategy

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Peptide-based nanomaterials are attracting increasing attention nowadays for biological and diagnostic applications due to their intrinsic biocompatibility and biodegradability. Among them, self-assembled nanoarchitectures arising from short synthetic amino acid sequences exhibit essential characteristics (such as chemical and mechanical stability, high water absorption capacity, accessible functional groups, responsiveness to external stimuli, electron transfer properties, fluorescence features...) for a wide range of applications in biochemistry, including nanovectorisation, surface functionalization and diagnostics (1).
Aromatic dipeptide building blocks represent major structural elements for future technological applications. Among them, diphenylalanine (FF) is particularly studied for its capability to self-assemble into well-defined nanotubes that paves the way for innovative surface modification strategies breaking with classical carbon nanotubes in sensing applications when combined with biomolecules. Our group recently explored the hierarchical self-assembly of FF and the passive adsorption of derived nanostructures on a carbon-based surface using a wet deposition strategy. Resulting peptide modified electrodes, screen-printed on paper, eventually showed improved electrochemical performance toward a model redox probe (2).

The present work aims at going further by probing the potentialities of a plasma assisted vapor deposition strategy for a better control of the surface modification of screen-printed carbon electrodes and consequently improved surface properties. Cauliflower-like agglomerates of FF peptide were characterized by TEM and ATR-FTIR, respectively. Nonetheless, the robustness of this surface modification process still needs to be improved concerning the homogeneity and thickness of deposits. Besides, FF deposits seem to rapidly reassemble in needle-like structures upon a wetting-drying cycle. Nonetheless, the electrochemical properties of peptide-modified carbon electrodes, screen-printed on paper, were characterized using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). If not as performant as electrodes modified by wet deposition, they showed improved response as compared to raw electrodes and were ap- plied to the quantitation of a redox probe in saliva as proof-of-concept.

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Keywords: nanostructured biomaterials, peptide selfassembly, plasma technology, surface engineer- ing, diagnostics

B38. Formulation and characterization of Patent Blue dye Nano-liposome labeled with ^{99}mTc-HMPAO, in-vivo and ex-vivo Sentinel Lymph Node mapping

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Sentinel Lymph Node Biopsy (SLNB) is a useful means for evaluation of axillary lymph nodes and decision on lymph node dissection in breast cancer patients, Since current methods which apply a dye and a radioactive compound separately are linked with high error rate in detection and uptake of primary nodules, a single radiolabeled dye-loaded nanostructure may significantly decrease such errors. The aim of this study was to prepare nano-liposomes loaded with patent blue and labeled with ^{99}mTc-HMPAO (a lipophilic compound) for mapping lymphatic system.

The liposomes were prepared using the solvent evaporation-extrusion method. Three formulations were designed:
(1) HSPC, m-PEG and cholesterol
(2) HSPC and cholesterol
(3) DSPC, DSPG and cholesterol.
Characterization was done in terms of particle size and zeta potential, percent of Patent blue dye encapsulation, yield of Technetuim labeling of HMPAO, yield of liposome labeling with ^{99}mTc-HMPAO and serum stability of radiolabeled liposomes. The liposomes were then tested in vivo by mice footpad injection and lymphoscintigraphy and visual imaging of the lymph nodes was carried out.
Third formulation was excluded from our study due to low encapsulation and stability. First formulation showed a significant decrease in lymph node uptake in second hours post injection compared to the first hours ($p< 0.05$). However, in the fourth hours, the lymph nodes were not stained and could not be detected.
In second formulation (non-PEG), the activity was increased by time and there was a significant increase in 4 hours as compared to the first hour ($p< 0.05$). However, it has less serum stability.
In conclusion, second formulation has a higher ability for SLNs identification, due to its higher encapsulation yield for Patent blue and a much higher lymph node uptake.

Keywords: Nano, liposome, sentinel lymph node, 99mTc, HMPAO

B39. New methodologies for Taylor Dispersion Analysis – Theory, Proof of Concept and Application to gold nanoparticle – protein interaction

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Since proteins can act as ligand targeting a specific receptor, and nanoparticles (NP) can be used as probe, the knowledge of the interactions between both agents is important for the design of an innovative sensor (1). The change in hydrodynamic radius (rH) induced by the formation of the nanoprobe can be followed using several technics, such as dynamic light scattering or Taylor dispersion analysis (TDA). TDA is a technique dedicated to the determination of the molecular diffusion coefficient (D) of species using band broadening of an analyte in a laminar flow. The advantage of TDA towards other methodologies is the possibility to measure molecules with lower sized (down to 0.2 nm), with the possibility to analyze multimodal samples. Two modes are commonly used: Pulse and Frontal modes. In each case, a fitting of the signal (denoted as Taylorgram) is required and limitations are occurring (2).
To extend the use of TDA beyond the classical requirements, we propose here two innovative approaches:

- Firstly, a third mode denoted as *Cross-frontal mode* (3) is proposed, combining two crossed sample fronts without modification of a classical capillary electrophoresis device for the rapid and accurate determination of D of caffeine, reduced glutathione, insulin from bovine pancreas, bovine serum albumin and citrate-capped gold NP (AuNP).

- Secondly, a *new mathematical description* of the Taylorgram obtained using Pulse mode is proposed, allowing for increased injection volume (up to 15 % of the volume of the capillary inlet, against 1%), which allows for higher sensitivity and a better signal fitting.

Theoretical aspects and methodology of classical and new TDA methodologies will be described, showing a good correlation between experimental results and measurements from DLS and TaylorSizer® device. Finally, the use of this new approach allows for the study of the interaction between AuNP and Concanavalin A (ConA), a lectin known for its interaction with sugar moi- eties like D-Mannose (4). Using TDA, formation of an AuNP-ConA nanoprobe is demonstrated with a D changing from 5.5 to 2.0.10-11 $m^2.s^{-1}$ between the native protein and the nanoprobe. These methodologies are promising for metallic NP-proteins interaction characterization and study, while extending the potential use of TDA as a conventional and reliable method for NP size determination.

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Keywords: Taylor Dispersion Analysis, Nanoparticle, methodology

B40. Microfluidic preparation of monodisperse perfluorocarbon nanodroplets and acoustic droplet vaporization assays

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Acoustically activated nanodroplets (NDs) are receiving increased popularity in both ultrasound diagnostic as contrast agents, and therapeutic application as ultrasound-sensitive drug delivery systems. Except for the core, often consisting of liquid perfluorocarbons, nanodroplets display similar composition to commercially available gas-filled microbubbles. Thanks to their smaller size compared to conventional microbubbles, nanodroplets display prolonged *in vivo* circulation and deep penetration into the tissues *via* the extravascular space. Moreover, they are able to vaporize into gaseous echogenic microbubbles via ultrasound energy beyond an Acoustic Droplet Vaporization (ADV) threshold.
A limitation of nanodroplets is their relatively limited physico-chemical stability over time, due to nanoemulsion ripening, which may affect their use in diagnostic and therapy applications. A possible strategy to overcome this issue has been identified in the selection of biocompatible surfactants constituting the NDs shell.

In this poster, we present the structure and synthesis of two families of biocompatible fluorinated surfactants stabilizing the NDs shell called ”F-TAC” (1) and ”Dendri-TAC” (2) and their use in the preparation of monodisperse perfluorocarbon NDs. Indeed, a bottom-up lab-on-a-chip approach using a microfluidic platform (NanoAssemblr® from Precision NanoSystems) is used for the preparation of various size-controlled NDs (3). Moreover, we discuss the effect of formulation parameters and process settings on the NDs properties, including size, monodis- persity and *in vitro* stability. Finally, the acoustic behavior of the generated NDs is addressed by determining the ADV threshold using a custom-made set-up (4).

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Keywords: Nanodroplet, Monodisperse, Microfluidic, Fluorinated surfactant, Biocompatible, Ultrasound

B41. Preparation, characterization and in vitro behavioral of condensed lipid-coated perfluorocarbon nanodroplets

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Phase-shift contrast agents, also known as perfluorocarbon nanodroplets (PFC-NDs), consist of a liquid perfluorocarbon core surrounded by a lipid layer that can be vaporized by ultrasound to generate echogenic microbubbles and thus a contrast with excellent spatiotemporal control. This phenomenon is called Acoustic Droplet Vaporization (ADV). These NDs hold a great promise for ultrasound biomedical applications including diagnostic and therapy.

In the present study, perfluorobutane nanodroplets are prepared by condensation of precursor lipid-coated microbubbles (MBs) and are thoroughly characterized using fit-for-purpose analytical tools such as Coulter Counter Multisizer, Nanoparticle Tracking Analysis (NTA), Zetasizer Nano and gas chromatography (GC). NDs/MBs features including size distribution, concentration, surface charge and gas core content are systematically determined. Moreover, the NDs *in vitro* stability diluted in blood matrix is also investigated using a GC analytical procedure by assaying the PFC core. Finally, the acoustical behavior of the generated nanodroplets is addressed by determining the ADV threshold using a custom-made set-up.

Collectively, these results highlight the usefulness of appropriate and orthogonal characterization procedures to better design and control the physico-chemical and acoustical properties of new sonoresponsive nano-sized contrast agents.

Keywords: Nanodroplet, Perfluorocarbon, Particle characterization, In vitro stability, Ultrasound

B42. Exploring the Potential of Exosomes as Diagnostic, Prognostic, and Drug Resistance Biomarkers: A Comprehensive Review of In Vivo Studies

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Exosomes are small extracellular vesicles that have recently emerged as promising biomarkers for the diagnosis, prognosis, and treatment of various diseases, including cancer. Exosomes have been shown to contain specific biomolecules that can be used as diagnostic and prognostic markers. For example, exosomes derived from cancer cells may contain specific proteins or nucleic acids that provide information about the cancer type, stage, or response to treatment. Furthermore, exosomes may be involved in drug resistance, and their analysis may provide insight into the mechanisms underlying resistance to chemotherapy.

The purpose of this review is to provide an overview of current knowledge regarding the use of exosomes as diagnostic, prognostic, or drug resistance biomarkers in in vivo studies. This review begins by discussing exosome biogenesis and composition, followed by a description of the methods used to isolate and characterize exosomes. This review focuses on the use of exosomes as biomarkers in cancer.
Overall, this review highlights the potential of exosomes as biomarkers for cancer diagnosis, prognosis, and treatment. However, further research is required to verify their clinical utility and develop standardized methods for their isolation and analysis.

Keywords: Exosome, Cancer, Diagnostic, Prognostic, Resistance

B43. Vivoptic, a preclinical optical imaging platform for the evaluation of diagnostic and therapeutic strategies

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Vivoptic is an approved platform for preclinical experimentation located at the Bordeaux Biomedical Imaging Institute. Labeled France Life Imaging (FLI), it offers not only access to optical imaging equipment for small animals after user training, but also experimental models (genetically modified lines, in vivo tumor models) as well as a set of therapeutic devices. This L1 biological level platform (no pathogens) has fully equipped surgery and animal preparation rooms (anesthesia stations, monitoring (ECG, T◦, respiratory rate), micro-injector, stereotaxic frame, etc.). Vivoptic can also help you design the *in vivo* experiment, support you throughout your project and analyze the results.

1 Vivoptic an optical imaging platform

Optical imaging is widely used in cancer research, cardiology and neurology and is a convenient tool for first pharmacological and biodistribution evaluations of nanoparticles labeled with fluorescent dyes, targeting assessment and new therapies. After an initial training, Vivoptic offers you free access to optical imaging devices for bioluminescence and fluorescence imaging.

2 Vivoptic, a complete offer for in vivo evaluation of your diagnostic and therapeutic agents or your innovative therapeutic strategy.

Vivoptic can provide you a library of optical imaging reporter genes (luciferase, NIR fluorescent proteins), vectors, and modified cell lines. Vivoptic also provide immunocompetent and immuno-compromised mouse models of sub-cutaneous, orthotopic and metastasis solid tumors especially dedicated for optical imaging monitoring. Generation of new biological models adapted to your own project is also possible.

3 Vivoptic, a place to share preclinical therapy devices

Therapeutic devices for in vivo gene therapies (electroporation), magnetic hyperthermia, pho- todynamic therapies (PDT), high intensity focused ultrasound and a clinical and preclinical echograph for imaging and image-guided surgery are also available at Vivoptic.
Vivoptic is a certified place for animal experiment, why not consider Vivoptic to install your own preclinical therapeutic setup?

Keywords: in vivo optical imaging, biological models, therapy devices

B44. Self-assembling properties of the new styrylpyridinium dyes and evaluation of their biological properties

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Styrylpyridinium salts are widely studied both as imaging agents for biochemical, biophysical, and molecular biology applications due to fluorescent properties and as prospective compounds with biological properties, for example antimicrobial and antifungal activities. The development of new compounds with desirable photophysical properties is a challenge for the researchers working in this field. The aim of the study was the synthesis and evaluation of self-assembling and biological properties of new styrylpyridinium derivatives as prospective theranostic agents.
Styrylpyridinium derivatives **3** were synthesized from the appropriate aldehydes **1** and 4-picolinium salts **2**. The mixture was refluxed for 24h in ethanol solution in the presence of piperidine.

Dynamic light scattering method was used for the estimation of self-assembly; samples were prepared by an injection method as aqueous solutions. Styrylpyridinium derivatives with longer alkyl chains at pyridinium nitrogen atom formed homogenous nanoparticles with an average diameter in the range of 118-597 nm depending on the structure of compound. Cell staining with one of compound revealed the strong fluorescent signal localised in the cell cytoplasm, whereas the cell nuclei were not stained. Cytotoxicity of compounds *in vitro* was assessed by the MTT test on tumor cell lines – HT-1080 and MH-22A and normal mouse fibroblasts NIH3T3. Compounds with longer alkyl chains at pyridinium have high basal toxicity. More details are described in our recent article.

Keywords: styrylpyridines, julolidine, self, assembly, near, infrared fluorescent dye, cell labelling, macrophages

B46. Interaction between Carbon Dots from folic acid and their cellular receptor: a qualitative physicochemical approach

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According to the World Health Organization, the number of cancers (all cancers, both sexes, all ages and worldwide) in 2020 reached a total of 19 292 789 new cases leading to 9 958 133 deaths during the same period. Many cancers could be cured if detected early. Preventing cancer or detecting it early are two essential strategies for controlling this pathology. For that purpose, several strategies have been described for imaging cancer cells. One of them is based on the use of Carbon dots, carbon nanoparticles.
The literature describes that cancer cells can be imaged using Carbon dots obtained from Folic acid and that the *in cellulo* observed photoluminescence probably results from the interaction of these nanoparticles with folic acid-receptor, a cell surface protein overexpressed in many ma- lignant cells. (1)

However, this interaction has never been directly demonstrated yet. We investigated it, for the first time, using (i) freshly synthesized and fully characterized Carbon dots, (ii) Folate Binding Protein, a folic acid-receptor model protein and (iii) fluorescence spectroscopy and isothermal titration calorimetry, two powerful methods for detecting molecular interactions.

Our results even highlight a selective interaction between these carbon made nano-objects and their biological target. (2)

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Keywords: nanoparticles, receptor, folate binding protein

B47. HPMA-based delivery systems conjugated with porphyrins used in photodynamic therapy and tumour imaging

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Photodynamic therapy (PDT) uses a light-sensitive photosensitizer (PS), such as tetraphenyl- porphyrin (TPP), in combination with illumination for the treatment of various malignant tu- mours. The light-activated PS reacts with oxygen in the tumour tissue leading to the formation of reactive oxygen species inducing cell death. (1) Unfortunately, the PS's possible use in PDT is restricted due to their limited solubility and/or low stability in physiological conditions or their lack of tumour selectivity.
Binding of PS to nano-carriers such as biocompatible, water-soluble, and non-toxic *N* -(2-hydroxypropyl)methac (HPMA) copolymers can improve its physico-chemical properties. Moreover, due to the En-
hanced Permeability and Retention (EPR) effect, the nano-carriers are passively accumulated in tumour tissue amplifying the therapeutic outcome. (2)

This study presents the synthesis, physico-chemical and biological characterisation of HPMA- based conjugates with up to 6 wt.% TPP. PSs were bound by pH degradable hydrazone bond via either aliphatic or aromatic spacer. The structure-release rate dependency was studied in conditions mimicking the healthy tissue and the acidic lysosome environment of the tumour cells. Remarkable increase of *in vitro* cytotoxicity was observed after illumination with λ=420 nm in comparison with no illumination. Fluorescence of free TPP vs conjugated with HPMA was measured.

Acknowledgement:
This work was supported by the Czech Academy of Sciences (project no. CNRS-22-01).

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Keywords: porphyrin, HPMA, delivery system, photodynamic therapy, tumour imaging

B48. Poly(2-ethyl-2-oxazoline)s as versatile platform for biomedical applications

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The biocompatible polymers have gained researchers' interest and become useful in drug delivery systems in a number of complex applications. These polymers might be directly attached to pharmaceuticals and thus can be a part of the drug substance. There are plenty of biocompatible and biodegradable polymers widely used in medicine such as poly(ethylene glycol) (PEG), *N* -(2-hydroxypropyl) methacrylamide (HPMA), another class of high-quality polymers is polyoxazolines. The poly(2-oxazoline)s (POx) are products of cationic ring-opening polymerization (CROP) of 2-oxazoline monomers. By varying the initiator and termination agents, both *α*- and *ω*-chain ends of POx can be functionalized. The physico-chemical properties can be modulated by varying the alkyl substituent. We herein report synthesis of star poly(2-ethyl-2-oxazoline)s (PEtOx) with three and four branches with maleimide function. The microwave assisted polymerization was performed in acetonitrile using corresponding triflates as initiators. The Diels-Alder furan protected maleimide was used as termination agent. The cleavage of protection group was done by retro Diels-Alder reaction in toluene. The structure of synthesized polymers has been confirmed from ^{1}H NMR, UV-VIS, IR, SEC, MALDI-TOF-MS spectra. Furthermore, we demonstrated that synthesized PEtOx with maleimide end-groups are accessible for the Michael addition reaction of small molecules like N-(tert-butoxycarbonyl)-L-cysteine methyl ester and are able to the conjugation of drugs, proteins, nanoparticles etc.

Keywords: star polymers, poly(2, ethyl, 2, oxazoline)s, polyoxazolines

B49. Development of EGFR targeted magnetic nanovectors co-delivering siRNA and doxorubicin for the treatment of triple-negative breast cancer.

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Triple-negative breast cancer (TNBC) represents 15 to 20% of breast cancers (1). Its poor prognosis resulting from its aggressiveness, heterogeneity, lack of therapeutic options and chemoresistance, makes the need for new treatments compelling.
Therefore, our aim is to develop targeted magnetic nanovectors (NVscFv), suitable for IV injection, co-vectorizing doxorubicin (DOX) and siRNA (NVscFv-si-DOX) in order to (re)sensitize TNBC cells to chemotherapy.
NVscFv are generated from superparamagnetic iron oxide nanoparticles (SPIONs), labelled with a near infrared fluorochrome (DyLightTM 680), coated with NHS-PEG5000-Maleimide to increase stealthiness and functionalized with antibody fragments (scFv) targeting EGFR that is overexpressed in around 70% of TNBC (2). NVscFv met the objectives of a hydrodynamic diameter (DH) around 100nm, a polydispersity index (PDI) lower than 0.3 and a surface charge close to neutrality. Moreover, indirect ELISA assay was used to verify successful grafting of scFv targeting EGFR.
Afterwards, DOX was loaded onto NVscFv (NVscFv-DOX) through a DOX/iron pH-sensitive complex developed by our team that interacts directly with the surface of NVscFv (3). DOX-loading analysis performed using spectrofluorimetry showed better results using a DOX/iron molar ratio of 1:1.5 and TRIS buffer, resulting in a loading of 154.3 mg DOX/g iron on NV.
The next step is to load the DOX/iron complex and siRNA targeting ABC transporters (ABCG2) and/or antiapoptotic proteins (Bcl-xl, survivine) onto the same NVscFv in order to formulate NVscFv-si-DOX suitable for IV injection. By decreasing chemotherapy efflux and/or restoring apoptosis, these nucleic acids will have a synergistic effect with doxorubicin, thus, increasing its cytotoxic effect. Through vectorization with NVscFv-si-DOX, the hope is to reduce side effects of DOX and to protect siRNA from degradation while ensuring proper delivery of both therapeutics agents to TNBC cells. Formulation of control siRNA with NVscFv was already successfully achieved through electrostatic interactions with cationic polymers (poly-L-arginine chitosan) (4).

In parallel, the obtained NVscFv-DOX and NVscFv-si will be incubated with TNBC cell lines (MDA-MB-468, MDA-MB-231) in order to perform cytotoxicity assays and to determine the most efficient *in-vitro* treatment protocol

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Keywords: magnetic nanovectors, SPIONs, doxorubicin, siRNA, EGFR, triple negative breast cancer

B50. AGuIX nanoparticle-nanobody bioconjugates to target immune checkpoint receptors

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This presentation relates to bioconjugates combining nanoparticles (AGuIX) with nanobodies (VHH) targeting Programmed Death- Ligand 1 (PD-L1, A12 VHH) and Cluster of Differentiation 47 (CD47, A4 VHH) for active tumor targeting. AGuIX nanoparticles offer theranostic capabilities and an efficient biodistribution/pharmacokinetic profile (BD/PK), when VHH's reduced size (15 kDa) allows efficient tumor penetration. Site-selective sortagging and click chemistry were compared for bioconjugation. While both methods yielded bioconjugates with similar functionality, click chemistry demonstrated higher yield and could be used for the conjugation of various VHH. The two methods resulted in NPs with similar physicochemical characteristics using the A12 VHH. However, click chemistry exhibited higher VHH conversion while using lower-cost reagents, making it a more favourable approach for further investigations. Interestingly, the two bioconjugation methods yielded AGuIX@VHH with strong binding affinities, suggesting that site-specific VHH modification, such as sortagging, may not be required when coupling to relatively small NP such as AGuIX NP. PD-L1 targeting of AGuIX-A12 prepared by click chemistry was validated by ex vivo autoradiograpy demonstrating substantially greater uptake and persistence than untargeted NP. The A4 nanobody was used to validate? the reliability and reproducibility of click synthesis for these constructs. Overall, click chemistry emerges as a favourable and promising approach for preparing a broad array of potential AGuIX@VHH bioconjugates that can be applied to multiple nanobodies, with diverse applications in combined targeted immunotherapies, radiotherapy, and cancer imaging.

B51. Introduction to the New Malvern Panalytical Nanosight Pro

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In 2023, Malvern Panalytical launched the new Nanosight Pro, the last generation of nanopar- ticle tracking analysers. Nanoparticle Tracking Analysis (NTA) is a method able to provide unique visualization of Brownian motion. As the speed of particles correlates with their size, this technique is able to give high resolution particle-by-particle analysis of size and concentra- tion.
This presentation will introduce the main features and innovations brought by this new analytical solution for the characterization of nanoparticles. Amongst them we can cite increased sensitivity and reproducibility for scatter and fluorescence mode. But also, drastic improvements in ease of use and standardization of measurements.

Please visit Malvern Panalytical website if you need more information (https://www.malvernpanalytical.com/en range/nanosight-range/nanosight-pro)

Keywords: nanoparticle tracking analysis, analytical solution, Malvern Panalytical, nanoparticle, Nanosight Pro

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