Wednesday, December 11th

YOUNG RESEARCHERS SESSION

Romanée-Conti Amphitheater

18h15 - 19h00

Abstracts

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanoparticules & therapeutic targeting

Keywords: Boron neutron capture therapy, Theranostic, Nanogel, BODIPYs

Polymer-based nanogels for image-guided Boron Neutron Capture Therapy

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Boron neutron capture therapy (BNCT) is a specific form of targeted radiotherapy. Its success relies on the sufficient and selective ^{10}B accumulation at the tumor site, which is followed by neutron beam irradiation. This binary system induces nuclear fission reaction that provokes huge damages in the ^{10}B-rich tissues. The efficacy of BNCT is still limited by the suboptimal distribution of clinically available ^{10}B agents. Thus, there is a fundamental need to design innovative theranostic ^{10}B nanocarriers, able to deliver ideal ^{10}B concentrations to the tumor tissues and to be evaluated using optical imaging.

To this end, biocompatible polymer-based nanogels were developed using functionalized hyaluronic acid moieties and selective core-crosslinking method. As a fluorescent probe for optical imaging in the near-infra-red (NIR) and source of boron, BODIPYs coupled to the clinically available ^{10}B-BSH were either encapsulated within the nanogel or evaluated as free compounds. In vitro, both formulations were evaluated in glioma cell lines. Their bio-distribution was observed in vivo on glioma-bearing mice. In perspective to BNCT, the different formulations were evaluated in cells and using in ovo glioma model.

The use of these theranostic formulations allowed monitoring the tumor accumulation, hence optimizing the treatment protocol for neutron exposure. Promising preliminary results regarding the tumor sizes were obtained, thus further studies will be conducted to confirm these data.

The SFNano C'Nano joint meeting 2019

Thematic Session: (Bio-inspired nanosystems)

Keywords: Polysaccharide nanoparticles; Fucoidan; P-Selectin targeting; Thrombolytic therapy

Thrombolytic therapy based on P-Selectin targeted polysaccharide nanoparticles

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Recombinant tissue Plasminogen Activator (rtPA) administration is the gold standard treatment for thrombolytic therapy, however, it causes high risks of intracranial hemorrhages. Thrombus-targeted drug delivery systems can limit these complications. The objective is to develop biocompatible and biodegradable nanocarriers suitable for the targeted treatment of thrombotic diseases.

Hereby, natural polysaccharide nanoparticles (NPs) were developed by w/o emulsification/crosslinking process. NPs were functionalized with fucoidan to target P-selectin, a thrombus molecular biomarker. NPs were spherical, homogeneous, and exhibited a size of 662 nm and a zeta potential of -30.3 mV. Fucoidan presence was confirmed by elemental analysis (9% w/w). NPs were cytocompatible at concentrations from 0.1 to 1.5 mg/ml by Resazurin assay on HUVECs. NPs remained stable for 30 days at 4°C.

Actilyse®, a clinical rtPA, was loaded onto NPs by adsorption (70% w/w). Flow cytometry monitored a classical rtPA release profile from NPs. The enzymatic and fibrinolytic activities of rtPA-loaded NPs were maintained, evidenced by *in vitro* assays. Microfluidic experiments proved high affinity of Fucoidan-NPs to P-Selectin and activated platelets in arterial and venous flow conditions, validating targeting strategy. Favorable biodistribution and safety profile of NPs were evaluated by histological analysis in healthy rats. Preliminary *in vivo* $FeCl_3$ mouse model of thrombosis experiments showed that Fucoidan-NPs were effective to induce thrombolysis.

To conclude, novel Fucoidan-functionalized polysaccharide NPs were loaded with rtPA, and their thrombolytic activity was tested in preclinical models of thrombosis. This proof of concept study underlines the potential of biomaterial-based targeted nanomedicine to treat acute thrombotic events.

The SFNano C'NANO joint meeting 2019

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

Noninvasive in vivo multimodal imaging of multiple bone metastases using a bimodal tumor targeting contrast agent for fluorescence and photoacoustic imaging

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Introduction: Photoacoustic imaging (PAI) is an emerging technology that provides unique opportunities to measure noninvasively both endogenous compounds such as oxy- and deoxy-hemoglobin and exogenous contrast agents. Although several inorganic compounds with photoacoustic contrast have been described, there is still a crucial need for organic contrast agents specifically targeting biomarkers of cancer development mechanisms.
In this study, we propose the use of a tumor-targeting tracer with bimodal fluorescence and photoacoustic properties for multimodal imaging of multiple bone metastases from human breast cancer.
Methods: A human aggressive breast cancer cell line (MDA-MB-231-ZNFrvluc2) was injected into the left ventricle of mice that led to the development of multiple bone metastases as previously described in [1]. Several days after cells inoculation, Angiostamp800 was injected intravenously and mice were imaged sequentially by bioluminescence, 3D fluorescence (fDOT), microCT and PAI. For the need of this study, we developed a multimodal animal-holder which can fit into the three last imaging systems in order to keep the animal in the same position and, therefore, combine the information coming from each imaging modality.
Results: in vivo bioluminescence showed multiple bone metastases in the skull, scapula, spinal column and legs. MicroCT imaging revealed multiple osteolytic lesions at the sites of these bone metastases. Both fDOT and PAI imaging then showed Angiostamp800 uptake in the metastasis locations and combination of fDOT and PAI reconstructed volumes with microCT bone volume demonstrated the collocation of the tracer's tumor uptake and the osteolytic lesions. Cross validation of fDOT and PAI was of particular interest since it is the first time that PAI is reported for bone imaging.
Conclusion: By using Angiostamp800, a unique tumor targeting contrast agent with bimodal fluorescence and photoacoustic properties and a dedicated multimodal animal bed, we were able to combine fDOT, PAI and microCT and this resulted in all in one 3D whole-body high-resolution morphological and high-sensitivity molecular images of multiple bone metastases bearing mice.

1. Bellanger, A., et al., *The critical role of the ZNF217 oncogene in promoting breast cancer metastasis to the bone.* J Pathol, 2017.

The SFNano C'Nano joint meeting 2019

Thematic Session: (Nanoparticles & targeting)
Keywords: (HPMA copolymers conjugates, arthritis, dexamethasone, biodistribution)

Drug delivery systems based on *N*-(2-hydroxypropyl) methacrylamide for treatment and diagnosis of arthritis

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The application of nanomaterials in medicine increased rapidly over the last years. They are often used for drug delivery purposes since they can improve drug pharmacokinetics and biological activity. The nanotechnology has been applied also in the field of inflammatory diseases, which represent a serious problem in contemporary medicine. The current treatment approach is generally accompanied by serious side effects on healthy tissues. Moreover, the therapeutic efficacy is only partial. Binding of the drug to the polymer systems can decrease or even eliminate the side effects since the active form of the drug is released in stimuli sensitive way at the desired site. Furthermore, the anti-inflammatory activity can be enhanced due to accumulation of polymer carrier in inflamed tissues caused by the ELVIS effect (Extravasation through leaky vasculature and subsequent inflammatory cell-mediated sequestration)[1].

This study is focused on the synthesis and study of physicochemical and biological properties of water-soluble (*N*- (2- hydroxypropyl)methacrylamide) (HPMA) copolymers and micellar poly(lactic-*co*-glycolic)acid-*block*-poly(HPMA) drug delivery systems containing active drug. The studied polymer systems determined for the treatment of acute arthritis were evaluated for their suitability for effective delivery and stimuli-responsive activation of drugs in inflamed joints. Fluorescently labeled polymers conjugates with dexamethasone bound via pH- sensitive hydrazone bond were accumulated in inflamed tissue in arthritic mice during the whole course of the experiment (4 days). The preliminary therapeutic experiment confirmed that described polymer systems are suitable as drug carriers, can be used in effective delivery to inflammation and have a great potential in the field of inflammatory diseases.

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The SFNano C'NANO joint meeting 2019

Thematic Session: Nanoparticle and targeting
Keywords: Triple negative breast cancer, theranostic, nanomedicine, anti-EGFR scFv, siRNA

Pegylated magnetic nanovectors functionalized with anti-EGFR scFv as a perspective siRNA vehicle for triple negative breast cancer targeting

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Triple negative breast cancer (TNBC) remains the poorest prognosis and the most limited therapeutic options subtype. TNBC is characterized by a chemotherapeutic resistance and by a lack of universal key biomarkers. To deal with the chemoresistance of TNBC, Pegylated Magnetic Nanovectors (PMN) based on superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with a targeting ligand were developed to deliver siRNA that inhibits expression of chemoresistance-linked protein (1). Anti-EGFR single chain variable fragment (scFv) was selected as the targeting moiety and grafted at the extremity of the PEG chains. siRNA association is performed by the complexation between cationic polymers, PMN and siRNA (2). Furthermore, PMN can also be used as imaging agent for theranostic properties.

This study focused on the optimization of the PEG layer density of PMN and its impact on 1) the amount of scFv grafted and its biological relevance, 2) the ability to complex and protect siRNA, 3) the delivery into the target cells. The increased of the polymer layer was achieved by increasing the ratio Fe/PEG and was confirmed by Dragendorff and Ellman methods. The active targeting with anti-EGFR scFv was highlighted by a factor of two in intracellular penetration of functionalized PMN with scFv compared to non-functionalized one. With higher density of PEG layer, the complexation of siRNA is not disturbed and the siRNA nanovectors have size around 100nm and a charge of +10mV. The potential delivery of siRNA into TBNC cell line expressing green fluorescent protein (MDA-MB231-GFP) was demonstrated with an efficient gene silencing effect.

(1) Alric *et al*, J of Nanobiotechnology, 2018

(2) Bruniaux *et al*, IJP, 2019

The SFNano C'Nano joint meeting 2019

Thematic Session: Nano for imaging, diagnosis & theranostics

Keywords: Nanobioparticles, Quantum dots, Nanothermometry, Nanophotonics

Towards a novel biocompatible probe allowing for real-time temperature measurements at cellular scale

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Biocompatible nanoheaters are of great interest as they can induce low and localized temperature gradients within malignant cells. Hyperthermia at cellular scale may significantly improve tumour response to current cancer-treatment gold standards, while reducing their invasiveness. To investigate this potential, a novel probe allowing for cellular scale temperature gradient measurements is required. Indeed, using currently available thermometers, thermal and spatial resolutions do not permit such measurements. In addition, abnormal temperature distribution at cellular scale is the first manifestation of health disorders. Thus, our novel temperature probe could surpass hyperthermia-therapy application and allow for early-stage diagnosis of diseases.

Silver sulphide quantum dots (Ag_2S Qds) were synthetized following two main routes and evaluated as potential nanothermometer to monitor temperature changes at cellular scale. Quantum dot's emission spectrum consists in a real-time fingerprint of its surrounding temperature. Indeed, temperature increase strongly quenches emission, allowing for concentration independent temperature sensing based on ratiometric measurements. Nevertheless, emission spectrum shape is strongly impacted by solvent or biological media optical behaviour (e.g photon reabsorption), preventing to perform one universal temperature probe calibration.

The talk details a method to figure out the best luminescent parameter for *in-vivo*-Ag_2S-Qds-based temperature sensing. Luminescent parameter selection is of crucial importance to perform a robust probe calibration and to move towards low uncertainties on nanothermometer's measurements. Our experimental approach involves different Ag_2S-Qds-containing biological media and the study of corresponding emission spectra thermal dependency for *in-situ* temperature going from 25°C to 60°C.

The SFNano C'Nano joint meeting 2019

Thematic Session: Nanochemistry: synthesis & functionalization of nanosystems for bioapplications

Keywords: magnetic hyperthermia, magnetic nanoparticles, surface chemistry, thermoablation

Modulation of thermal dose in tumors by using magnetic nanoparticles

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In magnetic hyperthermia experiments, huge quantities of magnetic nanoparticles (MNPs) are required to achieve complete tumor ablation, while preserving surrounding healthy organs. Moreover, quiescent cells-those that are present in the tumor environment and responsible for cancer relapse-are still present after the treatment. To face these problems, new approaches using alternative strategies must be developed, including those that target the tumor's microenvironment. Magnetic hyperthermia may be used not only for the thermoablation of solid tumors but also in mild hyperthermia conditions that can be synergistically combined with other therapeutic agents or to induce gene expression through thermosensitive promoters. Therefore, MNPs simultaneously exhibiting a high specific heating power for a better control of the released thermal dose and a modified surface chemistry to keep them in the tumor microenvironment, in the optic of longterm tumor treatment to prevent cancer relapse.

The present study reports the development of multimodal hybrid nanoparticles composed of iron oxide nanoflowers [1] surrounded by a NIR-fluorescent silica shell. These MNPs were surface-modified with quaternary ammoniums enabling them to have permanent positive charges in order to improve the residence time in the extracellular matrix of tumors and promote endocytosis [2]. Magnetic hyperthermia monitored by optical imaging was performed *in vivo*, after intratumoral injection of MNPs. Results obtained *in vivo* show that, by injecting a dose of iron oxide giving a concentration in tumors ten times lower than that used for clinical trials, core tumor thermoablation can occur while inducing thermosensitive gene expression in the peripheral microenvironment. This modulation of the microenvironment may be interesting to prevent cancer relapse in the case of incomplete tumor destruction.

References: [1] *Chem. Mater*. 2004, 16, 25, 5527-5534. [2] *Nat. Nanotech.* 2016, 11, 533-538.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanochemistry **or** Bio-inspired nanosystems

Keywords: Self-assembled nanoparticles, Elastin-like polypeptide, Hyaluronic acid, Targeted drug delivery

Design and self-assembly of HA-*b*-ELP block copolymers for cancer drug delivery applications

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The combination of natural polysaccharides and elastin-like polypeptides (ELPs) into block copolymers is anticipated to lead to materials with precise stimuli-responsive self-assembly properties and bioactivities. In this presentation, we will present the design, synthesis, characterization and self-assembly of macromolecular bioconjugates composed of Hyaluronic Acid (HA) as hydrophilic and bioactive (CD44 targeting) segment and of a 60 repeat unit-ELP containing periodically spaced methionine residues, ELP[M_1V_3-60]. (Figure 1A)

ELPs were produced recombinantly in *Escherichia coli* bacteria and post-modified at the *N*-terminal chain end to anchor an alkyne reactive group. HA was modified at the reducing end with a complementary azido group to allow the synthesis of the diblock copolymer by copper-catalyzed azide-alkyne Huisgen cycloaddition (CuAAC). The resulting bioconjugate was characterized by NMR and SEC and its temperature-triggered self-assembly was investigated by turbidimetry and light scattering techniques. HA-*b*-ELP[M_1V_3-60] was found to self-assemble into well-defined 50 nm diameter nanoparticles in TRIS buffer above a specific transition temperature (*Tt*) and to reversibly disassemble below the *Tt*.

Further tuning of HA-*b*-ELP bioconjugates self-assembly properties is currently explored using two different strategies. ELPs of longer chain length, namely ELP[M_1V_3-80] and ELP[M_1V_3-100], were produced and their thermal responsiveness experimentally determined to confirm theoretical predictions. ELP[M_1V_3-60] was also chemoselectively modified at methionine residues as an easy means to tune its *Tt* as well as to orthogonally conjugate drugs to access smart drug delivery nanocarriers.

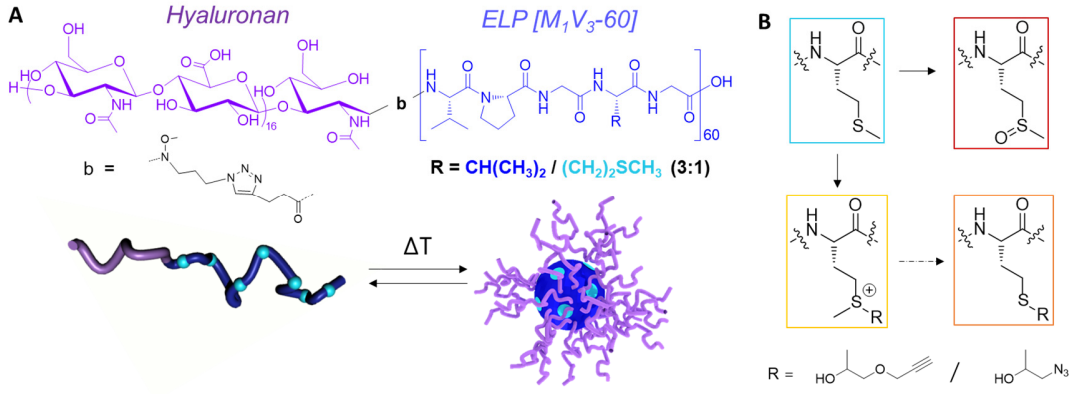

Figure 1: A) Chemical structure of HA-b-ELP[M_1V_3-60] and illustration of its temperature-triggered self-assembly. B) Examples of ELP chemoselective modifications at methionine residues for further tuning of physico-chemical properties or functionalization.

The SFNano C'NANO joint meeting 2019

Thematic Session: Nanochemistry: synthesis and functionalization of nanosystems for bioapplications
Keywords: Bioapplications, Synthesis, Nanoparticles, Phosphates, Peritoneal Dialysis.

Adsorption of Phosphates onto Iron Oxide Raspberry-Shaped Nanostructures for Peritoneal Dialysis

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The use of functionalized nanoparticles (NPs) for pollutant removal applications in a biological environment emerged recently as an important topic[1]. Nowadays, studies have shown the promising capacity of magnetic iron oxide (IO) nanoparticles for removing of pollutants from water or human body[2, 3, 4]. Magnetic nanoparticles can be easily separated from water under an external magnetic field. Moreover, they may have a high surface-area that allow high removal capacity of micro-pollutants with a small quantity.[5] In that context, we have designed iron oxide nanostructures to improve the phosphate removal during the peritoneal dyalisis process

At first, we have developed the synthesis of magnetic raspberry shaped nanostructures (RSN) by modified-polyol solvothermal methods.[5, 6, 7] This iron oxide nanostructures consist in orientated aggregates (150-500 nm) of iron oxide nanocrystals (10-25 nm) which preserve them from oxidation and provide a very high saturation magnetization (Ms ~ 85 emu·g^{-1})[8,9]. In addtion, they display a superparamagnetic behavior favoring their colloidal stability.

We have then investigated the adsorption of phosphates using these RSNs as function of pH and RSN concentration. We demonstrated thus that they can be used as phosphate recyclable adsorbent which can be extracted from the media by applying a magnet. I*n vitro* experiments have been further conducted in a home-made peritoneal dialysis model to evaluate the efficiency of dyalisis performed by adding such iron oxide nanostructures.

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