Wednesday, December 11th

Session: BIO-INSOPIRED NANOSYSTEMS

Romanée-Conti Amphitheater

14h30 - 16h30

Keynote speaker: Sébastien LECOMMANDOUX

From bioactive polymeric vesicles to autonomous cell-like reactors

Keynote speaker: Maria-José BLANCO-PIETRO

Treatment of pediatric osteosarcoma using nanomedicine

Abstracts

The SFNano C'Nano joint meeting 2019

Thematic Session: (Bio-inspired nanosystems)
Keywords: (extracellular vesicles, regenerative medicine, stenosis, thermo-responsive hydrogel)

Triggering high-yield scalable production of extracellular vesicles from adipose stromal cells and local administration in a hydrogel for regenerative medicine: proof-of-concept in an esophageal stenosis porcine model

Amanda Silva[1], Elise Coffin[2], Alice Grangier[1], Iris Marangon[1], Shony Lemieux[1], Imane Boucenna[1], Claire Wilhelm[1], Florence Gazeau[1], Gabriel Rahmi[2]

1. *Laboratoire Matière et Systèmes Complexes, CNRS UMR 7047 Université Paris Diderot, 10 rue Alice Domon et Léonie Duquet, Paris, France,*

2. Hopital Européen Georges Pompidou, APHP and PARCC, INSERM U970, Université Sorbonne Paris Cité (USPC), Université Paris Descartes, 56 rue Leblanc, 75015, 75015, Paris, France

The clinical translation of extracellular vesicle (EV) regenerative medicine is hampered by difficulties in producing EVs in large scale at high yield. Inspired by EV release induction by blood flow, we propose to boost EV release by exerting a controlled physical stress on cells during their 3D culture in bioreactors. Considering that EV administration is another critical issue, we propose EV delivery in a carrier gel. We capitalize on the off-label use of Poloxamer 407, a vessel occlusive thermoresponsive gel medical device authorized in Europe and USA. The proof-of-concept of these approaches was investigated in a porcine esophageal stenosis model. Our strategy relies on shear stress acting on cells to trigger EV release. We evidenced that flow tuning triggered massive EV release (10 times more and 10 times faster than classical starvation method). The frequency of alimentary troubles was statistically lower for the group treated by the gel and EVs 16.7% when compared to negative control and gel groups (83.3% and 66.7%, respectively). In conclusion, our results evidenced that EVs produced by our turbulence method and administered locally via a carrier gel have a therapeutic effect in a swine model of esophageal stenosis. The proposed turbulence method is expected to be advantageous in terms of high-yield, cost-effectiveness and time-saving. The proposed administration strategy is expected to transiently retain EVs in the site of interest, limiting their wash-out by digestive secretions.

The SFNano C'Nano joint meeting 2019

Thematic Session: Bio-inspired nanosystems

Keywords: polymer therapeutics, infection, internalization

Investigating the cytotoxicity, uptake and intracellular fate of dextrin-colistin conjugates as novel nanoantibiotics

Mathieu Varache[1], Sian Rizzo[1], Edward Sayers[2], Arwyn Jones[2], David Thomas[1] and Elaine Ferguson[1]

1. Advanced Therapies Group, School of Dentistry, Cardiff University, Cardiff, UK

2. School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK

Data collected by the European Antimicrobial Resistance Surveillance Network (EARS-Net) for 2016 show resistance to at least one class of antibiotic in many strains of Gram-negative bacterial pathogens[1]. Whilst resistance to colistin is rarely reported, its systemic administration is limited by dose-dependent nephrotoxicity, a consequence of reabsorption and accumulation in renal tubular cells. We developed dextrin-colistin conjugates, whereby colistin is released by amylase-triggered degradation of dextrin, thereby reducing colistin's clinical toxicity and improving targeting to infected tissues[2-4]. These conjugates demonstrated comparable antibacterial activity to the clinically used pro-drug, Colomycin® (CMS), but with reduced *in vivo* toxicity and prolonged plasma half-life[2-4]. Recently, we demonstrated that the degree of dextrin modification affects the nature of the released species, thus altering the biological activity[5]. Therefore, the aim of this study was to evaluate whether dextrin conjugation reduces cellular internalization, accumulation and cell death.

A multiplexed *in vitro* cytotoxicity assay showed that dextrin conjugation reduced colistin's toxicity in kidney proximal tubule epithelial cells (HK-2). In contrast to the cellular necrosis induced by colistin, the conjugates induced DNA accumulation indicative of cell cycle disruption. To study cellular uptake and fate, Oregon Green (OG) was conjugated to dextrin, colistin and dextrin-colistin conjugates. Flow cytometry showed significantly less cellular internalisation of the conjugates when compared to OG-colistin. In addition, live-cell imaging using confocal microscopy revealed preferential accumulation of both free and dextrin-bound colistin in lysosomes after long-term (16 h) incubation. Studies to confirm the mechanism of cellular uptake are ongoing.

1. European Centre for Disease Prevention and Control (2017) Surveillance of Antimicrobial Resistance in Europe 2016. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).
2. Ferguson EL, et al (2014) Dextrin-Colistin Conjugates as a Model Bioresponsive Treatment for Multidrug Resistant Bacterial Infections. *Mol Pharm*. 11: 4437-4447.
3. Azzopardi EA, et al (2015) Development and Validation of an *In Vitro* Pharmacokinetic/Pharmacodynamic Model to Test the Antibacterial Efficacy of Antibiotic Polymer Conjugates. *Antimicrob Agents Chemother.* 59: 1837-1843.
4. Roberts J, et al (2016) *In Vitro* Evaluation of the Interaction of Dextrin-Colistin Conjugates with Bacterial Lipopolysaccharide. *J Med Chem.* 59: 647-654.
5. Varache M, et al (2019) Polymer Masked-Unmasked Protein Therapy: Identification of the Active Species after Amylase Activation of Dextrin-Colistin Conjugates. *Mol Pharm.* 16: 3199-3207

The SFNano C'NANO joint meeting 2019

Thematic Session: Bio-inspired nanosystems

Keywords: hybrid nanosystems, synthetic biology nanosystems, artificial proteins, phage display screening, protein-Au(111) interface engineering, protein origami

Artificial repeat proteins evolved as habit modifiers and protein origami templates for the morphosynthesis of (111)-terminated gold nanocrystals

Janak Prasad[1], Sébastien Viollet,[2] Laureen Moreaud,[1] Agathe Urvoas,[2] Marie Valerio-Lepiniec,[2] Agnès Mesneau,[2] Philippe Minard[2] and Erik Dujardin[1]

1. *CEMES, CNRS UPR 8011, 29 rue Jeanne Marvig, F-31055 Toulouse, France*
2. *I2BC, Univ Paris Sud, CNRS, CEA UMR 9198, Bât. 430, F-91405 Orsay, France*

Two main strategies to control the morphology of nanoparticles are the growth inhibition by molecules with strong affinity for the growing material and the templated growth on a molecular matrix. Here, we demonstrate, for gold nanocrystals, that the full design of artificial proteins offers a universal and biocompatible approach to both strategies.

First, we demonstrate the *ab initio* design of a library of fully folded rigid and thermostable artificial repeat proteins (named alpha-Reps) [1] and the evolutionary selection of a subset of proteins showing high affinity and specific binding for Au(111) crystal facets. A seeded growth of gold nanocrystals in the presence of alpha-Rep reveals the exclusive formation of Au(111)-terminated nanostructures - icosahedrons, decahedrons and 2D nanoplates – in high yield, demonstrating the morphosynthetic efficiency of the selected proteins. Next, the protein-coated nanocrystals are coupled to active biomolecules conferring more functionality to the plasmonic nanocrystals such as biomolecule-driven self-assembly and surface confined catalysis.

An alternative approach is the design of shape-directing templates. One promising avenue is to expand the concept of DNA origami to proteins. We show how directed evolution of the alpha-Rep library leads to the selection of two artificial proteins that act as the supercoil unit and the staple to self-assemble into large helical superstructures. Their regioselective interactions are optimized to spontaneously form tubular protein origami helices as observed by cryoelectron microscopy.