Tuesday, December 10th

Session: NANOPARTICLES &TARGETING

16h00 – 18h15

Keynote speaker: Tomas ETRYCH
Targeted polyHPMA-based nanomedicines for cancer and inflammatory treatment

Romanée – Conti Amphitheater

Abstracts

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticules & therapeutic targeting & Nanochemistry: synthesis and functionalization of nanosystems for bioapplications

Keywords: Neuropeptide, painkiller, nanomedicine, squalene, pain

A new painkiller nanomedicine to by-pass the blood-brain barrier and the use of morphine

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Enkephalin is an endogenous pentapeptide producing potent analgesia by activating opioid receptors. However, its clinical use has historically been limited due to pharmacokinetic issues, including restricted plasma stability and blood-brain barrier permeability. This project describes a new enkephalin-based nanomedicine targeting pain, using biocompatible and biodegradable materials for drug delivery and targeting purposes, such as squalene. Here, we show for the first time, that the rapidly metabolized Leu-enkephalin (LENK) neuropeptide may become pharmacologically efficient owing to its simple conjugation with a lipid squalene derivative (SQ). LENK neuropeptide was included into nanoparticles (NPs) by conjugation to squalene using three different chemical linkers. This new squalene-based nanoformulation prevented rapid plasma degradation of LENK and conferred to the released neuropeptide a significant anti-hyperalgesic effect in a carrageenan-induced paw edema model in rats (Hargreaves test) which lasted longer than after treatment with morphine. Pretreatment with *opioid* receptor antagonists such as naloxone (brain-permeant) and naloxone methiodide (brain-impermeant) reversed the nanoparticles induced anti-hyperalgesia, indicating that the LENK-SQ NPs acted through peripherally located opioid receptors. Moreover, the biodistribution of DiD-fluorescently labeled LENK-SQ NPs showed a strong accumulation of the fluorescence within the inflamed paw as well as in the liver, spleen, and lung, while no signal could be detected in the brain, confirming the peripheral effect of LENK-SQ NPs. This study represents a novel nanomedicine approach, allowing the specific delivery of LENK neuropeptide into inflamed tissues for pain control associated with inflammatory events.

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Thematic Session: Nanoscience for Cancer

Keywords: TRAIL, Iron nanoparticle, magnetic hyperthermia, photothermia, Cancer

Nanoparticles to remote-control TRAIL-induced cell death in cancer cells

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Abstract

TRAIL (Tumor necrosis factor (TNF)-Related Apoptosis-Inducing Ligand) has long been considered a holy grail in cancer treatment owing to its ability to selectively eradicate tumour cells. However, the poor formulations evaluated so far in the clinic have all but one failed to provide evidence that this approach may be applicable to cure patients suffering from cancer. Yet, rationalized formulations of TRAIL or antibodies targeting TRAIL agonist receptors functionalized to nanoparticles could be proposed to achieve this objective. I will present here our most recent pre-clinical data demonstrating that the grafting of TRAIL or its derivatives onto iron oxide nanoparticles not only increases their antitumoral potential but could also allow, combined to magnetic hyperthermia (MHT) or photothermia (PT), to meet the grade for the clinic, thanks to the ability of remote-controlled “moderate hyperthermia” to further enhance TRAIL's antitumoral activity.

Belkahla H, Mazarío E, Sangnier AP, Lomas JS, Gharbi T, Ammar S, Wilhelm C*, Hémadi M*, Micheau O*. TRAIL acts synergistically with iron oxide nanocluster-mediated magneto- and photothermia. Theranostics. 2019 14;9(20):5924-5936.

Dubuisson A, Favreau C, Fourmaux E, Lareure S, Rodrigues-Saraiva R, Pellat-Deceunynck C, El Alaoui S, Micheau O. Generation and characterization of novel anti-DR4 and anti-DR5 antibodies developed by genetic immunization. Cell Death Dis. 2019 4;10(2):101.

Belkahla H, Herlem G, Picaud F, Gharbi T, Hémadi M, Ammar S, Micheau O. TRAIL-NP hybrids for cancer therapy: a review. Nanoscale. 2017 11;9(18):5755-5768.

Zakaria AB, Picaud F, Rattier T, Pudlo M, Dufour F, Saviot L, Chassagnon R, Lherminier J, Gharbi T, Herlem G*, Micheau O*. Nanovectorization of TRAIL with single wall carbon nanotubes enhances tumor cell killing. Nano Lett. 2015 11;15(2):891-5.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles and targeting
Keywords: Microfluidic, liposomes, microbubble, nanodroplets

Use of microfluidic technology to prepare nano/micro particles for diagnostic and therapeutic applications

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Several clinically used vehicle systems including liposomes, lipid nanoparticles and microbubbles have been already marketed for a large variety of diagnostic and therapeutic indications. In fact, numerous conventional preparation procedures are routinely applied; with each technique exhibiting strengths and weakness in terms of ease of use, cost effectiveness, production yield, control of size and polydispersity, etc. More recently, microfluidics has evolved as a powerful and scalable technique for the consistent manufacture of controlled assemblies

Design of Selectin-specific liposomes (LipoSelectin) will be presented using a microfluidic based system. LipoSelectin are applied to assess tissue biomarkers expressed in inflammation using Optical Imaging (OI) platform. To this aim, a recombinant P/E-selectin ligand is coupled to fluorescent liposomes. Liposomal formulations are thoroughly characterized and their ability to probe different inflammatory status is evaluated in clinically relevant Rheumatoid Arthritis (RA) mouse model.

Microfluidic flow-focusing technology will also be described for the preparation of monosize microbubbles (MSB). This new generation of gaseous particles displays a narrow calibrated and controlled size distribution compared to commercially available polydisperse microbubble ultrasound contrast-agents. These MSB are expected to result in increased imaging sensitivity and improved efficiency to deliver drug and gene to specific organs. Both acoustic and visco-elastic properties of MSB will be addressed.

The last example will be dedicated to the preparation of acoustically responsive phase shift nanodroplets. In contrast to microbubble, nanodroplets can target extravascular markers and extravasate into the tumor environment. The preparation and chemical/acoustic characterization of these PFC nanoconstructs will be highlighted.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticles & targeting
Keywords: PLGA, microparticles, bioadhesive hydrogel, perivascular delivery

Perivascular drug delivery system for prevention of vein graft failure

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Intimal hyperplasia (IH) is a pathophysiological process, which starts immediately after vascular bypass surgery and affects walls of the vein graft. The vessel walls thicken, decreasing the graft lumen to the point of restenosis (vein graft failure) with the need for revascularization. IH poses an increased risk for the patient, and as it occurs in as often as 50% of the cases, it is a health issue and an economical burden. We aim to develop a perivascular system delivering a local treatment for the prevention of IH.

The system made of a hydrogel-microparticle formulation will be applied locally to the exterior surface of the graft during surgery. The system loaded with atorvastatin has proved efficient against IH (1, 2). To have longer residence time on site, we aim to use mussel-inspired dopamine conjugation to the hyaluronic acid (HA) hydrogel. To increase the drug loading of the poly(lactic-co-glycolic acid) (PLGA) microparticles, a spray-drying technique was developed.

Dopamine was conjugated to HA hydrogel with a degree of substitution of 15% to promote adhesion to biological surfaces. Microparticles of 9 and 18% (w/w) drug loading have been produced with a spray-drying technique. Particle size was characterized by laser diffraction and drug loading and release studies were performed with U-HPLC. Having elaborated the 2 components of the hydrogel-particle system enables us to combine them for further characterization, subsequent *in vivo* studies and use in the clinics.

1. Mylonaki I, et al. Perivascular sustained release of atorvastatin from a hydrogel-microparticle delivery system decreases intimal hyperplasia. J. Contr. Rel. 2016;232:93-102.
2. Dubuis C, et al. Atorvastatin-Loaded Hydrogel Affects the Smooth Muscle Cells of Human Veins. J. Pharm. Exp. Ther. 2013;347(3):574-81.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticles and targeting

Keywords: Thiolate protected gold nanoparticles, bioconjugation, targeting, nanobody, electron microscopy probes

Novel synthetic routes for small and defined gold nanoparticle-nanobody conjugates

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Gold nanoparticles (AuNPs) with countable number of gold atoms and organic thiolates as surface covering ligands being in the size range of 2 nm were recently developed. These AuNPs diffuse well inside cells without getting trapped within the filamentous cytoskeleton. Furthermore, they do not trigger apparent cytotoxicity and the surface coordinated ligands can be exchanged to other thiol-containing molecules, affording the opportunity to generate AuNP bioconjugates in a straightforward manner. Our aim is to demonstrate that these AuNPs can be used to upgrade the currently used immunogold labeling probes and hence to complement the advancing electron microscopy (EM) technologies. The classically used EM probes are 5-10 nm colloidal gold-antibody conjugates. Their large sizes limit diffusion and the distance of ca. 10 nm between the binding site and the gold core limit spatial resolution. To solve these issues, we propose to switch from large antibodies to smaller nanobodies and to use thiolate-coated AuNPs having diameters of ca. 2 nm.

We set up the synthesis of 2.4 nm thiolate-protected AuNPs and an anti-GFP nanobody was genetically engineered to generate AuNP-nanobody conjugates using a site-selective conjugation strategy via thiol-Au coordination bonds. We synthesized AuNP conjugates with various numbers of nanobodies and passive ligands. Some of these conjugates demonstrated effectiveness for selective localizing of GFP-fusion proteins inside mammalian cells.

Altogether, we demonstrate that 2.4 nm AuNP-GFP nanobody conjugates can be synthesized and used as immunogold labeling probe, opening new ways to localize proteins inside cells at increased accuracy using EM.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanomedicine

Keywords: liposomes, cyclodextrins, temoporfin, photodynamic therapy, tumor spheroids

Hybrid liposomes for temoporfin delivery to the tumor tissue

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The present study is aimed at the development of drug-in-cyclodextrin-in-liposome (DCL) nanoparticles by coupling two independent delivery systems: cyclodextrin/mTHPC inclusion complexes and liposomal vesicles to improve the transport of mTHPC to target tissue and to strengthen its intra-tissue accumulation in tumor. Liposomes offer an excellent opportunity to achieve selective drug targeting what is expected to prevent local irritation and reduce drug toxicity. Cyclodextrins (CDs) have been utilized as independent carriers for improvement of pharmaceutical properties such as solubility, stability and bioavailability of various drug molecules, including mTHPC. Therefore, we assumed that encapsulation of CD-complexed drug into liposomes may increase drug loading capacity, entrapment efficiency, may restrain the dissociation of drug-CD complexes and prolong its systemic circulation.

We prepared DCLs with various compositions to optimize the structure. It was demonstrated that mTHPC-DCLs are stable and almost all mTHPC is bound to β-CDs in the inner aqueous liposome lumen. The influence of DCLs on mTHPC accumulation, distribution and photodynamic efficiency was studied in human adenocarcinoma HT29 cellular monolayer and spheroid models. Among all tested DCLs, double loaded DCL, which include mTHPC in lipid bilayer along with (CD-mTHPC) inclusion complexes in the inner aqueous lumen, displayed the highest potency for mTHPC delivery. Using 3D multicellular HT29 tumor spheroids we demonstrated that trimethyl-β-CD-based DCL provides homogenous accumulation of mTHPC across tumor spheroid volume thus supposing optimal mTHPC distribution.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer

Keywords: Active targeting, triggered delivery, magnetic nanoparticles, local heating, alternative magnetic field, molecularly imprinted polymer

Magnetic Molecularly Imprinted Polymer for Cancer Therapy

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Abstract

Molecularly imprinted polymers (MIP) are cross-linked polymer networks presenting very specific recognition sites for a template molecule. A great interest is nowadays focused on their use in nanomedicine, e.g. for *in vivo* protein targeting, because conventional methods employed to target cells have proved to be very effective but difficult to produce, requiring either animal hosts or expensive and time-consuming synthetic methods.

As magnetic nanoparticles can heat when they are submitted to alternative magnetic field (AMF), their combination to MIP could open doors to a novel class of synthetic materials for nanomedicine.

In this context, since several years, I developed magnetic MIP containing doxorubicin (DOX) with the objective to release this drug under AMF for cancer cell death without macroscopic temperature elevation and without passive diffusion. We showed that after internalization in cells, the nanoparticles did not induce cancer cell death demonstrating that when bonded to the MIP, DOX is inactive. By contrast, after AMF application, the cell viability is reduced to 60% after 1 h30 min treatment. It is very important to emphasize that this AMF-induced cancer cell death was achieved under athermal conditions.

We then became interested in printing a protein with a strong interest in nanomedicine, the differentiation complex 44, or CD44, to develop a system that specifically targets cells expressing this protein on their surface. CD44 is a protein especially present on cancer cells called "triple negative". We showed for the first time the possibility to specifically target cancer cells overexpressing CD44 using magnetic MIP nanoparticles.