NANO STEM

H2020-MSCA-ITN-2017 G.A. 764958

2nd NANOSTEM

International Meeting

An online event

ZOOM

Thursday 16th July 2020

Thursday 16th July 2020 (Time in BST)

9:00 – 9:15	The NanoStem project - Prof. Marina Resmini, QMUL, UK
9:15 – 10:15	Federico, Alena, Roberta – presentations
10:15 – 10:25	**Break**
10:25 – 11:25	Ines, Francesca, Angela – presentations
11:25 – 11:35	**Break**
11:35 – 12:35	Eleonora, Elisa, Georges –presentations
12:35 – 14:00	**Lunch**
14:00 – 15:00	Sara, Matteo, Sonia – presentations
15:00 – 15:10	**Break**
15:10 – 15:50	Patrick and Eirini – presentations
15:50 – 16:30	**Break**
16:30 – 17:30	ESRs networking session (“next set of transferable skills”)
16.30 – 17.30	Supervisory board meeting

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Abbreviation	Organisation
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CNC	Center for Neurosciences and Cell Biology, University of Coimbra, Portugal
UA	Universite D'artois, France
KI	Karolinska Institutet, Sweden
CHUC	Centro Hospitalar E Universitario De Coimbra, Portugal
HMGU	Helmholtz Zentrum Muenchen, Germany
MJR	MJR PharmJet GmbH, Germany
UIBK	Universität Innsbruck, Austria
UoB	University of Birmingham, UK
UC	Universidade de Coimbra, Portugal
HP	HCS Pharma, France

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ABSTRACTS

Nanoparticles for Brain Drug Delivery: Development of Novel Nanogels.

Federico Traldi – ESR1

School of Biological and Chemical Sciences, Queen Mary University of London, E1 4NS

Abstract

The incidence of neurodegenerative diseases has dramatically increased in the last few decades.[1] Despite the advances in drug discovery and drug development technologies, the number of drugs targeting neurodegenerative diseases is still relatively low.[2] This high rate of unsuccess is mainly due to the blood-brain barrier (BBB), a semipermeable cellular membrane separating the brain tissue from the blood compartment. Although the BBB offers protection to the brain from toxins and pathogens, it prevents most of developed pharmaceuticals to reach a therapeutic concentration in the brain tissue, leading to ineffective treatments.[3]

Nanoparticles for drug delivery have attracted increasing interest in the scientific community as they can be designed to encapsulate drugs and improve their permeation through the BBB. Among the plethora of different nanoparticles that are currently being investigated, polymeric crosslinked nanogels offer an attractive alternative.[4] The hydrophilicity of nanogels grant them high colloidal stability, while their high surface volume ratio and the possibility to introduce "smart" switches in their structure give them high drug cargo capacity and stimuli responsive release.[5]

In this work, progresses in the synthesis and characterization of nanogels incorporating fluorescent monomers is discussed. Fluorescence is employed to assess nanogels permeation through a BBB *in vitro* model designed by the University of Artois. Latest results regarding the study of protein corona formation on nanogels using model proteins fibrinogen, albumins and lysozyme are also presented.

References

1 Alzheimer's Disease International, *The global impact of dementia*, 2015.

2 W. . Pardridge, *J. Neurochem.*, 1998, **70**, 1781–1792.

3 C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira and L. Bernardino, *J. Control. Release*, 2016, **235**, 34–47.

4 K. S. Soni, S. S. Desale and T. K. Bronich, *J. Control. Release*, 2016, **240**, 109–126.

5 S. A. Papadimitriou, M. P. Robin, D. Ceric, R. K. O'Reilly, S. Marino and M. Resmini, *Nanoscale*, 2016, **8**, 17340–17349.

The effect of synthetic methodology on the morphology and properties of thermo-responsive nanogels

Alena Vdovchenko – ESR2

Alena Vdovchenko[1], Amanda Pearce[2], Rachel O'Reilly[2], Marina Resmini[1]

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Abstract:

Unique thermo-responsive behaviour of Poly(N-isopropylacrylamide)-based cross-linked nanogels make these materials particularly attractive for drug delivery to the brain. An increase in temperature induces rearrangement in the polymer network, which allows the transition of nanoparticles from a swollen to a shrunken state. Literature data report the use of different methodologies for preparing nanogels, including precipitation polymerization [1],[2], aqueous surfactant-free polymerization [3],[4],[5], high dilution radical polymerization in aprotic solvents [6] to cite a few. Understanding the impact of the synthetic method on the thermoresponsive behaviour of nanogels is a priority, in order to correlate synthetic parameters with particle morphology. Therefore, a library of PNIPAM-based nanogels were synthesized by various methodologies based on free radical polymerization. Our results show that the choice of radical initiator has a great influence on the morphology and characteristics of nanoparticles. By changing the nature of the initiator used, it is possible to suppress a microscopic aggregation of nanoparticles above the volume phase transition temperature. At the same time, it was shown that polymerization under homogeneous conditions, when all components are soluble during the reaction, allows to obtain smaller, but more polydisperse particles. Additionally, the impact of interactions between nanogels and surfactant / solvent molecules, occurring during polymerization, on the thermoresponsive behaviour of the final matrix is evaluated.

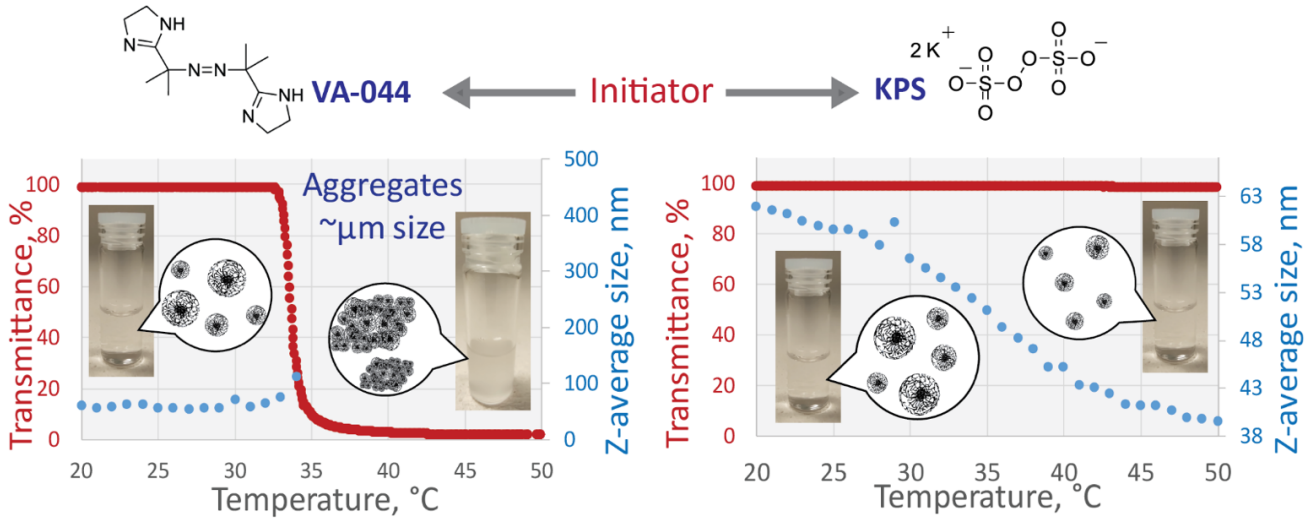

Fig. 1. Schematic representation of how the choice of an initiator can change the macroscopic properties of a nanogel

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Polymeric cross-linked nanogels for brain drug delivery: characterisation and *in vivo* studies on zebrafish

Roberta Biliardo – ESR3

Roberta Bilardo, Caroline Helen Brennan, Marina Resmini
School of Biological and Chemical Sciences, Queen Mary University of London, E1 4NS

Abstract

Polymeric covalently cross-linked nanogels are very promising candidates for the delivery of drugs to the central nervous system. Their properties, such as particle size, morphology and chemistry, can be easily tailored by the use of specific functional monomers. These particles' ability to form stable colloids, while retaining a high volume-to-surface ratio, allows enhanced loading capability. Moreover, preliminary *in vitro* studies on these nanogels have provided evidence of low toxicity [1].

Neutral and charged thermoresponsive N-isopropylacrylamide-based nanogels crosslinked with N,N'-methylenebisacrylamide were synthesised *via* high dilution radical polymerisation. The charged formulations were prepared by adding small quantities of charged monomers and their physicochemical properties compared with those of the neutral ones. The particle size and charge of the optimised nanogels were characterised by using dynamic and electrophoretic light scattering, respectively. While the thermoresponsive behaviour was assessed by UV-vis spectroscopy. In this talk, preliminary results on the nanogels' toxicity are showed. Toxicity studies were performed *in vivo* on early-aged zebrafish (Figure 1), using both neutral and charged nanogels. The main nanogels' characteristics evaluated against the response induced *in vivo* were (i) the degree of crosslinking and (ii) the presence of differently charged chemical groups on the surface. Additionally, the first results obtained from the exposure to fluorescently labelled nanogels are presented.

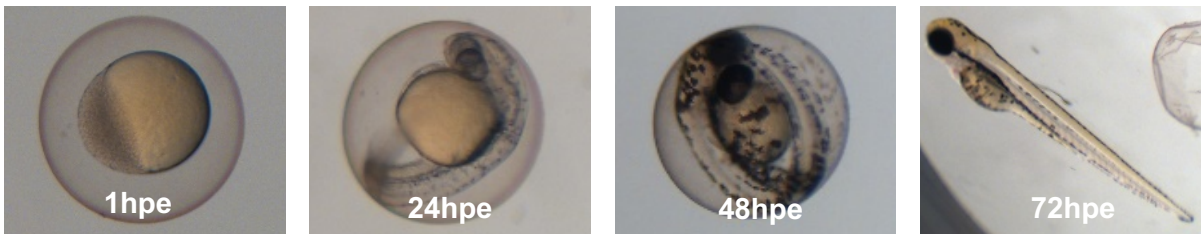

Figure 1. Zebrafish embryos exposed to a nanogel preparation, at different hours post-exposure (hpe).

[1]. S. A. Papadimitriou, M. P. Robin, D. Ceric, R. K. O'Reilly, S. Marino and M. Resmini, *Nanoscale*, 2016, **8**, 17340.

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Engineered extracellular vesicles for stroke therapy

Ines Albino – ESR4

CNC-Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal

Abstract

Ischemic stroke remains a leading cause of death and disability worldwide. Current treatments available in the clinic focus on restoration of the blood flow when administered within 4.5 h after stroke; however, only 2 to 10% of all stroke patients receive this treatment. Moreover, the complexity of the restorative pathways triggered after cerebral ischemia may require new therapeutic approaches involving different regenerative mechanisms. Extracellular vesicles (EVs) are promising candidates affecting several of these pathways. Preclinical stroke models have shown that EVs support brain restoration and induce repairing effects, including neurovascular remodeling. Despite the beneficial effects reported, these outcomes seem to be indirectly mediated by a systemic anti-inflammatory response rather than a local response and thus it remains to be determined potential side effects of this indirect therapeutic strategy. In addition, insufficient targeting capability and accumulation at the injury site may limit the therapeutic success of EVs. Here, we present a bioengineering platform for intra-arterial administration of EVs in the brain, which is able to recognize and interact with the brain endothelium increasing the accumulation of EVs in the brain parenchyma. EVs have been conjugated at their surface with hyaluronic acid functionalized or not with peptides targeting transcytosis or inflammatory receptors located in endothelial cells. HA interaction with blood plasma and the endothelial cell layer was investigated by *in vitro* studies. Our first results indicated a higher EVs uptake in the presence of HA as compared to EVs conditions alone. These results may provide preliminary cues of a strategy for EVs approximation to the brain endothelium.

Gene editing of neural cells by nanoformulations

Francesca Tomatis – ESR5

CNC-Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal

Abstract

The neuronal death is one of the consequences of brain disorders such as stroke and neurodegenerative diseases, conditions that have been related with ageing. However, both because of the post-mitotic properties of neurons and because of the reduced proliferative ability of aged tissues, the human body cannot provide for the loss of these cells. Therefore, the regeneration of the affected area can be achieved through the modulation of endogenous neural stem cells, that in the adult brain are located in the subgranular zone of the dentate gyrus in the hippocampus and in the subventricular zone. The stem cells can be genetically edited through the CRISPR/Cas9 tool, which can be coupled to nanoformulations that allow to better control the behavior of the system or its crossing of the blood brain barrier (BBB). Different approaches can then be explored: gold nanorods are useful to release the payload only once the nanoformulation is inside the cells, while modified extracellular vesicles can be exploited to improve the BBB crossing after intravenous or intra-arterial injection. The two solutions are under development both to reverse senescence in neural stem cells and to reprogram brain cell populations into neurons (in this case with the catalytically dead Cas9 enzyme instead of the classic Cas9), and tests are going to be performed *in vitro* before checking the systems also *in vivo*. Finally, as the crossing of the BBB is a fundamental step, static and dynamic models of the barrier are under establishment. They need to be fully characterized in order to be employed to investigate the ability of the nanoformulations to reach the brain even when injected into the bloodstream.

Engineered formulations for brain gene edition

Angela Berrera – ESR6

Angela Barrera[1], Susana Simões[1], and Lino Ferreira[1,2]

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Neurodegenerative diseases are a group of pathologies characterized by the progressive loss of neurons, synapses, and metabolic function in the brain parenchyma. Ageing at the blood-brain barrier (BBB) as well as neural stem cells, through gene alterations, may contribute to these disorders. Hutchinson-Gilford progeria syndrome (HGPS) is a rare, progressive premature aging disease resulting from a de novo point mutation in the *LMNA* gene that leads to the generation of an abnormal LMNA protein named "progerin". The accumulation of progerin in vascular cells lead to premature vascular ageing. The aim of our work is to genetically edit HGPS cells such as brain endothelial cells using CRISPR/cas9 technology. In the last months, we have performed initial studies to validate the sgRNA using a plasmid encoding Cas9 and sgRNA. Our results indicate that the plasmid internalization by HGPS cells was relatively low. So far, the gene editing efficiency is below 15% and progresses are being made to improve the efficiency. In parallel, we have started gene editing experiments using a non-viral formulation gold nanorods (AuNR). The efficiency will be determined by evaluating the decrease of progerin with RT-qPCR and Miseq, further nucleic abnormalities by immunofluorescence. Future experiments will evaluate the gene editing properties of AuNR formulation in HGPS cells to show the disruption of LMNA and progerin.

Permeation of nanoparticles in a disease *in vitro* Blood-Brain Barrier model

Eleonora Rizzi - ESR7

Universite d'artois, France

Abstract

Stroke is an acute neurological disorder whose ischemic subtype, prevalent across patients, is caused by the formation of a thrombus in a brain vessel. The consecutive decrease or blockage of blood flow induces the depletion of oxygen and nutrient to a portion of the brain creating a pathological cascade leading to cell damage and death whose extent and severity depends on the occlusion time. This pathology requires emergency care associated with a narrow therapeutic window, to restore the blood flow and minimize the extent of the damage. However, the restoration of the blood supply, represents itself a critical event that can also emphasize the cellular damages. Considering the narrow window of intervention of the only approved treatment for the acute phase of the pathology to restore the blood flow, therapeutic strategies aiming at reducing the infarct size, are now more focused on the latest events such as the differentiation of the neural stem cells (NSCs).

In this frame, nanomedicine is gaining more and more attention. Among the developed nanoparticles, nanogels represent a promising material based on polymeric networks, physically or chemically crosslinked. They held high drug encapsulation capacity with minimal toxicity associated with an active participation to the delivery processes. Hence the delivery of drugs stimulating the NSCs differentiation, vectorised by nanogels into the brain, can represent an innovative therapeutic approach to treat stroke. However, the access to the brain parenchyma is controlled and restricted by the blood brain barrier (BBB) which poses a great challenge in drug delivery to the brain. Located at the level of the capillary endothelial cells (ECs) the BBB ECs possess specific characteristics that distinguish them from ECs of the peripheric vessels. Moreover, the BBB has a specific architecture including close interactions and communications among the neurovascular unit comprising different cell types which are crucial for the maintenance of the BBB in physiological conditions and in response to pathological stress.

In this work, we have developed a human BBB *in vitro* model, consisting of a triculture system, and submitted to oxygen- glucose deprivation (OGD) and reoxygenation (R) to mimic the ischemic stroke. The genotype and phenotype of the BBB model following OGD-R was characterized. Moreover, as the model allows to study the crossing of nanogels through the BBB before and after OGD-R, the study protocol of nanogels transport through the physiological BBB was developed and preliminary data collected.

Development of Blood-brain barrier (BBB) assay that allows improved screening of higher number of compounds

Elisa Moya - ESR 8

Universite d'artois, France

Abstract:

The already well-established *in vitro* BBB model from *Cecchelli et al, 2014.*[1], formed with human brain like endothelial cells (BLECs) derived from human cord blood hematopoietic stem cells $CD34^+$, in co-culture with brain pericytes to induce the BBB properties, developed in 12 TransWells (TW) format system, can be used for toxicity, permeation, as well as mechanism and internalization pathway studies of compounds and nanoparticles (NPs) within the brain Endothelial cells (ECs).

On one hand, this 12 TW *in vitro* BBB model, has been used and adapted for the study of fluorescence NPs. Latest efforts were focused in the study of the mechanisms of transport of the PLGA NPs loaded with Lumogen, and coated with surfactants P188 (P188 NP) and PS80 (PS80 NP), developed by MyBiotech (MJR Pharmjet). The study has been performed by testing a range of selected inhibitors and antibodies against organelles involved in different metabolic routes of molecules internalization within the ECs. In preliminary results (obtained of triplicate samples (n=3)) has been found that; both NPs are able to internalize in the brain ECs, visualized within the cellular cytoplasm by fluorescence microscopy; being some NPs able to succeed in the ECs crossing, in a range between 2 to 4%; NPs present toxicity in concentrations higher than 50 µg/ml in a maximum 24 hours incubation tested; Both NPs internalization are metabolic dependent, visible when cell metabolism is stopped at 4ºC in temperature-dependent experiments for 1 hour; in addition, results show a tendency in the decrease of NPs uptake when ATP inhibitors were present in the cell media meaning a possible active transport mechanisms dependent; Due to some co-localization tests of NPs with specific antibodies against early endosomes, NPs seem internalize by endocytosis vesicles, since some co-localization was found after a NPs incubation of 30 min and 1 hour, visible by confocal microscopy; Moreover, both NPs could interact with membrane receptors which mediate transcytosis pathway, because of a NPs uptake decrease when NPs were co-incubated with Low Density Lipoproteins (LDL) and acetylated LDL (acLDL), suggesting a possible receptors competition for the cell internalization as well as in the endothelium crossing; finally, by using a set of inhibitors for caveolae, clathrin and macropinocytosis pathways, results showed a tendency in the decreased of P188 NP uptake by one clathrin

formed vesicle inhibitor; however, this tendency is different for PS80 NP, which shows a decreased in the uptake for inhibitors involved in caveolae and clathrin pathways.

Moreover, on the other hand, in base of this already well-established BBB *in vitro* model, during the secondment in HCS Pharma, the aim is to develop a miniaturized (96 TW system format) and automated BBB model, allowing a higher and faster reproducibility, as well as a screening of a higher number of compounds. To do so, 96 TW insert systems with different characteristics have been tested, by using a wide range of cell seeding densities ratio ECs / Bovine pericytes in co-culture. An optimum work cell seeding ratio for each plate type has been found. Permeability and immunofluorescence assays were adapted, and automated by using a robot, in addition to the cell seeding step in sterile conditions. As preliminary results, a good correlation between the usual *in vitro* BBB 12 TW format, which is used as control, and the 96 TW system has been found, in terms of BBB integrity, BBB phenotype (tight junction proteins), and permeability of some fluorescent compounds tested.

1. Cecchelli R, Aday S, Sevin E, Almeida C, Culot M, Dehouck L, et al. A stable and reproducible human blood–brain barrier model derived from hematopoietic stem cells. PLoS ONE. 2014;9(6):e99733

Blood-brain barrier permeation using DNA nanotechnology

Georges Kiriako – ESR9

Abstract

Receptor-mediated transcytosis (RMT) across the blood-brain barrier (BBB) is a key component in delivering drugs to the brain. Avidity, the accumulated strength of multiple protein-protein interactions, is a major regulator of RMT and has become a key factor in drug design. Previous research has shown that an optimal BBB permeation could be achieved by varying the avidity of nanoparticles. However, in these systems, only crude control of avidity levels could be achieved by changing the average surface density of ligands on the surface of nanoparticles. In this study, we used DNA nanostructures as scaffolds to manipulate the avidity and nanoscale spatial distribution of aptamers against the transferrin receptor (TfR). This tool allows for control of nanocluster spatial configuration and composition in the length scale of 100 nm and is unique in its high precision in presentation of aptamer spatial distributions. We combined WAZ, an aptamer specific for the TfR, with wireframe DNA nanostructures to produce aptamer nanocalipers with precise patterns and densities. This system will help us understand in the future the correlation between avidity and BBB permeation.

Permebality of the blood brain barrier along stroke phases and the transferrin receptor

Sara Bernardo Castro – ESR10

CHUC, Centro hospitalar e universitario de Coimbra

Abstract

1. Permeability of the blood brain barrier through the phases of ischemic stroke and relation with clinical outcome

When studying the bibliography regarding the permeability dynamics of the BBB, we noticed that were no systematic rereviews/meta-analysis (SR-MA), focusing on the development of the BBBP during the phases of AIS. Since SR-MA are statistical procedures that play a central role in evidence-based medicine and are the preferential approach to obtain and use medical information, performing one on the topic of BBB permeability dynamics will be central for our research topic inside the NANOSTEM project.

We registered the idea in the PROSPERO data-base with the **ID: CRD42019147314**. And following the recommendations of the head of the documentation department at CHUC, we created a protocol for the study which is currently awaiting reviewer's response for publication after first peer review.

The SR yield 19 eligible studies with near 1500 patients. The results reinforced the idea that the BBB permeability follows a continuous dynamic change, never closing completely. Moreover, it showed us an interesting result suggesting that the high peak of permeability may not occur in the acute stage of the disease but in the subacute. This systematic review provides therefore an insight on the evolution of the permeability of the blood brain barrier in patients affected by AIS through the different stages of the stroke and its relevance in the patient outcome and treatment. In the context of the NANOSTEM project this SR-MA supports the decision of scanning in the subacute stage. In the clinical field, ideally this knowledge would help amongst other things, in extending treatment windows.

2. Patient scanning

Due to the current pandemic situation the start on patient scanning have been delayed. Nonetheless, we are currently on terms to start again and have our first scan appointment ready to be conducted early in the month of July.

The ^{68}Ga-citrate tracer production and stabilization are currently being conducted at ICNAS. To our knowledge although this tracer has been accepted for human use and had been proven to specifically bind to the transferrin receptor (Tfr) in tumors, it has never been used to quantify the Tfr in the BBB, which grants us novelty in the field. We aim to conduct a dynamic PET-scan though instead of a fixed one. This means that instead of aiming to see the receptor binding or the tracer uptake into the brain, we will aim to see the whole dynamic process. This will allow us not only to detect and quantify the Tfr but to corroborate is correct functioning and uptake rate of the tracer.

We aim for a scanning rhythm of one patient per week, therefore despite of the substantial delay due to COVID-19 we believe that we are going to be able to fulfill the timing proposed in last year's meeting.

3. *Collaborative review on Frontiers for Neurology*

We are currently writing a review on the collaborative Frontiers issue on *"Intracranial Bleeding after Reperfusion Therapy in Acute Ischemic Stroke"* in the topic "*Patterns and implications of blood brain barrier disruption"*. The sight of this review is to give a molecular and cellular insight on the BBB permeability dynamics through the stroke phases in link them to the clinical outcome on patients and treatment decision. Publishing this review will give NANOSTEM visibility aside from granting us with a thoughtful review on the molecular insight of our research.

4. In vitro testing of tracer

Together with the CNC group, we are on terms of developing a model to quantify *in-vitro* the specificity of the Ga-citrate tracer for the transferrin receptor in the BBB. This *in-vitro* modeling will help us give support on the decision to use this tracer in our study, since the suitability of this tracer for BBB's Tfr in stroke has never being tested. Our intention with this, is to evaluate that the tracer binds specifically to the Tfr in the BBB and thus being able to confirm the PET-signal we are receiving in the scans comes from the correct uptake of the tracer and hence form the correct Tfr function in the BBB post-stroke.

Development of CRISPR-Cas9 Nanoparticles for the manipulation of mammalian Neural Stem Cells

Matteo Puglisi – ESR11

HMGU, Helmholtz Zentrum München, Germany

Abstract

During development, the vast majority of the cells residing in the nervous system are produced by the proliferation and differentiation of multipotent progenitors called Neural Stem Cells (NSCs). Some of these cells are maintained in adult mammalian brains and reside in specific areas called neurogenic niches. Unfortunately, these are restricted to very few niches in the adult mammalian brain and stem cell activity exhausts with age.

Our aim is to boost the neurogenic activity of adult NSCs with the CRISPR-dCas9 system delivered by nanoparticles (NPs). In particular, we use an enzymatically inactive version of the Cas9 (dCas9) conjugated to three transcriptional activator domains (Vp64, p65 and RTA = VPR) to induce the expression of critical genes *in vitro* and ultimately *in vivo*. In order achieve this final goal, we are working on different lines of research in parallel.

First, we have tested how to best induce neurogenic genes transcriptionally with the CRISPR-Cas9 system *in vitro*. About that, we are currently testing different gRNAs for the targeting of several neural fate factors. In parallel, we also cloned the gene coding for the dCas9-VPR inside a baculovirus expression vector, to produce the protein in insect cells. This system allowed us to obtain a high quality recombinant dCas9-VPR that could be loaded on different NPs. Finally, we also investigated how to culture NSCs best in non-proliferative media. Such optimization step was necessary since cell proliferation and neuronal differentiation are two processes that antagonize each other. For this, we developed a protocol to culture NSCs in media without grow factors. Now, we aim to use these culture conditions, dCas9 protein and gRNAs together.

PLGA nanoparticles for delivery through the blood-brain barrier

Sonia Lombardo - ESR 12

MyBiotech Gmbh, University of Saarland, Germany

Abstract

The goal of ESR 12 is to produce poly(ester) nanoparticles to deliver retinoic acid to the neural stem cells. During the past year, PLGA nanoparticle loaded with Lumogen® F Red 305 and coated with polysorbate 80 or poloxamer 188 were produced, to cross the blood-brain barrier (BBB) through receptor-mediated transcytosis. The nanoparticles were produced with the MicroJet reactor® technology and sent to Université d'Artois for *in vitro* test on their patented BBB model. To better understand the observed *in vitro* crossing differences between the formulations, protein corona analysis was performed, using DLS and BCA assay.

Nanoparticles loaded with retinoic acid were also developed. First, nanoparticles were produced, coated with polysorbate 80 or poloxamer 188, using the MicroJet reactor® technology. The nanoparticles size, loading and loading efficiency were characterized by DLS and HPLC. Their release was then characterized using a modified dissolution tester, coupled with tangential filtration.

Next, to try to obtain better release profile, nanocapsules were produced, loaded with retinoic acid in oleic acid and coated with polysorbate 80. Their size, loading efficiency, and release were also characterized.

The Thermosensitive Coil-Globule Transition of Acrylamide-Based Polymers

Patrick Quoika – ESR13

Department of Theoretical Chemistry, Universität Innsbruck, Austria

Abstract

This study focuses on the investigation of the Coil-Globule transition of thermosensitive polymers, such as e.g. Poly-*N*-Isopropylacrylamide. Understanding this conformational transition is crucial for being able to create thermosensitive polymers with tailored properties. In this talk, I will refer mainly to a manuscript, which we recently submitted to the ACS journal Macromolecules, and is currently under revision there:

In this study we systematically compared thermosensitive and non-thermosensitive polymers. We believe that drawing this comparison is of fundamental importance for understanding the driving force of the above-mentioned, counterintuitive conformational transition. Accordingly, we investigated the conformational dynamics of syntactic linear polymer chains of acrylamide-based polymers at different temperatures. Therefore, we performed long Molecular Dynamics (MD) simulations of linear 30mers of Polyacrylamide (AAm), Poly-*N*-Methylacrylamide (NMAAm), Poly-*N*-Ethylacrylamide (NEAAm) and Poly-*N*-Isopropylacrylamide (NIPAAm) at temperatures from 250 K to 360 K. Furthermore, we analyzed structural and conformational properties of these polymers at different temperatures in respect to the Coil-Globule transition in these simulations.

Based on the radius of gyration and the solvent accessible surface area, we classified the conformations of the polymers to be either in the Coil or the Globule state. Therewith, being able to determine the mean conformational state of the polymers at different temperatures, we characterized their thermosensitivity. Hence, we succeeded in identifying NIPAAm and NEAAm as thermosensitive, in contrast to AAm and NMAAm, which we can confirm to be non-thermosensitive. Therefore, we demonstrate the suitability of conventional MD simulations for profiling the Coil-Globule transitions of these polymers in atomistic detail, given sufficient sampling.

Moreover, we performed a detailed analysis of hydrogen bonding with the solvent, where we specifically took the change in solvent accessible surface area into account. In order to facilitate a systematic comparison of the conformational states, i.e. Coil and Globule, we separated the structural ensembles accordingly and analyzed these states separately at all applicable temperatures. We report distinct hydration dynamic profiles for Coil and Globule structures for thermosensitive and non-thermosensitive polymers.

Drug delivery for neural stem cell proliferation

Eirini Epitropaki – ESR14

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Abstract

Neurodegenerative diseases such as ischemic stroke, Alzheimer's and Parkinson's can cause cellular loss, which can be treated with the use of neural stem cells (NSCs)[1]. Nanomedicine is a non-invasive method that can enable delivery of differentiating agents to promote stem cells differentiation into functional neurons,[1] while preserving the blood and brain barrier (BBB)[2, 3]. In order for nanoparticles to achieve efficient delivery to the central nervous system (CNS) numerous physico-chemical parameters must be examined, and size, morphology and surface charge of the nanoparticles are the key parameters that determine delivery efficacy[4]. Based on the above, the current work aimed to produce amphiphilic nanoparticles with various corona chemistries, different sizes and a biodegradable nature, in order to explore the above parameters. Different morphologies such as diamond platelets[5] and molecular caterpillars, were formed by the exploiting the core crystallinity using the crystallization driven self-assembly (CDSA) technique[6]. After attachment of a small fluorophore, the fluorescence of the particles was evaluated for biological monitoring purposes. As the particles are developed as an intravenious carrier, the interaction between the self-assemblies and protein models must be investigated. The effect of the surface charge was explored by introducing the nanoparticles in various biological environments such as salts, cell culture medium, fish water and model protein solutions to monitor any interactions occurring.

References

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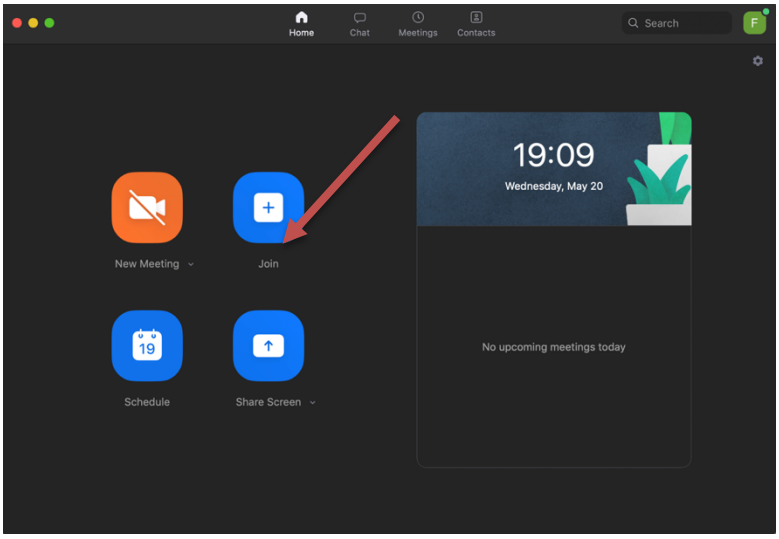

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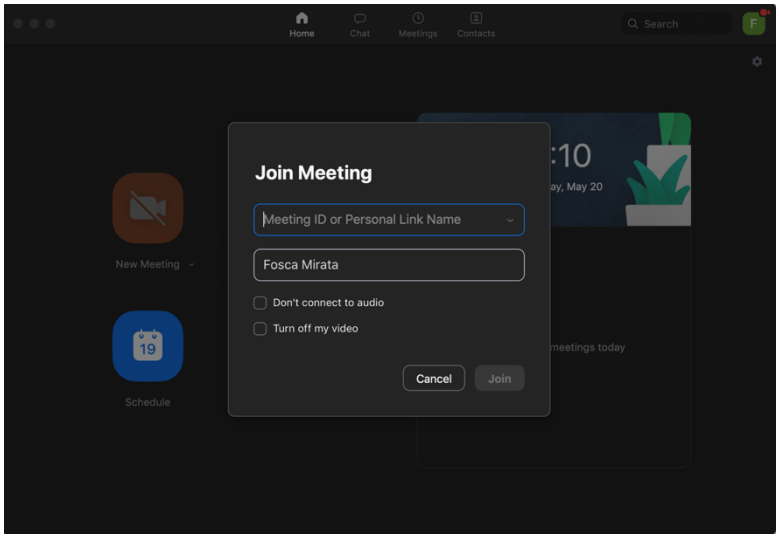

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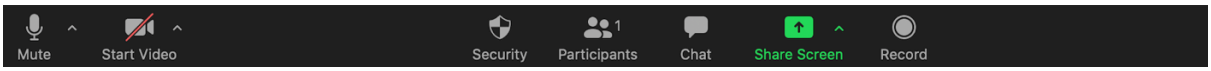

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