Abstracts

Poster Session

P1 - NANOPARTICLES & TARGETING

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticles & targeting
Keywords: *in vivo* targeting, inorganic nanoparticles, *in vivo* imaging,

In vivo targeting PSMA expressing cells in prostate cancer with scFv functionalized nanoparticles

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Multifunctional hybrid nanoparticles, offer promising applications in nanomedecine. Given their multifunctionality, hybrids NPs are developed for multimodal diagnosis or in therapy for theranostic approaches to follow the treatment by medical imaging. Despite these potentialities, clinical trial using hybrids NPs are scarce. These NPs administered by systemic route are not enough efficient due to a premature hepatic clearance and a low accumulation in tumors. Development of an optimized surface chemistry can improve tumor cells targeting by grafting a biomolecule which specifically recognized receptors overexpressed by tumoral cells. Fluorescent-labelled anti-PSMA scFv (single chain antibody fragment) was reported as an efficient probe for specific prostate cancer in a mouse model [1]. The aim of this study is to functionalize silica NPs with anti-PSMA scFv to improve specific targeting in prostate cancer.

Dually fluorescent silica NPs of 20 nm were synthetized by a Stöber method, and covalently covered with a dense PEG layer [2]. Anti-PSMA scFv fragments were grafted onto silica NPs. Flow cytometry analysis was performed in order to determine the optimal number of scFv per NPs to have the best binding properties. According to these results, functionalized NPs were injected in mice and tumor accumulation was monitored by fluorescence imaging. Results showed that NPs accumulated into both PSMA– and PSMA+ tumors but fluorescent signal from functionalized NPs specifically increases in PSMA+ tumors. Internalization of NPs in cancer cells was confirmed by histology. Grafting anti-PSMA scFv onto silica NPs enhance accumulation of NPs in PSMA expressing tumors but did not abolish non-specific accumulation in tumors that do not express PSMA.

Reference: [1] *Sci. Rep.* **2016**, 6, 23314. [2] *Biochim. Biophys. Acta*. **2017**, 1587-1596

The SFNano C'NANO joint meeting 2019

Thematic Session: (Aqueous synthesis, copper nanoparticles, transparent electrodes)
Keywords: (Nanoparticles, copper, optoelectronic, synthesis)

Copper-Based Nanoparticles Synthesis

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One-dimensional nanoparticles of transition metals, such as gold (Au), platinum (Pt), silver (Ag) and copper (Cu) nanowires (NWs) are important conducting materials for fabrication of electronic nano-devices. Compared with bulk metal, metal NWs exhibit unique electronic and optical properties due to their high aspect ratios and nanoscale diameters.

Cu NWs are of particular interest not only because of their relatively low cost but also because of their excellent electrical properties. We made contributions to this burgeoning topic in the important areas of production of metallic nanowires of controlled dimensions, electrode stability and electrode conductivity. Thanks to a hydrothermal process, we showed that we could synthesize copper nanostructures of controlled dimensions, in high yield.

The approach that we have developed offers many advantages in term of ease of mass production, product quality and simplicity over the prior art processes. Since the copper corrodes in the atmosphere, the conductivity of nanowire electrodes is well known to reduce over time. Furthermore, copper nanowires sold commercially are highly sensitive to daylight and humidity. It is necessary to passivate the Cu NWs to remove oxidation.

Yet, copper oxides also present interesting properties. Cu_2O is well known as a good catalyst (water splitting, macromolecules photodegradation). These catalytic properties are proportional to the specific surface area, the size and shape of Cu_2O NPs are a key parameter to these processes.
Then, Copper oxide nanoparticles (Cu_2O NPs) were produced through polyol synthesis, a technique well known and controlled by our team.

The SFNano C'NANO joint meeting 2019

Thematic Session: nanoparticles

Keywords: microparticles, pectin, shea butter, rutin, characterization

Influence of shea butter amount on the properties of calcium-pectin microparticles

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Low methoxyl-pectin (LMP) is a polysaccharide mainly used to obtain microparticles by ionotropic gelation with calcium ions. However, the applications of this biopolymer are limited due to its rapid hydration and dissolution in water producing an immediate release.
So to better control drug release, different amounts of shea butter from Ivory Coast (20, 30 and 40 %) were added to LMP and the mixture was used to produce microparticles containing rutin as a model drug. Then the properties of microparticles after drying were investigated by morphological characterization with optical microscope. The encapsulation efficiency and yield and moisture content were also determined. Rutin *in vitro* dissolution was performed using a paddle apparatus.
All amounts of shea butter allowed to produce microparticles with quite a round shape. Microparticle size increased as more shea butter were used (from 3.2 ± 0.3 mm to 3.7 ± 0.2 mm) and on the opposite, the moisture content decreased (from 7.1 ± 0.2 % to 2.1 ± 0.3 %). The encapsulation yield was high for all the microparticles (above 88%) but the encapsulation efficiency decreased when the amount of butter increased. Finally rutin release was also influenced by the amount of shea butter (with 20% of shea butter, 50% of rutin release were achieved in 7h while 100% with 30 and 40% at the same time).
To conclude, the amount of shea butter in pectin microparticles led to microparticles with different properties. From 30%, the microparticles were more sensitive to the environment with a loss of controlled release and drug protective effect.

Thematic Session: Nanoparticles & targeting

Keywords: Age-Related Macular Degeneration, omega-3 fatty acids, cholesteryl esters, triglycerides, nanoemulsion, intranasal delivery

Preparation of omega-3 polyunsaturated fatty acid-rich cholesteryl esters and triglycerides in nanoemulsions for intranasal delivery in AMD.

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Age-related Macular Degeneration (AMD) is strongly related with omega-3 polyunsaturated fatty acid (PUFA) metabolism, particularly with that of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as cholesteryl esters (CE), which have shown better incorporation in the retina compared to triglycerides (TG) rich in omega-3 PUFAs. The physiological efficacy of such components on the background of AMD is dependent on their physicochemical nature and bioavailability across biological barriers. In the present work, we have prepared DHA- and EPA-rich CE and TG in order to incorporate them into nano-sized emulsions for intranasal delivery in AMD. For that purpose, a three-step experimental design was conducted, consisting in 1) synthesis and purification of compounds, 2) analytical validation followed by 3) the preparation and the characterization of omega-3 PUFA-rich nanoemulsions. Considerable yields were obtained for the synthesis of CE- rich in EPA and DHA whereas a yield of 100% and 85% was achieved for purified TG. Final nano-formulations were prepared as nanoemulsions through Phase Inversion Temperature obtaining nano-droplets at 20-25 nm. Physicochemical studies showed stable DHA and EPA content in the nanoemulsions. Future experimental studies could be conducted on animal models through pharmacokinetic and pharmacodynamic analyses. This formulation with nano-sized droplets can be utilized as a smart delivery vehicle for designing intranasal administration therapies on the background of AMD.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & therapeutic targeting

Keywords: polyphosphate nanoparticles, mucus permeation, intestinal alkaline phosphatase, enzymatically-triggered cellular uptake, zeta potential change

Intestinal Alkaline Phosphatase Triggered Zeta Potential Changing Nanoparticles across Mucus and Epithelial Barriers

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Abstract

Mucosal drug delivery by nanocarrier systems is an engaging research topic in the field of nanomedicine. However, key parameters of these systems, such as surface charge, should be considered exhaustively taking barrier function of the mucus gel layer and underlying epithelium into account. The negative charge is required to avoid ionic interactions between substructures of the mucus gel layer and carrier system, whereas positively charged carriers are up-taken by epithelial cells to a higher extent. Therefore, it was aimed to develop zeta potential-changing polyphosphate nanoparticles (pp-NPs) utilizing the enzymatic stimuli by intestinal alkaline phosphatase (IAP). pp-NPs were obtained by in situ gelation and characterized concerning size and zeta potential. Phosphate release studies were carried out by incubation of pp-NPs with isolated as well as cell-associated IAP and quantified by malachite green assay. Change in the zeta potential was measured, and pp-NPs were analyzed by scanning electron microscopy studies. Mucus permeation was investigated with porcine intestinal mucus via the transwell insert method and rotating tube method. Cell viability and cellular uptake studies were performed on Caco-2 cells. Within 4 h, a remarkable amount of phosphate was released from pp-NPs incubated with isolated IAP as well as cell-associated IAP and zeta potential raised from −9.14 ± 0.45 to −1.75 ± 0.46 mV. Compared with dephosphorylated polyphosphate nanoparticles (de-ppNPs), an enhanced mucus permeation of pp-NPs was observed. Moreover, pp-NPs did not exhibit cytotoxicity. Cellular uptake increased 2.6-fold by conversion of pp-NPs to de-pp-NPs following enzymatic triggering by IAP.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & targeting
Keywords: Liposomes, cyclodextrins, chlorpromazine, AML, drug re-purposing

Encapsulating chlorpromazine in cyclodextrin-liposomes for treatment of myeloid malignancies

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Re-purposing of established drugs can be an efficient way developing new therapies against a range of diseases. This is common in cancer chemotherapy, where for instance thalidomide and analogues have shown effectiveness against myeloid diseases.

Chlorpromazine (CPZ) is identified as a potential drug against acute myeloid leukaemia (AML) based on its effects on haematopoietic tissues. We have confirmed the cytotoxic effect of CPZ against multiple leukemic cell lines. However, as CPZ was originally used as an anti-psychotic drug treating schizophrenia, its effect on the central nervous system (CNS) must be diminished if used against AML. Thus, CPZ necessitates a formulation to prevent it crossing the blood-brain barrier (BBB).

We loaded CPZ in the central cavity of various cyclodextrins (CD); Sugammadex (SGM), a γ-CD, hydroxypropyl-γ-CD (HP-γ-CD), and sulfobutyl ether- β-CD (SBE-β-CD). After verifying that CPZ-CD inclusion complexes were successfully formed, we encapsulated them within liposomes. Importantly, liposomes with or without cholesterol were stable in the presence of CD.

The CPZ-CD-loaded liposomes were tested for cytotoxicity on AML and normal cell lines. While liposomes with HP-γ-CD were similarly potent to free CPZ, liposomes using SBE-β-CD and SGM showed reduced cytotoxicity. When studying cellular internalization of fluorescent liposomes, no correlation between liposome uptake and cytotoxicity was found. Differences in cytotoxicity is thus likely to be connected to the close association between CPZ and CD.

In conclusion, drug-in-cyclodextrin-in-liposomes (DCL) appear to be a promising platform to encapsulate anti-cancer drugs with the aim of minimizing toxic side effects and enhancing therapeutic potential.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticles & targeting
Keywords: Dye-loaded nanoparticles, Targeting, Oxytocin receptor, Apoptosis

Ultrabright fluorescent dye-loaded polymeric nanoparticles for targeting lipids and proteins

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Dye-loaded polymeric nanoparticles are a powerful bioimaging tool because of their high brightness and capacity to bear multiple functional groups[1]. Our group developed a class of ultrabright dye-loaded polymeric nanoparticles based on hydrophobic cationic dyes and bulky hydrophobic counterions; these nanoparticles are brighter than quantum dots and nontoxic[2]. In this work we functionalized these nanoparticles for obtaining a targeting system.

Polymeric poly(methyl methacrylate-co-methacrylic acid) (PMMA-MA) based nanoparticles when incubated with HeLa cells readily get internalized by endocytosis, therefore the first step in this project is producing NPs that do not interact with cells. This was achieved by "clicking" PEG2000 bearing activated alkyne (DBCO) on nanoparticles functionalized with azide groups. PEGylated NPs were tested on HeLa cells via flow cytometry and fluorescence microscopy and we found that, unlike bare control particles, PEGylated nanoparticles do not aggregate in PBS and FBS and do not adsorb on the surface of HeLa cells. Afterwards PEG2000 chains bearing a DBCO group on one end and a targeting unit on the other were synthesized. Two targeting units were chosen: (i) an orthoacetyl boronic acid derivatives that binds to amine-bearing lipids[3], which are present on the outer leaflet of the plasma membrane in bacteria and eukaryotic apoptotic cells, and (ii) carbetocin, an oxytocin analogue that binds to G-protein coupled receptor of oxytocin[4]. NPs bearing the boronic acid unit were prepared and their ability to target apoptotic cells was tested both via cytometry and fluorescence microscopy and it was found that they are able to selectively target apoptotic cells, while pure PEG2000 NPs do not display any significant difference in interaction between healthy and apoptotic cells.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & targeting
Keywords: nanoparticle ; design ; delivery ; mucosa ; vaccination

Impact of the inner structure of maltodextrin nanoparticles on the development of a mucosal vaccine

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Infections cause billions of diseases every year. They can lead to serious complications and even to death for weakened people, especially in developing countries. In order to efficiently decrease their spreading, the development of preventive vaccines remains the most effective way.

The administration route is an important choice in the design of a vaccine, because it directs the type of immune response against the pathogen. It is well known that parenteral routes can trigger humoral and systemic responses, but much studies have demonstrated that mucosal routes of administration (oral, nasal, vaginal and rectal) can also trigger mucosal responses. Since many infections are principally transmitted through mucosa, mucosal vaccination should then be a promising strategy.

There is a growing interest in the use of nanoparticles (NP) in vaccines. Indeed, the encapsulation of antigens inside vectors leads to in an increase of their biodisponibility and immunogenicity towards mucosal antigen presenting cells (APC), giving furthermore the possibility to reduce the administered doses.

Here, we compared different starch NP that differ in their density, to determine the most efficient antigen delivery nanovector in APC and finally to evaluate in vivo their ability to trigger the immune system through different administration routes: intradermal, nasal and rectal.

Reticulated and non-reticulated NPs have been synthesized. Their diameter, surface charge, colloidal stability and their ability to associate antigens were probed, and their ability to deliver antigens within APC and epithelial cells has then been evaluated. Finally, the immunostimulatory potential of the NPs/Antigen formulations have been evaluated on BMDC.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & targeting
Keywords: glioblastoma, canine, NFL-peptide, lipid nanocapsules, interaction

Effect of the NFL peptide on canine glioblastoma

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Glioblastomas are the most frequent and aggressive cancer of the nervous system. The standard treatment (Stupp protocol) is composed of neurosurgery followed by radiotherapy and chemotherapy, but the median survival remains very low (14 to 16 months). The NFL-TBS.40-63 peptide (NeuroFilament Low subunit-Tubulin Binding Site 40-63), also known as NFL-peptide, is able to specifically penetrate in all glioblastoma cell lines tested (rat, mouse and human), where it binds to tubulin, and blocks its polymerization into microtubules. Consequently, the peptide inhibits selectively the *in vitro* cell division of glioblastoma cells and their tumor development *in vivo* (Bocquet et al. 2009, Berges et al. 2012). When lipid nanocapsules are functionalized with the NFL-peptide, their uptake is targeted into glioblastoma cells both *in vitro* and *in vivo* (Balzeau et al. 2013). Similarly, the NFL-peptide enters massively in glioblastoma stem cells isolated from human patients, where it induces their death by apoptosis, and also targets lipid nanocapsules into these cells (Lépinoux-Chambaud and Eyer 2019). Here, we evaluated the impact of the NFL-peptide on glioblastoma cells from dogs using flow cytometry and microscopy. The presentation will document the capacity of NFL-peptide to interact with these cells, the cytotoxicity of the peptide, and the possible impact of lipid nanoparticles functionalized with NFL-peptide. This investigation reveals some similarities as well as major differences between canine and murine or human glioblastoma cells. This work is supported by Plan Cancer Inserm and Ligue contre le Cancer 49 to J. Eyer.

The SFNano C'NANO joint meeting 2019

Thematic Session: (Nanoparticles & targeting)
Keywords: (nanoparticles, curcumin, inflammation, IBD)

Study of mechanisms allowing nanomedicines To treat inflammatory bowel disease

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Curcumin is a natural polyphenol and essential curcuminoid isolated from the rhizome of the medicinal plant Curcuma *longa,* which exhibits pharmacologic properties owing to its antioxidant and anti-inflammatory activities. Despite its pleiotropic activities, the poor aqueous solubility and consequently, the rapid clearance and the low stability have limited its clinical application. However, studies proved that nanoparticles loaded with curcumin exhibited an increased solubility and bioavailability. The use of these nanovectors provides a better anti-inflammatory effects without the associated side effects, and could be useful in the treatment of inflammatory bowel diseases (IBD), that are a long-term illnesses due to intestinal self-sustained inflammation. The aim of this project was to demonstrate that association of curcumin to cationic porous NP with lipid core (NPL) improves the curcumin anti-inflammatory effects. Here by using TNF- α treatment, we compared healthy and inflamed *in vitro* intestinal epithelial cell models in order to mimics inflammation that occurred in IBD. We investigated the curcumin loading in NPL, the cellular uptake of curcumin and its effect on the secretion of cytokines. The results showed that the curcumin had a strong association to NPL that highly improves the uptake of curcumin by cells. A decrease of the production of pro-inflammatory cytokines and an increase of the anti-inflammatory ones is observed, associated with an absence of toxicity. This project could prove the concept of a better delivery of anti-inflammatory drugs like curcumin to reduce inflammation in IBD through nanotechnology.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticules & therapeutic targeting
Keywords: EGF receptor, HPMA copolymer, tumor imaging, endoscopic surgery

Actively targeted HPMA copolymer-based nanoprobes for head and neck cancer imaging

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Specific accumulation of the fluorescently labeled probe on the periphery of tumor mass is a prerequisite for successful fluorescence-guided endoscopic surgery as the fluorescence signal from peripheral malignant cells or tumor-associated endothelial cells enables more precise resection of the tumor with low level of the healthy tissue damage. This accumulation could be achieved by active targeting of the probe to EGF receptor plenteously represented on the surface of specific cancer cell lines (e.g. human hypopharyngeal carcinoma cells - FaDu). The presented construct is composed of *N*-(2-hydroxypropyl)methacrylamide based copolymer carrier decorated either with synthetic oligopeptide GE-11 (YHWYGYTPQNVI), oligopeptide GE-7 (NPVVGYIGERPQYRDL), both natural ligands for human epidermal growth factor (hEGF), their scrambled versions or commercial available antiEGFR monoclonal antibody cetuximab (Erbitux) and fluorescent dye Dy-633 or Cy-7.
Flow cytometry was employed for examination of the binding efficacy of targeted conjugates to epidermal growth factor receptor (EGFR) on the cell membranes of the malignant cells. The results showed that the highest binging efficacy was achieved with polymers bearing Cetuximab. *In vivo* experiment confirmed observation from flow cytometry and showed that nanoprobes targeted with GE-11 and cetuximab had the best targeting profile, but differing in the kinetics of the accumulation in tumor. While cetuximab probe was highly accumulated in the tumor after 15 min, GE 11 targeted nanoprobe required at least 4 h to show significant accumulation. We summarize that both these targeted probes are good candidates for further study within the fluorescence-guided endoscopic surgery.

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The SFNano C'Nano joint meeting 2019

Thematic Session: nanoparticles and targeting
Keywords: liposomes, CD44, lung delivery, mucus, everolimus

Mucopenetrating liposomes with CD44 targeting ability as a platform for the treatment of fibrotic and cancer lung disease.

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The use of nanomedicine for the local treatment of lung disorders, such as fibrosis and cancer, represents a promising approach to obtain a specific release near the target cells and reduce drug systemic effects. However, nanoparticles delivery to the lungs is challenging due to the presence of mucus that drastically limits cellular uptake. Currently, the best strategy to overcome the mucus barrier is nanoparticle PEGylation. Once PEGylated, nanoparticles can cross the mucus layer but the presence of hydrophilic coating can impair the cellular uptake.
To solve this issue, hyaluronic acid decorated PEGylated liposomes (HA/PEG liposomes) containing everolimus were prepared to obtain mucopenetrating liposomes with CD44 targeting ability. Hyaluronic acid was chosen as a targeting agent because it is the natural ligand of the CD44 receptor that is commonly overexpressed in fibrotic and lung cancer cells.
Liposomes were prepared by an ethanol injection method dissolving everolimus and hyaluronic acid-phospholipid conjugates (HA-DPPE) in the organic phase. The mucopenetrating properties were assessed by DLS, UV-VIS spectroscopy and nanoparticle tracking analysis (NTA). HA/PEG liposomes showed low mucin interaction, like those of PEGylated ones. Uptake studies performed on $CD44^+$ fibrotic and lung cancer cells showed higher uptake of targeted liposomes in comparison to the PEGylated ones. Preliminary *in vitro* studies demonstrated a cytotoxic effect comparable to that of free everolimus both on fibrotic and cancer cell lines.
Overall these results suggest that the combination of mucopenetrating properties and targeting ability can improve the lung delivery of nanomedicine.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticules & therapeutic targeting
Keywords: Polysaccharides, Nanogels, Boron neutron capture therapy, Cancer cells, BODIPY

Tailored polysaccharides based nanogels as boron delivery systems for boron neutron cancer therapy

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ABSTRACT

Boron Neutron Capture Therapy (BNCT) is an innovative cancer treatment therapy derived from radiotherapy using low energy neutrons to create a fission reaction with a boron isotope: ^{10}B. This reaction produces an alpha particle, a lithium ion and ɣ-radiation, causing selective damage to cancer cells (diameter of distraction: 15 µm)[1,2]. Currently, this treatment modality raises interest due to its accurate targeted action. Indeed, only ^{10}B-containing cells will be destroyed during the irradiation time. Today, 2 boron-rich compounds are being used in clinical BNCT trials: sodium borocaptate (BSH) and L-boronophenylalanine (BPA). However, these compounds do not demonstrate selective accumulation in tumor. Therefore, researches to find new compounds or carriers for these molecules are currently in progress. To overcome this issue, various strategies can be considered such as incorporation of boron into nanocarriers or by grafting boron to other molecules. Polysaccharides such as hyaluronic acid or heparosan self-assembled into nanogels seem to be interesting carrier candidates due to their biocompatibility, their biodegradability and especially their ability to accumulate in tumor[3,4]. The objective of this project is to encapsulate boron clusters in these polysaccharide nanosystems to develop boron-rich nanocarriers for BNCT. BODIPY (fluorescent boron-containing molecule) was also encapsulated to monitor the biodistribution of the polysaccharide nanocarriers after their *in vivo* administration. Subsequently, preliminary BNCT experiments have been recently conducted with a neutron beam on U87 cells as well as on tumorous eggs.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & therapeutic targeting

Keywords: Liposomes, placenta, HPLC, *ex-vivo* model, microscopy

Evaluation of cationic and neutral liposomes' uptake by human villous placental explants

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Pregnant women are still considered as drug orphans, so there is a real need to develop new medications for pregnancy complications. Nanomedicines seem to be a promising approach to control the biodistribution of drugs to ensure both the mother's and the baby's safety[1]. Understanding the interaction between nanoparticles and the placental barrier is the key to develop medication for pregnant women. For this purpose, a protocol has been developed to analyse qualitatively and quantitatively the liposomes' uptake by an *ex-vivo* model, the villous placental explants[2].

In this study, neutral and cationic liposomes were labelled with a fluorescent lipid, the DOPE-NBD. Liposomes were incubated at three different concentrations with villous placental explants for 24 hours. The liposome' uptake was qualitatively evaluated using confocal microscopy and quantitatively determined through the dosage of the fluorescent lipid, extracted from the villi, by HPLC.

No neutral liposomes were observed inside the villous explants with confocal microscopy and traces of fluorescent lipid were detected by HPLC (< 0.1% for the three concentrations). An Uptake of cationic liposomes inside the villous explant was observed by confocal microscopy. This result was confirmed by HPLC quantification and the amount of DOPE-NBD rose while increasing the incubation concentration.

These results highlight the correlation between the surface charge of liposomes and their placental uptake. Further experiments, using other relevant placental models, are needed to gather more knowledge about the placenta's behaviour towards nanomedicines.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & therapeutic targeting

Keywords: Liposomes, cross flow, microfluidic, mono-dispersity, reproducibility

Reproducible production of liposomes by an automated microfluidic based apparatus

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Liposomes are drug delivery systems which are largely studied for clinical applications, their potential are confirmed by the entry of several liposomal drug products into market. However, robust production of liposomes, with high reproducibility and the ability to scale-up, is still a major challenge. Conventional preparation of liposomes is usually a multi-steps process and requires a qualified experimenter. In order to produce liposomes with minimum influence of the experimenter, we have designed an apparatus that combines ethanol injection method and microfluidic concepts by using syringe pumps and micro-sized tubes[1].

In this study, several liposomal formulations with different lipid composition were produced, using our microfluidic based technique. Ethanol evaporation was performed under a controlled flow of compressed air. Size distribution of resulting liposomes was assessed by dynamic light scattering (DLS) and tunable resistive pulse sensing (TRPS). Liposomes prepared by ethanol injection technique were used as a control.

Liposomes composed of one lipid (DOPC) were produced with optimised process and showed monodispersed distribution and high reproducibility. Ethanol evaporation by compressed air and by rotavapor resulted in liposomes with similar size distribution. Liposomes composed of DOPC and cholesterol showed an increase in mean size and poly dispersity index while increasing the cholesterol percentage. Finally, monodispersed Pegylated liposomes were reproducibly formulated with a size of 70 nm and PdI of 0.2.

These results showed the ability of our customized apparatus to provide a controlled and reproducible production of liposomes with different lipid compositions. Further experiments, using charged lipid, are planned to extend the application of our microfluid based apparatus.

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The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoscience for Cancer

Keywords: multicellular tumor spheroids, fibroblasts, liposomes, photodynamic therapy

The influence of stroma content on the behavior of liposomal-based Temoporfin formulation in 3D tumor spheroids

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Conventional 3D multicellular tumor spheroids of head and neck squamous cell carcinoma (HNSCC) consisting exclusively of cancer cells have some limitations. They are compact cell aggregates that do not interact with their extracellular milieu, thus suffering from both insufficient extracellular matrix (ECM) deposition and absence of different types of stromal cells. In order to better mimic *in vivo* HNSCC tumor microenvironment, we have constructed 3D stroma-rich *in vitro* model of HNSCC, using cancer-associated MeWo skin fibroblasts and FaDu pharynx squamous cell carcinoma. The developed spheroids were optimized, characterized by fluorescence microscopy and immunohistochemical analysis of spheroid cryo-section and appeared to reproduce sufficiently a stroma-rich HNSCC tumors. The expression of stromal components in heterospheroids was confirmed by immunochemical staining. The generated co-culture FaDu/MeWo spheroids were applied to study the behavior of Temoporfin, clinically approved second-generation photosensitizer, and its liposome-based nanoformulation. It was demonstrated that in stroma-rich spheroids both free and nanoformulated drug better were accumulated by spheroids with deeper penetration depth. Overall, the developed stroma-rich spheroids enlarge the arsenal of *in vitro* pre-clinical models for high-throughput screening of anti-cancer nanomedicines.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles and targeting

Keywords: Cationic liposome, microfluidization, thin film, large scale

Characterization of cationic liposome formulated by microfluidization combined with thin film method

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Abstract

Background: Cationic liposomes havr been widely used in the treatment of different diseases as drug delivery vector. The outer lipid bilayer and hydrophilic core enable it to encapsulate small molecular drugs, especially siRNA for gene targeting therapy. The size reduction and stability during large scale production still remain obstacles. Microfluidization is one of the methods to form homogenous and scalable batch of liposome.

Material and method: We use DMAPAP, DOPE, and DSPE-PEG2000 lipids to form cationic liposome with thin film method and reduce its size by microfluidization for large scale batch. The parameters of microfluidization include: the number of passes (1-5) and pressure (5000 psi-20000 psi).

Result: The Z-average (nm) and PDI of liposomes are significantly reduced after microfluidization, as compared to the not processed liposomes ($p<0.001$). The Zeta potential is not significant changed. With pressure of 20000 psi, the Z-average ranges from 150nm to 180nm and PDI range from 0.2 to 0.3.

Conclusion: Microfluidization is effective to reduce the size of cationic liposomes without modifying their positive surface charge. The distribution of liposome is monodisperse. The characteristics of liposomes maintain stable within one month. These original microfluidized formulations could be used for further studies as siRNA and gene delivery vectors.

The SFNano C'NANO joint meeting 2019

Thematic Session: nanoparticles and targeting

Keywords: liquid crystalline nanoparticles, cubosomes, catalase, curcumin, BioSAXS.

Cubic liquid crystalline nanostructures involving catalase and curcumin: A BioSAXS study and bioassays with neuronally derived cells

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Nanomedicine development for treatment of neurodegenerative disorders requires innovative nanocarriers for combined loading of multiple neuroprotective compounds [1-3]. We report dual-drug loaded monoolein (MO)-based liquid crystalline architectures designed for the encapsulation of a therapeutic protein and a small molecule antioxidant. Catalase is chosen as a metalloprotein, which can provide enzymatic defense against oxidative stress by the decomposition of hydrogen peroxide (H_2O_2). Curcumin, solubilized in fish oil (FO), is co-encapsulated as a chosen drug with multiple therapeutic activities which can recover from the diseased state. The prepared self-assembled biomolecular nanoarchitectures are characterized by biological synchrotron small-angle X-ray scattering (BioSAXS) at multiple compositions of the lipid/co-lipid/water phase diagram. Constant fractions of curcumin and a PEGylated agent are included with regard to the lipid fraction. Stable cubosomal architectures are obtained for several ratios of the main lipid (MO) and the co-lipid (FO) ingredients. The impact of catalase on the structural organization of the cubosome nanocarriers is revealed by the variations of the cubic lattice parameters deduced by BioSAXS. The outcome of the cellular uptake of the dual drug-loaded nanocarriers is assessed by performing a bioassay of catalase peroxidatic activity in lysates of nanoparticle-treated differentiated SH-SY5Y human cells. The obtained results reveal the neuroprotective potential of the *in vitro* studied cubosomes in terms of enhanced peroxidatic activity of the catalase enzyme, which enables the inhibition of H_2O_2 accumulation in degenerating neuronal cells.

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The SFNano C'Nano joint meeting 2019

Thematic Session: (Nanoparticles & targeting)
Keywords: (siRNA, inflammation, glucocorticoids, solid lipid nanoparticles)

Designing A Novel Nanocarrier For combining the delivery of SiRNA and Glucocorticoids in The Treatment Of Atopic Dermatitis

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ABSTRACT

Atopic Dermatitis (AD) is a common chronic eczematous skin disorder that affects up to 20 % children and 3 % adults. It targets different organs and causes inflammation. Patients with AD also have elevated tumor necrosis factor alpha (TNF-α) levels in the skin which is mainly secreted by macrophages. Therefore, regulation of TNF-α is believed to take an important part to treat AD. Most treatments of AD contain topical corticoids (TCs) as an anti-inflammatory drug. Unfortunately, upon administration TCs have local and systemic adverse effects. Small interfering RNAs (siRNA) are considered as a promising anti-inflammatory strategy due to the ability to specifically and efficiently inhibit the expression of complementary RNA transcripts. However, siRNA-based therapy remains limited by the delivery system. Indeed, siRNA delivery still suffers from several drawbacks due to its high molecular weight and hydrophilicity that restrict entry across the cellular membrane. Moreover, siRNAs are sensitive to serum nucleases and have poor pharmacokinetic properties. Here we report a novel cationic nanocarrier to assemble and protect anti TNF-α siRNA together with a corticoid prodrug for the treatment of AD. Solid lipid nanoparticles (SLNs) were formed by using cationic lipid (DOTAP) and dexamethasone palmitate by emulsion evaporation. Then lipoplexes were obtained by complexing siRNA to SLNs through electrostatic interactions. Both nanoparticles and lipoplexes were characterized in terms of size, polydispersity, zeta potential, binding efficacy, quantification, TEM observation and cytotoxicity, cellular uptake and anti-inflammatory efficacy on RAW 264,7 macrophage cell line.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & therapeutic targeting

Keywords: Magnetic nanoparticles, Cell penetrating peptides, endocytosis, Magnetic hyperthermia

Cell penetrating peptides to improve magnetic nanoparticles cellular uptake

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The activation of magnetic nanoparticles (MNPs) is a promising anticancer strategy. Upon exposure to an alternating magnetic field, localized heat is generated resulting in intracellular hyperthermia and cell death. However, a major limitation to this approach is the endosomal trapping of MNPs following their internalization by endocytosis, as it has been shown that the heating properties of MNPs are completely abolished when they are aggregated in endosomes1. The aim of this project is to promote direct access of MNPs to the cytosol by conjugation with cell-penetrating peptides (CPPs). These are typically short amphiphilic or purely cationic peptide sequences that are able to carry cargo (such as peptides, proteins, or nanoparticles) across biological membranes via the translocation pathway as well as the endocytosis pathway2.

Poly-Arginine peptide containing an azido group was synthesized on a solid support using Fmoc solid-phase method. With core-shell particles (Fe2O3@SiO2, diameter 40 nm) and azido-CPP in hand, future work will be focused on the construction of CPP-MNP conjugates and the evaluation of their internalization pathway and heating properties upon activation.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & targeting

Keywords: Cell-penetrating peptide, ferrocifen, self-assemblies, light scattering, NMR diffusometry

Is dynamic light scattering a good method to characterize self-assemblies of cell penetrating peptide-ferrocifen conjugates?

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The objective of this study was to create a nanoplatform in order to deliver a hydrophobic drug, belonging to the ferrocifen family, called p54, into cancer cells. For thus, three strategies were used in the same nano-object: a combination of cell-penetrating peptides (CPPs), prodrug and self-assembly. To this end, a hydrophilic CPP (RLW, Arg_n, n=6-9) was covalently coupled to a hydrophobic ferrocifen in order to form an amphiphilic conjugate, that could potentially self-assemble into nanostructures[1].

CPP-p54 conjugates were synthesized, purified and characterized by mass spectrometry (LC-MS/MS) and ^{1}H NMR spectroscopy. Self-assemblies were formulated by a solvent displacement technique using a microfluidic mixer. The formed nanostructures were fully characterized using several complementary methods: dynamic light scattering (DLS), static light scattering (SLS), cryogenic transmission electron microscopy (Cryo-TEM) analysis, nanoparticle tracking analysis (NTA), small angle X-ray scattering (SAXS), and NMR diffusometry.

CPP-p54 were synthesized with a yield between 30 % and 70 % and a purity of 90 %. Interestingly, depending on the method used, two populations could be observed: one with a hydrodynamic diameter close to 2 nm (corresponding to more than 99 % of the matter) and another with a size around 100 nm (less than 1 % of the matter). This study highlighted the importance of the methods used to characterize the suspension and the complementary contribution of each experimental approach. It is indeed essential to perform orthogonal measurements such as SAXS, quantification after filtration or NMR diffusometry to obtain a reliable characterization of self-assemblies.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & targeting

Keywords: Oxidative stress, antioxidant nanoparticles, PEG coating, biocompatibility

ANTIOXIDANT PROPERTIES AND IMPROVED BIOCOMPATIBILITY OF CERIUM OXIDE NANOPARTICLES WITH INNOVATIVE POLYMER COATING

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Introduction: The deleterious role of oxidative stress is well established in many pathologies including cerebrovascular alterations. Cerium oxide nanoparticles (CNPs) hold interesting antioxidant capacities via scavenging free radicals and mimicking antioxidant enzymes [1]. To improve their biocompatibility, CNPs have been coated with multifunctional, PEG-based polymers. The present study evaluated the toxicity, antioxidant abilities and internalization of bare and polymer-coated CNPs.

Materials & Methods: Murine endothelial cerebral cells (bEnd.3) were incubated with glutamate to induce oxidative stress. Toxicity was evaluated through metabolic activity and mortality. Oxidative stress was detected with the H2DCF-DA probe, MitoSOXRed Probe and 8-OHdG antibody. Cyanine labelled CNPs were used to study internalization by FACS and fluorescence microscopy. Subcellular localization was determined by transmission electron microscopy. *In vivo* toxicity and biodistribution was assessed in healthy mice by blood formula, histology and quantification of cyanine.

Results: Decreased metabolic activity was only observed with CNPs at the highest dose. Bare CNPs induced cell death while no mortality arose with coated CNPs. All CNPs exhibited antioxidant effects at 100 and 1000 µg/mL. FACS and fluorescence microscopy showed internalization and perinuclear localization of coated CNPs in bEnd.3 cells. Electron micrographs showed the CNPs to be located in the endosomes. Histology and blood formula were not altered at both 24h and 1 month following coated CNPs' IV injection in healthy mice.

Conclusion: Coating CNPs increases their biocompatibility while maintaining their antioxidant properties and allowing their internalization.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer
Keywords: magnetic particle; mechanical treatment; rotating magnetic field; glioblastoma; cancer

Magnetic particles for magneto-mechanical treatment of glioblastoma cells

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In nanomedicine, treatments based on physical mechanisms are more and more investigated and are promising for challenging tumor therapy. One of these approaches is to trigger cell death via the vibration of anisotropic magnetic particles (MPs), under low frequency (<100 Hz) rotating magnetic field, without hyperthermia. In this work, we study how MPs surface functionalization influences the treatment efficiency, and its mechanism.

We prepared magnetite particles (MP-Fe_3O_4) by liquid-phase ball milling of a magnetite powder. Then, these particles were PEGgylated (MP-Fe_3O_4@$PEG_{2000}OH$) by a 4-step functionalization. Due to their magnetic properties, these MPs vibrated when exposed to a 0.4 T rotating magnetic field.

The MP-Fe_3O_4 and the MP-Fe_3O_4@$PEG_{2000}OH$ particles showed no intrinsic cytotoxicity on human glioblastoma U87-MG cells. To optimize the magneto-mechanical treatment efficacy, the cell viability was measured with 2 assays (WST-1 and LDH tests) while varying the magnetic field frequency (2-20 Hz) and exposure time (1-60 min). Our results indicated that 1) the magneto-mechanical treatment with MP-Fe_3O_4 particles induced a fast decrease in cell viability (14% of viability after 1 min at 20 Hz) whereas the effect with MP-Fe_3O_4@$PEG_{2000}OH$ particles was slower (55% after 60 min at 20 Hz); 2) the ratio of apoptotic cells after treatment was higher with MP-Fe_3O_4@$PEG_{2000}OH$; 3) a lower magnetic field frequency (from 20 Hz down to 2 Hz) favored apoptosis. These results highlight a difference in the cell death mechanism according to the type of particle used – the rapid cell death observed with MP-Fe_3O_4 particles pointing to necrosis, while MP-Fe_3O_4@$PEG_{2000}OH$ particles induced apoptosis.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & therapeutic targeting

Keywords: Block copolymer, oxazoline, cationic ring opening polymerization, gene delivery

Synthesis of polyplexes with double hydrophilic block copolymers lPEI-*b*-POx for gene delivery

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Double hydrophilic block copolymers (DHBCs) consisting of a binding block and a solvating block have a great interest in different domains. In the biomedical field, research on these copolymers is growing for new drug carrier systems.[1] Among them, the poly(ethyleneglycol-*b*-ethylenimine) (PEG-*b*-PEI) copolymer revealed to be a very effective candidate to condensate DNA into stealth polyplexes.[2]

Nevertheless, various debates discuss the use of PEG in medicine due to the complement activation.[3] The control of the grafting of the PEG moieties onto the linear PEI (lPEI) is still an issue, and large molar mass diblock copolymers are difficult to synthesize. Many neutral hydrophilic polymers were suggested to advantageously replace the PEG block. Poly(2-oxazoline)s (POx) are extensively studied for their good polymerization control, the large library of available monomers and their easy functionalization.[4] Moreover, the hydrolysis of POx constitutes the main method to synthesize lPEI. Thus, the synthesis of DHBC having one POx linked to lPEI is a challenging issue.

In this study, synthesis of news DHBCs of lPEI-*b*-POx was performed by selective hydrolysis of block copolymers of poly(2-oxazoline)s (POx), synthesized by cationic ring opening polymerization via sequential addition of monomers. Nanoparticles were formulated by mixing these DHBCs and a pDNA, for subsequent gene delivery applications.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticle and targeting
Keywords: gene delivery; phospholipid; mucus; lung

Impact of mucus on gene delivery efficacy by phospholipid carriers in the lung

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Gene therapy brings hope for treatment of pulmonary diseases. Our team developed biodegradable DOPC-based cationic lipids that exhibited potent transfection efficiency in healthy mice. However, in the lung, the mucus that covers the airway epithelium may represent a significant barrier to transfection particles, especially under pathological conditions characterized by mucus hypersecretion. To address this issue, phospholipids with a labile phosphoester substituent of increasing length (*i*-C_3 to C_{12}) were specifically designed, and the human bronchial epithelial cell lines Calu-3 and NCI-H292 were selected for evaluating how mucus hypersecretion, induced by treating NCI-H292 cells with allergen extract or by growing Calu-3 cells at the air-liquid interface (ALI), could impair the pDNA delivery activity of these carriers. The transfection rate of the lipids decreased from C_{12} to *i*-C_3 in untreated NCI-H292 cells. When mucus production was induced (20-fold) by treatment with allergen extract, the C_8-lipid exhibited greater transfection efficacy when compared to the other carriers. This phospholipid also tended to outperform the other lipids in immerged cultures of Calu-3 cells that produced more (7-fold) mucus than induced-NCI-H292 cells. However, transfection activity of all the lipids was totally inhibited in Calu-3 cells cultured at the ALI for 14 days (13 times more mucus than in immerged cultures). This inhibition was partially prevented by removing mucus before lipoplex exposure, showing that mucus is a barrier for transfection. In the future, the transfection activity of these gene carriers will be assessed in diseased mice, and thiol-releasing analogs will be evaluated as mucolytic gene carriers.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & therapeutic targeting
Keywords: Hybrid liposome/polymer, Syringe pump, Doxorubicin, Poloxamer

Development of hybrid liposome/poloxamer nano-particles to delay hydrophilic drug delivery by thickening the interior core of liposomes

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Liposomes are one of the most used nano-system to encapsulate the drug molecules for clinical application. However, liposomes suffer in terms of leakage of small hydrophilic drugs. In order to control the release, we designed a system with lipid shell and polymeric viscous core, namely Hybrid liposome/polymer inside (HLP_{in}) or lipogel.
Microfluidic based technique, using syringe pump was applied to produce nano-sized Hybrid liposome/poloxamer particles. The objectives of this study are i) to optimize the parameters for formulation preparation and ii) to optimize the formulation composition by adjusting the ratio of lipid (*i.e.* DOPC) and polymer (Poloxamer 407). The optimal formulation was characterized by Dynamic Light Scattering and Transmission Electron Microscopy. The lipogel organization was confirmed by physico-chemical studies based on thermal analysis and density analysis.
The lipogel formulation consisting of poloxamer (5% w/v) was found to be optimal when produced at injection rates of 5 mL/min and 0.5 mL/min for poloxamer solution and lipid solution, respectively. The selected conditions and composition resulted in nano-objects which are highly reproducible with mono-disperse size distribution of 206 ± 4.8 nm and polydispersity index of 0.15 ± 0.015. The HLP_{in} formulation was stable over 2 months, produced no cytotoxicity. Encapsulation efficiency and Loading content was improved with Poloxamer incorporation to the system. HLP_{in} formulation exhibited slow release of Doxorubicin in comparison to liposome formulation.
Our customized microfluidic system achieved reproducible, inexpensive, precisely controlled production of the liposomes and HLP_{in} formulations. HLP_{in} formulation showed a potential to be a new drug delivery system providing controlled drug release.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticles & targeting, Nanoscience for Cancer
Keywords: Glioblastoma, hydrogel, lipid nanocapsules, targeting

New generation of glioblastoma-targeted lipid nanocapsule hydrogel: a sustained and specific drug delivery system

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The standard of care of glioblastoma (GBM), malignant brain tumours, consists in a tumor resection, followed by the Stupp protocol (chemotherapy and/or radiotherapy) 4 to 6 weeks later. This non-specific and non-curative protocol allowed a slight increase in the median survival, but without preventing tumor recurrences, leading to the death of the patients. One of the factors associated with the recurrences is the gap between surgery and Stupp protocol, but necessary for good tissue healing and recovery of the patient. The objective of this project is to develop an implantable therapeutic hydrogel which will bridge this gap to ensure continuity in treatment for the patients.

A hydrogel of self-associated lipid nanocapsules (LNCs), without polymer matrix, was designed and allowed the gradual release of gemcitabine-loaded LNCs. Promising results have shown the therapeutic efficacy *in vivo* of this implant in murine GBM resection models.[1-3] However, the released LNCs were not specific to GBM cells. One of the opportunities to improve the targeting is the use of NFL-TBS.40-63 (NFL) peptide, able to associate with LNCs in suspension.[4-5]

The LNC hydrogels were formulated with the NFL peptide. It was totally and instantaneously adsorbed at the LNC surface, without modifying the hydrogel mechanical properties, and remained totally adsorbed after the hydrogel dissolution. In addition, *in vitro* studies on three GBM cell lines showed a faster internalization of the LNCs in the presence of NFL. Finally, when LNCs were loaded with gemcitabine, their cytotoxicity increased with the NFL adsorbed at their surface, proving a better specificity.

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The SFNano C'Nano joint meeting 2019

Abstracts

Poster Session

P2 - NANOSCIENCE FOR CANCER

SFNano cnrs C'Nano

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoscience for Cancer
Keywords: liposomes, nanoparticles, pentamidine, anticancer activity

A comparative study of the encapsulation of pentamidine into lipid and polymer nanocarriers

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Initially developed as a synthetic analogue of insulin, pentamidine (PTM) is an antimicrobial drug that has recently shown *in vitro* and *in vivo* anticancer activity [1,2]. Nevertheless, systemic administration of PTM causes severe side effects, especially nephrotoxicity. Here we propose the association of PTM to different non-toxic, biocompatible and biodegradable nanosystems in order to compare the physico-chemical characteristics of the loaded nanocarriers and their influence on the drug cytotoxicity towards cancer cells. In particular, PTM (as free base or with different counterions) was encapsulated into liposomes and poly(lactide-*co*-glycolide) (PLGA) nanoparticles and all the formulations have been deeply characterized concerning mean diameter, polydispersity index, zeta potential, stability, morphology, PTM loading and drug release profile. The anticancer activity was evaluated on a human ovarian cancer cell line over 72 h. Results showed that PTM is efficiently loaded into liposomes with a transmembrane citrate- or sulfate-gradient; concerning PLGA nanoparticles, important association occurred thanks to ionic interactions between the drug and the polymer. The *in vitro* studies confirmed the anticancer activity of PTM, which was gradually released with different profiles depending on the drug form and the nanocarrier structure tuning its cytotoxic activity.
In conclusion, the nanocarriers here proposed could be considered as a platform for PTM delivery. Moreover, the encapsulation of PTM is also a proof of concept that could enlarge the field of application of these PTM drug delivery systems to other pathologies for which PTM has been shown to be active other than *Leishmania*.

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Tuning self-assemblies morphologies by variation of the phopholipids polar heads: the case of the vitamin B2 (riboflavin)

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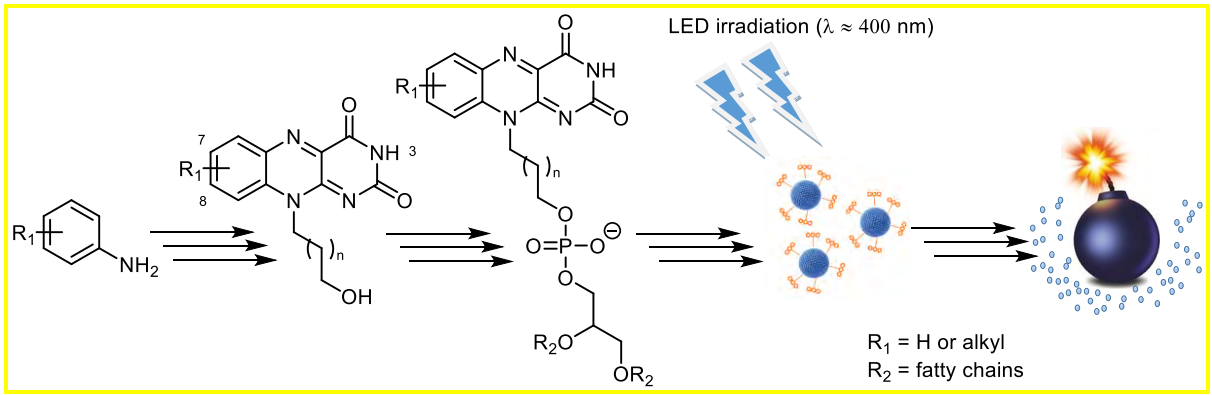
Riboflavin (RF) is an essential vitamin which, along with its derivatives (flavin mononucleotide and flavin adenine dinucleotide) participate in fundamental physiological events. The presence of isoalloxazine ring in their structures induce redox, photosensitizing and fluorescence properties that can be exploited in tissue engineering and cancer therapies. Moreover, numerous preclinical studies indicate that RF is internalized through RF transporters, which are highly upregulated in prostate, breast cancer cells and neovasculature.[1] By irradiation with low energy blue light (*e.g*. LED), RF is also able to generate reactive oxygen species (ROS).

Our group is interested in exploiting the physico-chemical properties of RF and its cancer targeting abilities. We are particularity focused on developing self-assembled nano-systems for biomedical applications starting from RF (or analogs)-conjugated phospholipids.[2] We already synthesized an amphiphilic RF phospholipid (*e.g.* RfdiC14), which, formulated with other lipids, was able to generate targeted liposomes with applications in photo acoustic imaging of tumor. [3] However self-assemblies of this amphiphile used alone induce a compact lamellar morphology because of the π-stacking between the isoalloxazine rings that quenches fluorescence and ROS production.

Our current hypothesis is to prevent the π-stacking between the isoalloxazine rings by incorporating bulky substituents in 3, 7 or 8 positions on the flavin moiety in order to enhance ROS generating properties and create "**ROS nano-bombs**" (Schema).

Starting from commercially available 4-*tert*-butyl aniline, we synthesized the desired analog with a *tert*-butyl group in 7 position and without hydroxyl functions on its ribityl chain. The flavin moiety has been conjugated to artificial phospholipids using the phosphoramidite chemistry. The current communication will present the synthesis, the formulation and physico-chemical characterization of the obtained nanoparticles. These are compared with previously obtained RFdiC14 self-assemblies to determine the influence of the bulky group on isoalloxazine ring, their morphologies and the ROS productions.



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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for cancer

Keywords: Liposomes, peptide vaccine, antitumor vaccine, immune response

Induction of a strong and persistent antitumor immune response using liposomal vaccines in the HPV-transformed orthotopic lung tumor model TC-1

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Conventional cancer therapies have the major drawback of triggering numerous side effects. Currently, a challenging goal in this area is the development of innovative targeted antitumor immunotherapy with a long-term efficiency. We took advantage of liposomal nanoparticle properties for the conception of therapeutic cancer vaccines, which contain all the elements needed for the induction of an efficient antitumor response i) a peptide able to activate $CD4^+$ T helper cells ii) a tumor peptide (E7) expressed by the cancer cells, recognized by $CD8^+$ T cytotoxic cells and iii) Toll or Nod-Like receptor (TLR and NLR) agonists which act as adjuvants for the activation of the innate immune response. The aim of our project is to conceive liposomal vaccines, which would be effective even against tumors in later stage.

In our work, liposomal vaccine containing TLR4 agonist was the most effective treatment against E7-expressing tumor cells. This vaccine induced a potent Th1-oriented antitumor immunity, which led to a significant reduction in tumor growth and a prolonged survival of mice, even when injected after pulmonary tumors apparition. TLR4 liposomes antitumor efficacy was dependent of $CD8^+$ T-cells presence. TLR2/6 liposomes induced a Th1-immune response but weak and not sufficient for a prolonged antitumor activity. Surprisingly, whereas NLR liposomes resulted in the control of early tumor growth, they did not extend survival.

We obtained a therapeutic strong and persistent antitumoral efficiency only using TLR4 liposomes highlighting the importance of immune monitoring of vaccine therapy. This modulable platform can be used for the development of vaccines.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoscience for Cancer

Keywords: water-soluble nanoparticles, prodrug, paclitaxel, glioblastoma

Preclinical evaluation of a paclitaxel nanoprodrug for glioblastoma therapy

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Glioblastoma (GBM) is a malignant central nervous system tumor with a median survival of 15 months despite standard treatment protocols combining surgery, chemotherapy and radiotherapy. Although it is active on GBM cells *in vitro*, paclitaxel (PTX) is not used against brain tumors because of its limited cerebral availability. Moreover, aqueous solubilization of PTX requires the use of excipients such as Cremophor EL®, occasionally causing serious allergic reactions.

These hurdles may be overcome by a PTX nanoprodrug obtained by covalent grafting of PTX on water-soluble nanoparticles. Our results show these nanoparticles are safe and internalized in different cell lines, making them interesting drug carriers for hydrophobic drugs. The anticancer activity of nanoparticles-PTX conjugates was confirmed *in vitro* on GBM cell lines, albeit at several hundred-fold higher concentrations than free PTX. *In vivo*, paclitaxel nanoprodrug did not show anticancer activity in murine models at usual equivalent PTX doses (1 mg/kg, intratumoral administration) due to slow drug release not affording sufficiently high intratumoral PTX concentrations. However, these nanoparticles may be useful as a sustained-release form if used at higher doses. Moreover, these PTX nanoprodrugs are water-soluble, thus avoiding the use of potentially harmful excipients, and afforded concentrations equivalent to at least 1 g/L of PTX in saline, when the free PTX molecule would have been virtually insoluble.

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Low Energy Electron Dissociative Attachment to DNA : an Experimental and Theoretical Study

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Low-energy electrons (LEEs, typically 0-20 eV) are ubiquitous in nature and play an important role in natural phenomena as well as many potential and current industrial processes. It is now well-established that LEEs are copiously released in any high ionization event (high energy photons, ions, electrons) ; they constitute a major fraction of the complete set of secondary electrons (4.10^4/1MeV by ionizing radiation) [1].
These last decades, along with conventional radiation therapy (high energy photons), new techniques such than hadron (heavy ions) and proton-therapy emerge and start to be used in several countries. The strength of hadrontherapy lies in the unique physical and radiobiological properties of these particles ; they can penetrate the tissues with little diffusion and deposit the maximum energy just before stopping.
Whatever is the type of incident radiation used in radiation therapy (photons, ions, ...), its final objective lies in the inactivation of cancer cells while keeping healthy cells as much as possible unaffected. Ionizing radiation works by damaging the DNA of cancerous tissue leading to cellular death. Therefore, studying the damaging effect of LEEs on DNA constitute an essential prerequisite.
LEEs, known as "sub-ionization electrons", interacting with a molecule can bind to a molecular orbital and be trapped, giving a temporary transitory negative ion (TNI) [2]. This anion is attached to the name *resonance*. The later is classified in several gender following the pattern of electron occupancy in the unoccupied orbitals of the molecule and the concept of rearrangement of the excited : AB^*, resonant : AB^{-*} and fundamental parent state : AB.
These TNI being temporary will disintegrate in several ways. The fragmentation undergone in neutral fragment and negative ion is defined by the process of dissociative electronic attachment, DEA. Plasmid DNA complexed with diaminopropane (Dap) as a thin layer (4-10 nm) is used as a target. This is mainly studied in the analysis work of anion desorption under LEEs impact by mass spectrometry. Two numerical tools are also used : one to make an adjustment of the yield curves of the desorbed anions of the DNA-Dap films as a function of the incident electrons energy (**Origin**) and the other to calculate the electron affinities (EAs) of the fragments (**Gaussian**).
This consists in explaining the results of the resonant structures in the yield functions of the fragments found in DSE (Low Energy Electron-stimulated desorption) and which are attributed to the disintegration of the TNI by DEA.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for cancer

Keywords: Gold nanoparticles, PEGylation, radiotherapy and cancer theranostic

Influence of PEGylation on the in vivo behavior of radiosensitizing gold nanoparticles

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Gadolinium coated gold nanoparticles have a promising potential for magnetic resonance imaging (MRI) guided radiotherapy. However this potential is not exploited plentifully because of a too fast renal elimination. In order to postpone the renal clearance, which is essential for non-biodegradable nanoparticles, gold nanoparticles coated with PEGylated chelators were synthesized and characterized. The PEGylated chelators are composed of an anchoring site, a thioctic acid moiety used for the immobilization onto the gold cores and a macrocyclic chelator, DOTA or DOTAGA, well known for their ability to form stable complexes with gadolinium ions (T_1-weighted MRI) or radioisotopes (nuclear imaging). In between, a polyethylene glycol (PEG) chain with various length (0, 4, 11 ethylene glycol units) was inserted to study their influence on biodistribution. The reduction of gold salts in presence of PEGylated chelators provides ultra-small nanoparticles Au@TAPEG$_n$DOTA and Au@TAPEG$_n$DOTAGA ($Ø_{core}$ < 3 nm). This strategy which rests on the use of PEGylated macrocycles appears attractive because it does not require, in contrast to the classical route of PEGylation, the post-functionalization of the nanoparticles by PEG chains. Preliminary results showed the potential of Au@TAPEG$_4$DOTA to improve the treatment of 9L Gliosarcoma bearing mice by radiotherapy in comparison to non-PEGylated nanoparticles.

Thematic Session: Nanoscience for Cancer
Keywords: nanoMOF, 3D tumors models, spheroids, lung cancer

A 3D model of lung cancer for in vitro preclinical prediction of in vivo behavior of nanoMOFs

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Despite a decline incidence in the last 30 years[1], lung cancer remains the second most common cancer and the first cause of cancer death in the world. In the last decades a variety of nanomedicines has been designed to improve the efficacy of anticancer drugs nevertheless, their application in lung cancer therapy remains challenging. Although particle lung filtration has been proposed for passive lung targeting, several toxicity issues are associated to their pulmonary retention.
In this context, the pH-responsiveness and the reversible aggregation behavior of Nanosized Metal-Organic Frameworks (NanoMOF) have raised interest for lung targeting. We demonstrated that, after intravenous administration, gemcitabine-monophosphate-loaded nanoMOF are retained in the lung, then degrade and release their payload leading to a higher antitumoral efficacy than the free drug on an experimental lung tumor.[2]
Nevertheless, how nanoMOF interact with cancer cell and how their physico-chemical features affect such interaction remains matter of question.
To face this issue, a small library of nanoMOF, starting from the ones whose surface has been functionalized with PEG chains either covalently or not covalently (i.e., Graft-Fast)[3], has been investigated on a 3D heterotype model of lung cancer. The latter mimics the tumor and its microenvironment and represents a more relevant tool that 2D cultures to identify the factors which play a crucial role in (i) the penetration/accumulation of the nanoMOFs through the whole tumor tissue and (ii) the effectiveness of treatments resulting from a better drug availability in the tumor.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer
Keywords: cancer therapy; magnetic particle; mechanical treatment; glioblastoma

In vitro and in vivo assessment of magnetic particles for magneto-mechanical glioblastoma treatment

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Glioblastoma is a brain cancer with a very poor prognosis. Existing therapies improve only slightly the median survival. In this work, we study the treatment by magneto-mechanical actuation of particles (TMMAP). With this, a low frequency (20 Hz) rotating magnetic field is applied to vibrate magnetic particles localized near or within cancer cells. Magnetic particles consist in permalloy disks with a vortex configuration, produced by UV lithography.

TMMAP efficiency is tested in-vitro on glioblastoma cell line, allowing for the optimization of the parameters of the experimental protocol. A large decrease in the number of living cells and the affected behavior of the remaining cells are observed after treatment. TMMAP is then adapted to an in-vivo study on glioblastoma orthotopic model on nude mice, where the intratumoral injection of the particles is developed. Few differences are observed between tissues submitted to TMMAP or injected with particles, and survival is slightly increased. To mimic mechanical properties of the brain in a more relevant model, an in-vitro 3D model based on cells spheroid encapsulated an agarose gel is proposed and validated. Such a model opens new perspective for the realistic assessment of the effects of mild mechanical stress.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer
Keywords: Platinum Nanoparticles, Breast Cancer, Radio-enhancement, EMT

Platinum nanoparticles to impair radiation-resistance in breast cancer stem cells: From physical theory to applied biology

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Breast cancer is the most common cancer among women with almost 60 000 new cases every years in France. Radiotherapy is often chosen as complement of a chemotherapy or surgery. Focusing on tumor heterogeneity, we identified a mesenchymal sub-population, characterized with a pair of markers (CD24/44), which has the dual ability of tumorogenicity and resistance to radiation.

As this aggressive cell population must be involved in occurrence of relapse, we propose to use PEGylated platinum nanoparticles to impair their radiation resistance.

From a theoretical perspective, one assumes that metallic nanoparticles may improve the probability of interaction between a radiation beam and matter. However, when a cascade of biologic effects is intended in response to a nanoscale dose deposition, demonstrate a proper radiation enhancement effect *in vitro* remains challenging, knowing that potential involved mechanisms are not fully understood.

Thus, we explored different expected effects of combined High-Z nanoparticles with gamma-irradiation (reactive oxidative stress production, DNA double strand breaks, cell cycle arrest, cell death, clonogenic survival…) in breast cancer cell lines of different EMT states and different levels of sensitivity to radiation.

Despite significant efforts, the demonstration of a radiation enhancing effect *in vitro* is not manifest. In literature, conclusions vary according to nanoparticles (nature, quantity and coating), radiation beam (nature and energy), cell-line and but also to performed assays.

We suggest the need for very specific conditions to raise a biological significant effect and highlight the importance of the nanoparticle uptake and diffusion into cells.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanomaterials

Keywords: Nanosheets; Brain tumor; graphene oxide; mucoadhesion; thiolation

Design and development of thiolated graphene oxide nanosheets for brain tumor targeting

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Abstract:
The present investigation emphasizes on synthesis and characterization of thiol functionalized reduced graphene oxide (TrGO) as a novel platform for loading of anticancer drug methotrexate (TrGO-MTX), through amide bonding. Thiolation of graphene oxide (GO) was achieved by transesterification process. The introduction of sulfur containing chemical groups and the partial reduction of GO to TrGO were proven by analytical techniques. Thiol content was found to be 6.98mM by Ellman's method in a quantitative manner. Furthermore, antineoplastic action of TrGO-MTX against human glioblastoma astrocytom U-373MG cell line was studied, wherein TrGO-MTX demonstrated significant inhibition rate as compared with pure MTX.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer, Nanoparticles & Targeting
Keywords: Microfluidics, precision medicine, oncology, polymer, scale-up

Polymeric nanoparticles: microfluidics parameters influencing encapsulation of oncology drug

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Pharmaceutical industry is continuously looking for innovative and scalable nano drug delivery systems (DDS). Despite the sustained scientific interest in the nanotechnology field, this approach is confronted to elevated risks of development failure when reaching the scale-up phase. Microfluidics applied to nanoDDS can address this challenge by providing scalable processes from the early development stage to the GMP manufacturing. Microfluidics has been developed for the formulation of various nanocarriers including polymer nanoparticles, well described in the field of nanotechnologies. In this work, an oncology compound was selected as model drug to evaluate its encapsulation efficiency and drug loading in polymeric nanoparticles produced by a bench-top microfluidics platform. The selected small molecule was efficiently encapsulated in the nanoparticles up to 20%w/w and the drug:polymer concentration ratio was investigated to optimize the drug loading. Throughout this study, an in-depth evaluation of the microfluidics opportunities for polymer nanoparticles development was successfully conducted. An automatized microfluidics equipment using the staggered herringbone mixer technology was used to manufacture nanoparticles via nanoprecipitation. The optimization of the microfluidics parameters was carried out to obtain appropriate characteristics for intravenous administration and potential *in vivo* tumor passive targeting (mean size 100 nm, PdI < 0.2).

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer

Keywords: targeted nanotherapy, magnetic nanoparticles, magnetic hyperthermia, cell death, resistance, HSP70

Combination of HSP70 inhibition with Magnetic Hyperthermia promotes synergistic anti-tumoral activity

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One major difficulty in anti-cancer therapy is the multidrug resistance appearing during treatments. Recently, studies showed that cancer cells resistant to traditional therapies are sensitive to agents that induce lysosome membrane permeabilization (LMP) causing lysosomal cell death. To date, lysosomal cell death is obtained using lysosomotropic agents, which could not selectively target lysosomes of tumoral cells. In this context, targeted nanotherapy based on Magnetic Intra-Lysosomal Hyperthermia (MILH) generated by magnetic nanoparticles (MNPs) grafted with ligands of receptors overexpressed in tumors appears to be a promising therapeutic option. As a proof-of-concept, we previously showed that MNPs targeting gastrin receptor internalized, accumulated into lysosomes and killed cancer cells by MILH through a lysosomal and non-apoptotic Capsase-1-dependent pathway. Since MILH induced cell death by ~30%, we hypothesize that certain mechanisms could inhibit this effect. Heat Shock Protein 70 (HSP70) over-expression in cancers is correlated with poor prognosis and treatment resistance. Additionally, HSP70 is a guardian of lysosome integrity and its downregulation/inhibition induces LMP, thereby promoting cell death. We showed that HSP70 overexpression prevents cells against LMP and death induced by MILH. In contrast, combination of MILH with low doses of PES (Pifithrin-μ), a HSP70 inhibitor, increases the efficiency of eradication of cancer cells with synergism, killing 50 to ~100% of cancer cells. This effect was associated with an increase in LMP and in the activation of the Caspase-1-dependent and of apoptosis death pathways. HSP70 exerts a protective role in MILH-induced cell death and emphasize the benefit of targeting HSP70 for combinatorial treatments, with the prospects of overcoming treatment failure and therapeutic resistance.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer

Keywords: Ewing sarcoma, biodistribution, siRNA, nanodiamonds

Delivery of siRNA to Ewing sarcoma tumor xenografted on mice, using hydrogenated detonation nanodiamonds: treatment efficacy and tissue distribution.

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Nanodiamonds of detonation origin are promising delivery agents of anti-cancer therapeutic compounds in a whole organism like mouse, owing to their versatile surface chemistry and ultra-small 5 nm average primary size compatible with natural elimination routes. However, to date, little is known about tissue distribution, elimination pathways and efficiency of nanodiamonds-based therapy in mice.

We have studied the capacity of cationic hydrogenated detonation nanodiamonds to carry therapeutic small interfering RNA (siRNA) in cell culture and in Ewing sarcoma tumor xenografted on mice. Ewing sarcoma is a bone cancer of young adult due in the vast majority to the *EWS-Fli1* junction oncogene. We used a siRNA sequence that interferes with this junction oncogene, preventing the production of the associated oncoprotein responsible for the cell proliferation.

In order to investigate the nanodiamond vector distribution throughout mouse organs and their excretion in urine and feces we replaced the hydrogen gas by its radioactive analog tritium gas, leading to the formation of labeled cationic nanodiamonds. Nanodiamonds were predominantly found in liver, lungs, spleen and kidneys 24 h after intravenous injection, with a small fraction in the tumor. Despite this result, we could measure a 50% inhibition of the oncogene. Moreover, some ND were also found in the feces, which indicates a possible elimination pathway. This work is a significant step to establish cationic hydrogenated detonation nanodiamonds as an effective agent for *in vivo* delivery of active siRNA.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoscience for cancer

Keywords: Photo Dynamic Therapy, polymer self-assembly, passive targeting, model membranes

Dynamic processes induced on cell membrane models by photodynamic therapy

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Polymer based nanocarriers have great potential to incorporate and transport photosensitizers commonly used in photodynamic therapy (PDT) to locally generate Reactive Oxygen Species subsequently leading to the death of the cells. PDT is used either in dermatology, ophthalmology or oncology. In the last years we worked with polymer based nanovectors and we showed a strong improvement of the therapeutic efficiency of the encapsulated photosensitizer, pheophorbide a.[1-3]
These results stimulated us to assess more closely the mechanisms of release and possible internalization of photosensitizers inside cells. For this purpose we used simple model membranes, a valid experimental tool in order to help interpreting the results of *in cellulo* studies, often complicated by the intrinsic variability of cell culture. Photosensitizers-loaded polymer nanocarriers were incubated with lipid vesicles and further irradiated. Leakage assays and confocal microscopy on the lipid vesicles give us experimental evidence of important modifications in lipid membranes (Fig1). We demonstrated that these modifications are related to singlet oxygen production and lipid damages, which were quantified and followed upon irradiation. All our results help to rationalize the influence of the nature and composition of the carriers on their efficiency in PDT.

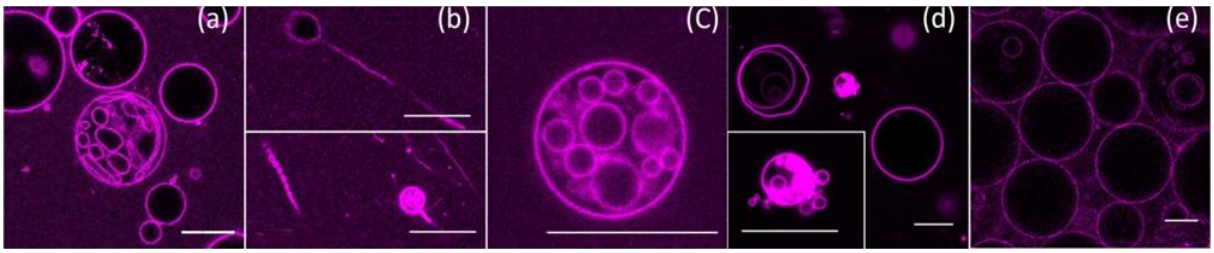

Figure 1. CLSM images of GUVs after interaction with pheophorbide a inside a)-c) poly(ethyleneoxide-b-ε-caprolactone) 5k-4k; d) poly(ethyleneoxide-b-D,L-lactide); e) poly(ethyleneoxide-b-ε-caprolactone) 5k-4k cross-linked. Scale bar: 25µm.

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The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoscience for cancer/Nanoparticles and targeting
Keywords: Ion oxide nanoparticles, hyperthermia, theranostics, local drug delivery

SPION-mediated hyperthermia: which administration route for an efficient minimally invasive, multimodal tumor treatment ?

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Conventional hyperthermia is an established adjuvant in oncology, combined with radio- or chemo-therapy. Using superparamagnetic iron oxide nanoparticles (SPIONs), hyperthermia may help to treat difficult-to-reach and deep-seated tumors. A challenge towards clinical translation lies in the high SPION concentrations required at the tumor site to effectively deliver heat to the tumor. We discuss two distinct routes of administration, local injection to the tumor site vs subcutaneous injection of targeted nanoparticles with respect to their therapeutic potential.

Local deposition of SPIONs formulations capable of forming a depot at the tumor site may allow repeatable, mild hyperthermia. In mice xenografted with human Co112 tumor cells, 45 °C hyperthermia was obtained leading to a 45% one-year survival. For bone tumors, we embedded SPIONs in a cement used for spine stabilization and demonstrated the safety of the procedure in sheep. Sustained delivery of doxorubicin was also shown, in view of a combined chemo-thermotherapy of vertebral metastases.

In order to diagnose and potentially treat early prostate cancer metastases, SPIONs might be injected subcutaneously, trafficking towards lymph node metastases. In this view, we decorated SPIONs with an aptamer and a small urea-like molecule targeting PSMA (prostate surface membrane antigen). Specific binding to PSMA-positive cells (LNCaP) was demonstrated for both ligands. The nanocarriers could be detected by magnetic resonance imaging (MRI) *in vivo,* holding promises for the detection of specific cancer metastases. Still, in order to achieve therapeutic hyperthermia at the tumor site, efforts towards more effective heating and/or higher SPIONs accumulation are needed.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoscience for Cancer

Keywords: Breast cancer, immunoliposome, vascularization, trastuzumab, taxane

Turning poorly vascular tumors into highly vascular tumors with nanoparticles: pharmacometric analysis

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Taxanes and trastuzumab are the gold standard of chemotherapy in HER2 positif breast cancer. The aim of this study was to highlight was to compare the efficacy and the biodistribution of stealth liposome encapsulating docetaxel grafted or not with trastuzumab.

Immunoliposomes and liposomes were synthesized using the thin film method. Swiss nude mice were orthotopically grafted with MDA-MB-231 and divided in 3 groups. Immunoliposomes and liposomes were administered by retro-orbital injection once a week over 4 consecutive weeks. Tumor growth was monitored twice a week using caliper and 3D bioluminescence analysis. Vascularization was evaluated in each group in vivo by angiosens (vascular density) and ex vivo on tumors slice with anti-CD31 antibody (central vs peripheric vascularization). An algorithm was developed in MatLab for tumor image analysis.

After 4 weeks of treatment, tumor growth was similar in each group ($p=0.798$) and angiosens studies showed no significant difference of vascularization between each group ($p=0.628$). Fluorescent microscopy studies demonstrated an increase of vascularization on central tumors vs peripheric tumors slice after immunoliposome vs liposome or vehicle injection. Immunoliposome significantly increased CD31 distribution in central tumors vs peripheric one by Matlab analysis ($p=0.00023$).

In this work we studied tumor vasculature and permeability and demonstrated with angiosens similar vascularization in each group consistently with tumors growth however CD31 antibodies highlight vasculature normalization due to the treatment, and a differentiation between central and peripheric tumor vasculature. This reshaping was more efficient to improve perfusion in central tumor, biodistribution of liposome and should lead to greater efficiency.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoscience for Cancer
Keywords: glioblastoma, fisetin, cisplatin, liposomes

Co-encapsulation of fisetin and cisplatin into liposomes: optimization of formulation and process and *in vitro* evaluation

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In order to fight against glioblastoma, the antiangiogenic fisetin and the anticarcinogenic cisplatin were proposed to be co-encapsulated in liposomes, aiming at a synergistic effect. As the two active substances have different physico-chemicals properties, the challenge was to determine the best compromise between the incorporation of fisetin into the phospholipid bilayer and the prevention of cisplatin release during storage. After optimization of the formulation and the process of preparation, a molar ratio of cholesterol between 18 and 21% and a preparation method by film-hydration followed by extrusion were determined as optimal for the co-encapsulation. To improve the stability, we optimized a freeze-drying process. The optimal amount of fisetin incorporated into the phospholipid bilayer was also investigated using DSC. *In vitro* experiments in PBS demonstrated an interesting sustained release of the encapsulated cisplatin and showed a protection of fisetin by the liposomes in aqueous medium. Experiments on human endothelial cells EA.hy 926 proved that encapsulated fisetin had the same morphological effect and IC50 than free fisetin. MTT experiments on glioblastoma cells U87-MG showed a higher IC50 for encapsulated cisplatin than free cisplatin; the association with liposomal fisetin or the use of the co-encapsulating liposomes allowed to reach the same IC50 than free cisplatin. We observed for the first time on these cells a cytotoxic effect of fisetin. These results are promising for a first *in vivo* study and future use in human treatment of glioblastoma.

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The SFNano C'Nano joint meeting 2019

Thematic Session: nanomaterials

Keywords: gold nanorods; photosensitizer; PDT; glioblastoma; NRP-1; targeting

Hybrid Conjugates of Gold Nanorods: A Drug-Delivery Vehicle for Targeted Photodynamic Therapy

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The use of porphyrin photosensitizers (PSs) and their derivatives is common in the photodynamic therapy (PDT). Yet, these are hindered by their hydrophobicity and lack of tumor-selectivity. This work describes the synthesis of cancer cell-specific nanoparticle (NP)-based system as vehicle for the efficient and selective drug delivery. Gold nanorods (AuNRs) have been used in different medical sectors including cancer diagnostics and therapy[2]. Gold absorbs strongly in the visible and near-infrared ranges, possesses a high X-ray absorption capacity and serves as a radiosensitizing agent. In our work, AuNRs are synthesized and modified with polyethylene glycol (PEG) to limit their dark toxicity[3]. They are covalently conjugated with a PDT agent, pyropheophorbide-a (Pyro), and with a peptide moiety (PP) as a targeting agent. The physicochemical properties of the AuNRs-PEG-PS-PP system are studied. The photophysical characterization of the hybrid NRs revealed that they acquired the characteristic properties of Pyro concerning the absorption profile, the fluorescence intensity and singlet oxygen emission upon excitation at 412 nm. The hybrid AuNRs showed good molecular affinity for NRP-1 recombinant protein (IC_{50} = 1.1 µM). *In vitro* assays were conducted on glioblastoma U87 cells exposed to hybrid AuNRs at different Pyro concentrations and irradiated at 652 nm. The NRs did not display any cytotoxicity even at high Pyro concentrations. However, they efficiently suppressed the cell viability by 67% under light exposure. This nanosystem possess a good efficiency in vascular targeted PDT and a potential effect in a combined photodynamic/photothermal therapy guided by NIR fluorescence imaging of the tumors due to the presence of both the hyperthermic agent, AuNRs, and the fluorescent active phototoxic PS.

The SFNano C'Nano joint meeting 2019

Abstracts

Poster Session

P3 - BIO-INSPIRED NANOSYSTEMS

SFNano cnrs C'Nano

The SFNano C'Nano joint meeting 2019

Thematic Session: (eg. Nanophotonics&nano-optics, nanomaterials, …)
Keywords: (4-5 keywords are required)

Evaluation of mesenchymal stem cells derived small extracellular vesicles as a vector for intracellular protein delivery

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Based on their natural properties to vectorize proteins, extracellular vesicles (EVs) are promising candidate to be used as vectors for intracellular protein delivery. We therefore chosen to evaluate nanosized EVs produced from murine mesenchymal stem cells (mMSC) (sEV-mMSC) for antibody fragments (single-chain variable fragment, scFv) transfer in cell cytoplasm. To establish the proof of concept of scFv encapsulation into sEV-mMSC, as well as effective intracellular release of functional scFv, we chosen to use a GFP-tagged scFv directed toward intracellular tubulin (GFP-scFv/αtub) as a reporter protein. Indeed, this GFP-scFv/αtub could allow dual tracking of protein loaded (GFP) and evaluation of its activity by the tubulin staining ability. To associate GFP-scFv/αtub into sEV-mMSC we evaluated several physical methods inspired by the liposome field (extrusion, bath sonication cycles, freeze drying, freeze-thaw cycles and several combinations of these methods). Impact of these processes was evaluated on sEV-mMSC (concentration, size, structure) and GFP-scFv/αtub (stability) individually before evaluating scFv loading into sEV-mMSC. We identified protocols leading to less than 20% lost in terms of sEV-mMSC and GFP-scFv/αtub concentration. Finally, GFP-scFv/αtub internalisation and ability to link intracellular tubulin was evaluated in human MSC by fluorescence microscopy. Interestingly, scFv alone was shown as unable to enter target cells, while for one of our combinatory protocols, a tubulin staining was observed. These preliminary results confirm the interest of associating scFv with sEV-mMSC for intracellular delivery of a functional, biologically active protein.

The SFNano C'NANO joint meeting 2019

Thematic Session: Bio-inspired
Keywords: Gold nanoparticles, polyelectrolyte, nanoparticles assemblies, photothermal properties, biocompatible system

Controled Assembly of Gold Nanoparticles With Quaternized Chitosan for Photothermal Therapy

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Gold nanoparticles (AuNPs) can behave as nanosources of heat under light irradiation with a frequency close to the surface plasmon resonance frequency. In medical domain, gold nanoparticles are already commercially[1] exploited for applications such as photothermal therapy, bioimaging or drug delivery. The heating efficiency of gold nanoparticles is especially determined by: (1) the amount of light intensity reaching NPs inside the body, and (2) the absorption cross section.
A viable option to maximize the heating power per NP would be to assemble NPs in nanometric clusters[2][3]. Indeed, as pointed by recent simulations[4], linear "colloidal oligomers" or opened structures including linear chain fragments could enable to maximize the normalized light absorption efficiency per NP at $\lambda > 700$ nm. To our knowledge, few experimental studies were dedicated so far to this kind of systems because of the difficulty to assemble nanoparticles in nanometric clusters of finite size by using biocompatible compounds.
I will present a new strategy to assemble AuNPs with long chains of quaternized chitosan. I will first show the methodology used for the synthesis of quaternized chitosan chains. I will then present a structural study based on cryoTEM of the electrostatic complexes AuNPs/Quaternized chitosan. The structure of the electrostatic complexes will be correlated with UV-Vis-NIR spectroscopy and photothermal measurements for typical nanometric complexes. In the last part, I will show how neutral complexes with the best photothermal properties, but also the lowest colloidal stability, can be stabilized over a period of one month by adding an excess of quaternized chitosan

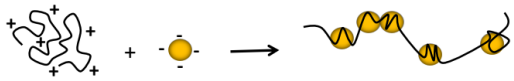

Figure 1: ***Schematic representation of quaternized chitosan coupled with gold nanospheres***

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The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticles and targeting, Bio-inspired nanosystems.
Keywords: nanotechnology, biomaterials, chitosan, oral delivery.

Tailoring structural properties of nanoemulsion-loaded chitosan scaffold for sustained intestinal delivery

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In this work, the development of a novel delivery system made of chitosan scaffold loaded with nanoemulsions (NEs) is presented. The aim is to achieve a sustained intestinal release of NEs from the chitosan (CH) matrix following oral administration [1-3].
CH and NE-loaded CH scaffolds, with varied porosity, were fabricated through a temperature-controlled freeze-casting process [4]. In order to select the system which best fitted the final application, scaffold structural and mechanical properties were modulated by varying the concentrations of CH (0.1% to 1% w/w) and NE (2.5% to 10 % w/w). Structural characterization of scaffolds was performed by scanning electron microscopy (SEM) and optical microscopy. From SEM images, it was shown that at high CH concentration scaffolds with defined cell-wall structure were obtained. Moreover, in presence of NE, scaffolds exhibited denser pore structure and enhanced mechanical strength due to hydrophobic interactions occurring between particles and polymer chains. Then, to investigate the impact of CH matrix on nanoemulsion release kinetics, scaffolds were rehydrated in biorelevant intestinal fluids (FaSSIF-V2). The water uptake capacity and NE release rate were highly dependent on scaffold structure and composition. A sustained NE release was achieved from scaffolds at high CH concentrations (50% of NE released after 8 h). While at low polymer concentration the NE release was faster (65% of NE released in 2 h and 100% after 72 h). Overall, these results demonstrated that nanocomposite scaffolds are promising systems for the development of sustained intestinal drug delivery.

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The SFNano C'Nano joint meeting 2019

Thematic Session: Bio-inspired nanosystems

Keywords: Membrane proteins, Parkinson's disease, nanodiscs,

Comparing detergent micelles and polymer nanodiscs for solubilization and purification of the integral membrane protein Vesicular Monoamine Transporter 2

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Vesicular Monoamine Transporter 2 (VMAT2) is the integral membrane protein responsible for transport of monoamines such as dopamine into vesicles for storage. Due to its role in dopamine regulation in the neurons the protein is an interesting target for treatments of dopamine deficiencies, such as Parkinson's disease and Tyrosine hydroxylase deficiency.

The main strategy used for disassociating and purifying integral membrane proteins from the membrane is the use of detergents, which disrupts the lipid bilayer, encasing the proteins in detergent-micelles. One of the drawbacks with detergent solubilization is that removing the protein from its native membrane may affect the structure and function of the protein, which are usually dependent on its lipid environment. In later years another strategy has also emerged, i.e. nanodiscs (ND), which are lipid bilayer systems encircled by either lipoproteins or specialized amphipathic co-polymers such as styrene/maleic acid (SMA) or diisobutylene/maleic acid.

In this work we explore the methods of solubilizing VMAT2, comparing detergent-based methods with NDs. We expressed VMAT2 in SF9 insect cells with GFP and 10x Histidine as fusion tag. , collected membranes by ultracentrifugation and solubilized VMAT2 using either detergents or SMA-NDs. Size of the resulting micelles or discs were confirmed with fluorescence size exclusion chromatography and dynamic light scattering, and morphology was examined using transmission electron microscopy. VMAT2 presence in the samples was confirmed using western blot, and functionality of the protein was determined using a known inhibitor of VMAT2, in a ^{3}H binding assay.

The SFNano C'Nano joint meeting 2019

Thematic Session: Bio-inspired nanosystems

Keywords: Cantilever, sensor, organophosphorus compounds

Nanostructured cantilevers for detection of organophosphorus compounds

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Organophosphorus compounds (OPs) used as pesticides or Chemical Warfare Agents (CWAs) such as sarin (GB), tabun (GA), soman (GD) and VX represent a real threat for civilians or armed forces. The detection of these molecules is a real challenge because no detection system can detect these products below their lethal threshold. Fast, selective and sensitive sensing methods are required to reduce the threat against CWAs [1].

This work is based on the concept of bio-inspired micromechanical cantilever sensors for fast and selective detection of low traces of nerve agent simulants such as dimethyl methylphosphonate (DMMP) in vapor phase [2]. The first step is dedicated to the surface nanostructuring of cantilevers with two kinds of one-dimensional nanomaterials (TiO_2 nanowires or ZnO nanorods). This strategy is essential to improve their sensitivity due to an increase of their specific surface areas and thus surface capture. TiO_2 nanowires and ZnO nanorods are synthetized by hydrothermal synthesis and soft chemistry [3] in a batch reaction, respectively. They are fully characterized (XRD, SEM) and transferred onto cantilevers. They are oriented vertically on the surface. In a second step, the cantilevers are functionalized with different organic molecules in order to improve their response and selectivity. Cantilevers were finally exposed to different concentrations of DMMP in order to evaluate their responses in terms of limit of detection (LOD) and selectivity.

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The SFNano C'Nano joint meeting 2019

Thematic Session: (Nanomaterials)
Keywords: (Cyclosoprine A, Colitis, Lipoproteins, targeting, inflammation)

The use of biocompatible Cyclosporine A loaded nanosystems ameliorates murine experimental colitis

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Cyclosporine A is a potent immunosuppressive agent, widely used for the prevention of graft rejection or the management of autoimmune diseases. Due to its blocking effect on interleukin-2, the drug was found to offer potential help to ulcerative colitis (UC) patients. Intravenous Cyclosporine A injections (2mg/kg/day) proved effective in 50-80% of severe steroid refractory UC patients, however, systemic risk of opportunistic infection and subsequent complications arise from the systemic use of the immunosuppressive drug. The use of nanotechnology would help targeting the drug to inflamed tissues and minimize the healthy tissues exposure. Interestingly, cyclosporine A has shown a high affinity to different lipoproteins and especially LDL. A strong correlation between Lipoproteins and inflammatory conditions indicates how they are involved in the pathogenesis of various inflammatory diseases. LDL immunoreactivity could enhance antibody-mediated clearance from blood and reduces vascular inflammation. The oxidized LDL recognition and uptake by macrophages is known to be abundant in case of atherosclerosis. HDL also had shown some anti-inflammatory, and anti-oxidant properties. Therefore, in the current work three lipoprotein formulations (HDL, LDL and VLDL-based) loaded with cyclosporin A were evaluated for the treatment of colitis in a murine model. The effect of variable concentrations of the different lipoproteins components on loading efficiency was evaluated. It was found that protein content of lipoproteins played a significant role in their loading capacity. After an intravenous injection of a drug dose of 2 mg/kg, clinical activity and the inflammatory markers MPO and TNF-**α** were compared to the untreated colitis control group.

A study of the properties of the D-Mannose in the natural treatment of urinary infections caused by *E- Coli*

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D-Mannose is a natural sugar; it is present in various foods, and binds to *E. coli*, which is then discharged in urine.

The purpose of this research is to prove the efficiency of D-Mannose on the urinary infection caused by E-coli bacteria.

First, we try to provide an approach to the mean duration of treatment by performing tests on laboratory rats, by provocation of UTI contaminating rats by different ways then, administration of D-Mannose orally. A bacteriological examination of urine was carried out and the interpretation of results was based on the sterility of the culture media.

Secondly, we study a protein-protein interactions, witch have an important role to understand the process of pathogenesis of bacterial and viral infections.

We have study the interaction between D -Mannose and the Fimh protein by the use of molecular dynamics method. Initially, several structural calculations and optimizations by Hyperchem8 software were conducted on D- Mannose to understand how this natural sugar attack the Escherichia coli bacterium.

Then, the Docking calculations were performed by Hex 6.3. Interpretation of results is based on the energy of interaction formed by ligands Alpha -D- mannose and Beta -D- mannose. The lowest energy of interaction of complex probably present a greater inhibition of Fimh protein.

Key words : D-Mannose, Urinary tract infection, Molecular Modeling, Molecular Docking, E. coli uropathogenic strains.