RÉUNION ANNUELLE PROGRAMMES FRANCE 2030

« Biothérapies et Bioproduction de Thérapies Innovantes »

25 et 26 novembre 2024

ABSTRACT BOOK

ORGANIZING COMMITTEE

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ANNUAL MEETING
FRANCE 2030 PROGRAMS

Biotherapies and Bioproduction of Innovative Therapies

November 25th - 26th, 2024

November 25th, 2024

8:30 am
Welcome coffee

9 am
Institutional session
Thomas Lombès (Inserm) - Deputy CEO Strategy
Vincent Lebon (CEA) - Deputy Director, Fundamental Research
Anne Jouvenceau (AIS) - Biotherapies Bioproduction National Strategy Coordinator

9:30 am - 10:20 am
- Presentation of the PEPR Biotherapies and Bioproduction of Innovative Therapies
- Presentation of the Biotherapies Bioproduction Integrators Network

10:20 am - 10:50 am
Coffee Break

Session 1: 10:50 am - 12:30 pm
Anticipate the scale-up of cell therapies and the emergence of tissue therapies

- **iPSC-France: PEPR project**

Stable universal edited pluripotent stem cells for allogeneic cell biotherapies - Annelise BENNACEUR-GRISCELLI

- **PIBT: Integrator**

MERIT Trial: From idea to clinical trial - Clémentine GAMONET and Syrine ABDELJAOUED

- **Bioengineered Skin-France: PEPR project**

Bioengineering of next-generation skin substitutes for skin regeneration - Gilles LEMAITRE

- **MEARY: Integrator**

PRIUM Cell: In Utero Myelomeningocele Repair Program using Mesenchymal Stromal Cells - Lucile GUILBAUD and Anne DELACOURT

- **iCHONDRO: PEPR project**

Generation and Functional Characterization of Induced Neural Crest Cells - Farida DJOUAD and Mareike EIS

12:30 pm - 2 pm: Lunch break and Poster session

Session 2: 2 pm - 3:40 pm
Engineering for biotherapies et bioprocesses

- **ACCREDIA: PEPR project**

Update of ACCREDIA and Cross-reactivity engineering in an anti PD-L1 Antibody - Bernard MAILLIERE and Hugo DORISON

- **MTInov: Integrator**

Multiscale modeling of monoclonal antibody production processes using CHO cells and development of a γδ T lymphocyte amplification process for off-the-shelf immunotherapy - Eric OLMOS and Emmanuel GUEDON

- **RNAvac: PEPR project**

RNAvac : New generation of mRNA-based vaccines - Chantal PICHON

- **TIBH: Integrator**

TIBH: Unlocking the future of biopharmaceutical production - Fayza DABOUSSI

- **THERA-B: PEPR project**

Update on THERA-B activities with a focus on genome editing in B cells - Anne GALY and Michel COGNE

3:40 pm - 4:10 pm
Coffee Break

Session 3 - Part 1: 4:10 pm - 5:10 pm
Accelerate the deployment of gene therapies

- **QualAAV: PEPR project**

Analysis and characterization of AAV vector batches - Alexis BEMELMANS

- **ART-TG (MAGENTA): Integrator**

ART-TG integrator: Accelerating Cell and Gene Therapies Towards Clinical Trials - Anne GALY

- **EDITO: PEPR project**

Development of breakthrough technologies for editing of neuronal DNA and RNA - Thérèse CRONIN and Laetitia HENG

5:10 pm - 6 pm: Presentation of the Consortium for Maturation in Biotherapy and Bioproduction (COMBIO)
Florence ALESANDRINI, Clément MINEZ, Séverine BARTH, Aude HYARDIN et Marie-Thérèse RUBIO

6 pm - 8 pm: Networking cocktail and Poster session

ANNUAL MEETING
FRANCE 2030 PROGRAMS

Biotherapies and Bioproduction of Innovative Therapies

November 25th - 26th, 2024

November 26th, 2024

8:30 am
Welcome coffee

Session 3 - Part 2: 9 am - 9:40 am
Accelerate the deployment of gene therapies

- **BIOSCALE: PEPR project**

Improving the whole chain of large scale AAV Bioproduction through disruptive technologies - Oumeya ADJALI

- **ViVeM (CPV): Integrator**

From AAV viral vector design to large scale pre-industrial bioproduction - Oumeya ADJALI

Session 4 - Part 1: 9:40 am - 10:20 am
Emerging fields in biotherapies

- **STROMAEV: PEPR project**

From engineering of iPS-derived mesenchymal stromal cell-derived extracellular vesicles to clinical translation - Marie MAUMUS

- **IVETh: Integrator**

Production, engineering and characterization of extracellular vesicles and other nanovectors for personnalized therapy and diagnosis - Kelly AUBERTIN

10:20 am - 10:40 am
Coffee Break

Session 4 - Part 2: 10:40 am - 11:40 am
Emerging fields in biotherapies

- **Bacter-EV-Booster: PEPR project**

Establishment of production, isolation and purification of F. duncaniae extracellular vesicles for therapeutic application - Liliia PANIUSHKINA

- **OBBI: Integrator**

Presentation of the OBBI integrator - Danièle NOËL

- **CARN: PEPR project**

Development of biotherapies based on the local delivery of therapeutic RNAs by hybrid functionalized extracellular vesicles (EVs) for musculoskeletal regeneration - Lucile ALEXANDRE

11:40 am - 12 pm: Presentation of France Biolead - Laurent Lafferrère

12 pm: Closing remarks - Poster awards

Microfluidics, automation and artificial intelligence for liposomes and lipid nanoparticle development and production

Joanna Duffrene, Pauline Chavrier, Karine Andrieux, Rabah Gahoual, Nathalie Mignet, and Khair Alhareth.
Unité des Technologies Chimiques et Biologiques pour la Santé (UTCBS), Université Paris Cité, CNRS UMR8258, INSERM U1267, Paris, France

Microfluidics technology is wieldy used for nanoparticles preparation thanks to its reproducibility and scalability. The high control over the process and the quality of data produced by automated microfluidics systems are in perfect synergy with advanced data analysis by mathematical and computational approach, as Quality by Design, Design of Experiments, and artificial intelligence (AI) tools, including Machine Learning (1). In this project, we aim to establish a pipeline for the production and the optimization of lipid nanoparticles. This workflow considers the incorporation of a standardized protocol, advanced technology, and automation, and insures an optimal analysis of data via a data management plan and AI tools. The pipeline is technically divided in 5 steps: solvent preparation, mixing, solvent removal, characterisation and biological evaluation. Every step should be standardized, validated and automated to collect high quality data that could be analysed using AI tools.

In this study, a series of quality control method were developed to strength our pipeline and to provide robust data regarding physical and chemical characterisation of liposomes and LNPs. For this propose, protocols and methods to quantify the residual solvent were established. A fast enzymatic and UV-based method for ethanol quantification was developed to check if the ethanol percentage is within the specification, as well as optimising the experimental conditions of the purification process (dialysis, ultrafiltration, evaporation). Additionally, an electrophoresis method was developed to assess the residual traces of DMSO in the final suspension of Liposomes. Finally, a standardised document for data collection was designed and used to build a database, and AI tools were developed for data transformation, visualisation and analysis.

Automation, advanced technology, and AI could accelerate nanomedicine and drug delivery research, as is the case in the chemistry field. A further evolution of our pipeline is planned to achieve a fully automated system with integrated AI for optimal development of liposomes and lipid nanoparticles

1- R. Rebollo, F. Oyoun, Y. Corvis, M. M. El-Hammadi, B. Saubamea, K. Andrieux, N. Mignet, K. Alhareth, Microfluidic Manufacturing of Liposomes: Development and Optimization by Design of Experiment and Machine Learning. ACS Appl Mater Interfaces 2022, vol. 14, no 35, p. 39736-39745, https://doi.org/10.1021/acsami.2c06627

Keywords:

- **Microfluidics technology**
- **Lipid nanoparticles (LNPs)**
- **Artificial Intelligence (AI)**
- **Quality control**

Innovative approaches for quality control of AAV-based gene therapies

Agache V, Aron Badin R, Aurégan G, Belot M-P, Bemelmans A-P, Constantin O, Dufour N, Fourmy D, Gaillard M-C, Gaston C, Gross D-A, Hantraye P, Hernandez C, Jaszczyszyn Y, Lecourtois S, Naquin D, Perrois M, Petit F, Ronzitti G, Tavella G, Van Dijk E

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2, LETI (Laboratoire d'électronique et de technologie de l'information), CEA, 17 avenue des Martyrs 38054 Grenoble, France.

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AAV are the most used vectors for gene therapy treatments due to their ability to transduce post-mitotic cells and their relatively low immunogenicity. As AAV are now used for gene therapy of systemic disorders, the effective therapeutic dose increased dramatically leading to immune response-related severe adverse events (SAE). The viral load administered to patients is largely a function of the quality of the produced batches, as current production and purification techniques still lead to a large portion of AAV with partial genomes or empty capsids. This argues in favor of a better characterization of the immune response induced by AAV and improvement of quality control (QC) to improve the safety/efficacy balance. The QualAAV project aims to define a pipeline of QC of AAV vectors by comparing production and purification processes using currently available and disruptive QC methods and evaluating their impact on the immune response in vivo. Standard QC methods established in the laboratory (qPCR, ddPCR, ELISA) will be compared with disruptive methods (Nanopore sequencing, transmission electron microscopy (TEM), mass sensors) in their ability to predict the immune response against AAV batches of known purity, genome length and integrity, and empty /full ratio. Preliminary results include the assessment and automation of TEM images analysis for the detection of empty/full ratio, as well as the comparison between qPCR and ddPCR in AAV genome titration. By determining the essential quality criteria to ensure efficacy and safety of AAV vectors, this project will accelerate the deployment of AAV-based gene therapies.

Keywords:

- **AAV (Adeno-Associated Virus) vectors**
- **Gene therapy**
- **Quality control (QC)**
- **Immune response**

dSTORM super-resolution microscopy applied to extracellular vesicles characterization

Nicolas Kuszla, Lucile Alexandre, Léa Jabbour, Laura Fouassier, Stéphanie Mangenot et Florence Gazeau.
MSC-med, UMR 7057, Université Paris Cité.

Extracellular vesicles (EVs) play critical roles in cell-cell communication and show a significant potential as biomarkers for disease diagnosis. However, their nanoscale size presents challenges for conventional optical microscopy techniques. In this work, we present an approach using super-resolution microscopy to achieve nanometric-resolution imaging of EVs, enabling high-throughput characterization at the single-vesicle level.

We developed a specific immobilization surface to capture EVs, ensuring EV-specific capture and stable imaging conditions. We utilized direct Stochastic Optical Reconstruction Microscopy (dSTORM) to acquire nanoscale-resolution images of immobilized EVs. By collecting thousands of individual EVs and extracting multiple features per EV—such as size, morphology, and intensity distribution—we provide a robust dataset for in-depth analysis of EV subpopulations.

Our workflow allows for the high-throughput acquisition of quantitative morphological data, enabling the exploration of EV subpopulations, and will offer potential applications in diagnostics and in understanding intercellular communication mechanisms.

Keywords:

- **Extracellular vesicles (EVs)**
- **Super-resolution microscopy**
- **Single-vesicle analysis**
- **Biomarkers**

Development of a HITI-based gene correction strategy for Stargardt's disease

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Stargardt disease (STGD1) is an autosomal recessive genetic disorder affecting the gene coding for the ABCA4 protein, leading to a juvenile form of severe macular dystrophy. To date, over 1000 pathogenic mutations have been reported in ABCA4, making the development of mutation-specific therapies highly impractical.

To address this challenge, we propose a novel approach focused on replacing an entire functional domain of ABCA4 -NBD2 (Nucleotide Binding Domain 2), necessary to catalyse ATP hydrolysis and thus power the activity of the ABCA4 transporter. By targeting NBD2, we aim to correct multiple pathogenic variants simultaneously, offering a broader therapeutic option that does not rely on individual mutation correction. Combining CRISPR-Cas9 and a Homology-Independent Targeted Integration (HITI) strategy, an engineered copy of the NBD2 domain is inserted upstream of the ABCA4 transmembrane domains in both human cells and a rat model of STGD1 using respectively RNP-Cas9 strategy and AAV vectors coding for SaCas9-GFP and guides.

Sanger-sequencing verified the insertion of the mini-domain fragment at the junctions of interest in vitro in human cells. To test this in human retinal cells, we developed advanced cellular models derived from induced pluripotent stem cells (iPSCs), including retinal pigment epithelium (RPE) cells and whole retinal organoids (RO). These models provide a relevant platform to test the efficacy of our approach.

For in vivo proof of concept, five distinct AAV vectors carrying SaCAS9 and a guide RNA were compared in a Stargard rat model until a 50% cutting efficiency was measured by the TIDE software, with one specific AAV vector and guide RNA combination showing partial insertion of the NBD2 domain in vivo.

This strategy has the potential to overcome the limitations of mutation-specific therapies and offer a more comprehensive solution for STGD1 treatment.

Keywords:

- **Stargardt disease (STGD1)**
- **ABCA4 protein**
- **CRISPR-Cas9**
- **Nucleotide Binding Domain 2 (NBD2)**

Design and evaluation of mucus penetrating lipoparticles for mRNA vaccine delivery

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The success of mRNA vaccines against SARS-CoV-2 relies mainly on the choice of LipoNanoParticles (LNPs) as a delivery system, and the careful design of mRNA coding sequences. However, using mainly lipid components, their soft nature implies that a nebulization/spray process could alter their composition and thus poor delivery of mRNA into the lungs or nasal cavities due to mucus entrapment and altered lipid composition. The addition of a solid core in the lipid assemblies such as polylactic Acid as recently described by our laboratory (Ayad C et al, 2021, 2022) for mRNA delivery has prompted us to develop a new class of mucus-penetrating lipoparticles (mucLPs). By playing with lipid components and their ratio, we designed several classes of mucLPs and compared their properties regarding i) their colloidal properties ii) their capacity to carry and express mRNA in different cell types using mRNA reporter genes such as luciferase or e-gfp mRNA iii) their capacity to cross the mucus barrier using air-liquid interface cell cultures. Through a screening process, we were able to select two promising mucLPs regarding their capacity to penetrate the mucus and express mRNA in mucus-secreting epithelial cells (Calu3) or alveolar (A549) cells and will then assess their biodistribution upon pulmonary or nasal administration in mice by in vivo imaging.

Keywords:

- **mRNA vaccines**
- **Lipid nanoparticles (LNPs)**
- **Mucus-penetrating lipoparticles (mucLPs)**
- **mRNA delivery**

Impact of dissolved oxygenation concentrations on hWJ-Mesenchymal Stromal Cells (MSCs) growth and secretome production in stirred bioreactors

Kevin Audoux, Jessica Schiavi-Tritz, Eric Olmos
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IBB MTInov

This project aims to understand the impact of oxygenation environments on MSCs to improve the production yield of extracellular vesicles (EVs) while ensuring quality of therapeutic product. The impact of MSCs culture conditions and bioproduction process on the quantity and quality of the secretome is poorly described in stirred pilot-scale bioreactors(1). Here, the impact of precisely controlled oxygenation concentrations is investigated. Secretomes from hMSCs, including EVs, are emerging as a promising cell-free therapeutic. However, the scale-up and the standardization of the bioproduction process remain major challenges. This work aimed to test different oxygenation conditions on cells physiology and characterise EVs. Wharton's Jelly hMSCs were seeded on Synthemax II at 5 cells/microcarrier in Ambr® 250 bioreactors in controlled microenvironments of hypoxia (2%, 10%) or normoxia (19% oxygen). Until day 7, cell physiology was monitored daily by counting cells after trypsinization or fluorescence staining, and checking their metabolism (glucose, lactate, glutamine, lactate dehydrogenase). Then, the cells were starved for 48 hours to produce particles which were characterized by nanoparticle tracking analysis. In all oxygenation conditions, no latency was observed while from day 4 there was a significant increase of cell concentration under hypoxia compared to normoxia ($p<0.05$). However, 4.75±1.151011 particles were produced at 2% of O2 versus 8.49±1.251011 and 7.28±1.151011 at 10 and 19% of O2 respectively. Similarly, one cell produced 5.39±1.22104 particles (at 2% of O2) but 9.84±1.1104 and 8.12±2.16104 particles with 10% and 19% of O2, respectively. In addition, different particle sizes were obtained with 172.15±4.03 nm, 187.5±7.77 nm and 143.2±0.14 nm (2, 10 and 19% of O2, respectively) suggesting a significant effect of oxygenation on specific production. Data demonstrated that hypoxia promoted cell proliferation but reduced particles bioproduction in dynamic culture. Interestingly, 10% of oxygen increased cell proliferation while maintaining high EVs bioproduction.
(1)Yuan X, and al.2022

- **Keywords:**
- **MSCs (Mesenchymal Stem Cells)**
- **Extracellular vesicles (EVs)**
- **Oxygenation conditions**
- **Bioproduction**

Evaluation of the neuro-inhibitory therapeutic properties of extracellular vesicles-containing bacterial supernatant from Faecalibacterium genus in a preclinical model of non-inflammatory colonic hypersensitivity

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A.1
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Objective:
Advances in high-throughput sequencing have enabled the identification of bacterial strains with potentially beneficial health effects. Among them, the genus Faecalibacterium stands out as a promising probiotic due to its anti-inflammatory properties, generating increasing interest. Additionally, Faecalibacterium has been shown to play a key role in the regulation of colonic hypersensitivity (CHS) in a chronic stress model induced by neonatal maternal separation. Moreover, patients suffering from irritable bowel syndrome (IBS) or inflammatory bowel diseases (IBD) often exhibit gut dysbiosis characterized by reduced microbial diversity, notably a decrease in Faecalibacterium. This study explores the therapeutic potential of this bacterial genus to modulate colonic sensitivity and associated neuro-inhibitory mechanisms. In addition, various bacteria are known to produce extracellular vesicles (EVs), which may impact intestinal function, inflammation, as well as cognitive, behavioural, and neuro-inflammatory processes. Indeed, bacterial-derived EVs could represent a breakthrough in the field of acellular biotherapies, playing an essential role in intercellular or interorgan communication involved in various physiological and pathological processes.

Methods:
Several Faecalibacterium strains were provided by Dr. Chatel to represent the phylogenetic diversity of this genus. The in vivo anti-hyperalgesic effect was assessed using a mouse models of chronic stress induced by neonatal maternal separation (NMS) model. Colonic sensitivity was measured using a colorectal distension test, coupled with a pressure sensor to quantify intracolonic pressure variations. Additionally, the in vitro neuromodulatory properties of Faecalibacterium strains were evaluated using calcium imaging approaches. Different bacterial fractions, including soluble and insoluble proteins as well as culture supernatants, were tested on the immortalized neuronal cell line ND7/23, as well as on primary cultures of neurons derived from dorsal root ganglia (DRG).

Results:
Similar to the reference strain Faecalibacterium duncaniae A2-165, this study revealed that several, but not all, tested Faecalibacterium strains significantly reduced NMS-induced CHS. Furthermore, by examining neuronal responses to nociceptive stimuli from various algogenic substances, it was observed that culture supernatants from certain strains exhibited a notable inhibitory effect on neuronal activation, highlighting their neuro-inhibitory potential.

Conclusion and Perspectives:
Several bacterial supernatants from Faecalibacterium genus show significant therapeutic potential for the treatment of CHS in a non-inflammatory IBS-like model. Since bacterial culture supernatants contain EVs and various secreted products, including metabolites, proteins, it is possible that these supernatants exert their in vitro neuro-inhibitory and in vivo anti-hyperalgesic effects in part through the EVs released by the bacteria. Further research into the specific components of EVs and their impact on biological pathways involved in pain could lead to the development of new therapeutic strategies for managing chronic abdominal pain observed in patients suffering from IBS or IBD in remission.

Keywords:
- **Faecalibacterium**
- **Extracellular vesicles (EVs)**
- **Chronic hypersensitivity (CHS)**
- **Irritable bowel syndrome (IBS)**

High throughput assessment and selection of antibodies based on stability

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Developability describes the multiple features of an antibody, which are essential to determine if it is safe, effective and manufacturable. Notably, antibodies can undergo chemical and structural changes, which can affect their binding affinity, specificity, and overall function. The assessment and selection based on these features are critical in ensuring antibodies production, manufacturability, and efficacy in therapeutic applications. To evaluate antibody developability, candidate molecules are evaluated one by one and subjected to physical and chemical stresses to identify the best sequences for manufacturing, storage, or delivery within the body. This is however done at a low throughput (typically 1-100 antibodies) and at the final stages when the candidate is already selected.

Our approach aims to perform a high-throughput analysis of antibodies, exposed to stressful environments and, correlate the developability features directly to the sequences. Several antibody formats will be studied (i.e., scFv, Fab and IgG), used either as soluble protein or on surface-display systems. The objective is to build a large database of millions of sequences with associated stability and expression data. This database will be used to train deep-learning models to develop tools to predict some aspects of antibody developability only based on sequences. This work will also facilitate the development and optimisation of new antibodies libraries.
This vast data collection and associated deep learning based tools will significantly accelerate and streamline the future development of therapeutic antibodies, saving invaluable time and driving innovation forward.

Keywords:

- **Antibody developability**
- **High-throughput analysis**
- **Deep learning models**
- **Therapeutic antibodies**

Alternative mRNA encapsulation methods for the preparation of cost-effective small volumes of LNPs

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b Neovacs SA, Suresnes, France.

Microfluidic systems have become the gold standard method for LNPs formulation by allowing fast, scalable, reproducible and robust production of nanoparticles. This method allows for the controlled mixing of lipids and nucleic acids, resulting in well-defined particle sizes and efficient encapsulation of payload. However, despite these advantages, the production of small LNP volumes at the laboratory scale, using conventional microfluidic mixing protocol is associated with significant material waste. Given the high cost of synthetic mRNA, this waste can be a major limitation, particularly for early-stage screening of formulations.

In this study, we explore alternative methods for mRNA-LNP formulation that could reduce the material waste associated with microfluidic production while maintaining the key physicochemical and biological properties of the LNPs. Specifically, we investigated post-encapsulation of mRNA into pre-formed vesicles (PFV) obtained by microfluidic mixing. These PFV were then mixed with mRNA solution by: (1) microfluidic or (2) manual pipetting method.

The resulting mRNA-LNPs produced using both post-encapsulation methods exhibit similar physicochemical properties and morphologies to those obtained by conventional microfluidic mixing, and have comparable in vitro/vivo activities.

These findings indicate that the post-encapsulation of mRNA into microfluidically produced PFV offers a cost-effective alternative to conventional microfluidic mixing protocol for producing mRNA-LNPs, with the potential to significantly reduce waste during small-scale production. These methods could be applied to encapsulate tailored doses of mRNA and various mRNA constructs to achieve an optimal and personalized therapy.

Keywords:

- **Lipid nanoparticles**
- **mRNA delivery**
- **Microfluidic**
- **Post-encapsulation**
- **Laboratory scale**

Multimodal characterization of turbulent extracellular vesicles from human adipose derived stem cells for therapeutic applications

Lea Jabbour, Sylvain Cam, Sarah Razafindrakoto, Nicolas Kuzla, Estelle Surply, Kelly Aubertin, Nathalie Luciani, Amanda Brun, Florence Gazeau
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Extracellular vesicles (EV) secreted by stem cells have gained significant attention due to their pivotal role in intercellular communication, tissue regeneration, and potential therapeutic applications. These vesicles carry a diverse cargo of proteins, lipids, and nucleic acids, reflecting their cells of origin. Comprehensive characterization of EVs is critical to understanding their biological function, therapeutic efficacy, and safety. A multimodal approach, integrating techniques such as asymmetric flow field-flow fractionation (AF4), nanoparticle tracking analysis (NTA), electron microscopy, flow cytometry and superresolution microscopy enables accurate characterization of EVs' size, shape, concentration, and molecular identity. These methods provide complementary data, capturing both structural and biochemical characteristics, crucial for establishing a standardized profile for therapeutic EVs.

In addition to physical characterization, potency assays are key for evaluating the functional capabilities of EVs. These assays assess the biological activity of EVs in promoting tissue repair or modulating immune responses, offering insights into their therapeutic potential.

Together, this multi-faceted analysis ensures a more rigorous understanding of EVs derived from stem cells, paving the way for their safe and effective use in regenerative medicine.

Keywords:

- **Extracellular vesicles (EVs)**
- **Stem cells**
- **Therapeutic applications**
- **Characterization techniques**

HEK293 cell concentration and viability prediction from holographic imaging: acquisition and analysis

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As part of the PEPR project Bioscale, that is dedicated to cell monitoring during Associated-Adeno Virus (AAV) bioproduction processes, our team developed a generic and fully automatic pipeline to predict HEK293 cell concentration and viability from low-cost holographic imaging for commercial and Inserm-TaRGeT lines. Our method includes five main steps:

·Scanning of microscope slides containing samples picked up from the bioreactor with our unique multimodal bench (see Figure 1). It allows to acquire a same field of view with holographic lens-free (LF) at three wavelengths, holographic microscope at currently one wavelength (DF), brightfield imaging under blue spatially semi-coherent illumination, and fluorescence of the GFP protein expressed by transfected cells.

·Phase and amplitude reconstruction from lens-free and microscope images is performed using a combination of in-house algorithms: Gerchberg–Saxton algorithm, an inverse problem-based reconstruction and a dedicated convolutional neural network (CNN) to estimate the phase

·Segmentation of the phase image using the Cellpose model (Figure 2) followed by standard post-processing (small objects and incomplete cells removal, phase threshold)

·Feature extraction on the filtered-out cells. The most relevant were found to be cell absorption, phase, and size features

·Feature analysis throughout the study (n timelapses), starting with a 2D Gaussian mixture to cluster cells between viable and dead populations. The linear decision boundary is established in the feature space by placing a parameterized line between the centers of both clusters, as seen on Figure 3

Several solutions were compared to reconstruct the images and to separate the two cell populations. Our final solution produce accurate predictions of both variables of interest on LF and DF images when compared to ViCell measurements. Furthermore, the results on both modalities were coherent with each other. More validations will allow in the close future to ensure the robustness of our method and evaluate its potential to avoid biologists costly manipulations.

Our next objective will be to estimate the AAV titer on holographic images. For that purpose, we will upgrade the bench to enable acquisition of microscope images at three wavelengths, and we will combine holographic images with the fluorescence images of the GFP protein expressed by transfected cells.

Keywords:

- **Holographic imaging**
- **HEK293 cell concentration and viability**
- **Adeno-associated virus (AAV) bioproduction**
- **Convolutional neural network (CNN)**

Interplay of nanoparticles transport and nano rheology through interferometric light microscopy

Lucile Alexandre1, Florence Gazeau1, Amanda K. A. Silva1, Stéphanie Mangenot1 and Kelly Aubertin1
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Background
Extracellular vesicles (EVs) are nanometric objects, from tens to hundreds of nanometers, encapsulating proteins, lipids and nucleic acids. They play a crucial role in intercellular communication. In biological tissues, EVs are crossing complex matrices (CM) with particular mechanical properties, such as high viscosity, where pores can be smaller than the average size of EVs. This transport is governed by the physicochemical properties of both the matrix and EVs, but the interplay of EVs with their biological environment is rarely studied.
Material & Methods
We develop an innovative approach investigating the interactions of EVs with a CM relying on the use of nanoparticle tracking by interferometric light microscopy (ILM-NTA) in a chip. The analysis of interference patterns allows the tracking of particles' motions at the submicrometric level, giving access to their trajectories and allowing to calculate the mean square displacement (MSD) and diffusion coefficients. In case of Brownian motion, the Stoke-Einstein equation allows to link the particle's hydrodynamic diameter and the media's viscosity. To mimic EV's transport inside CM, we embedded nanoparticles within Newtonian (Glycerol) and non-Newtonian (Poloxamer407, a therapeutic thermosensitive copolymer) matrices, at various concentrations and therefore viscosities, and evaluated their transport properties by ILM-NTA.
Results
The analysis enabled to (i) measure the viscosity at the nanoscale for Newtonian and non-Newtonian environment via calibration beads of known size, achieving viscosity levels that are beyond the capabilities of standard EV characterization instruments; (ii) analyse data to determine the effective size distribution of EVs in non-Newtonian complex matrix such as poloxamer formulation and (iii) highlight the interactions of EVs with poloxamer-407. Difference in the diffusion coefficients and size distributions of EVs in comparison to beads in poloxamer formulation compared to PBS suggest significant interactions between the poloxamer and EVs. This study was complemented by cryo-electron microscopy imaging of EVs embedded in CM, shedding light on the mechanisms of interactions. If the bilayer structure of EVs was clearly observed, however, in poloxamer, we observed evidence of tension on some EVs, leading to modifications in size distribution and, consequently, alterations in transport properties.
Conclusion
Our approach provides a valuable tool for quality control test for EV formulations intended for clinical use. ILM-NTA demonstrates significant potential as tracking device and nanorheometer, exhibiting high effectiveness in quantifying mechanical properties and transport within viscous matrices, with direct application in EV drug delivery. Mechanical stiffness, a key property of solids, is crucial in nanocontacts, suggesting our approach to measuring nanovector rheology could align with regulatory standards for EV-based therapies.

Keywords:
- **Extracellular vesicles (EVs)**
- **Nanoparticle tracking analysis (NTA)**
- **Complex matrices (CM)**
- **Poloxamer 407**

Hybrid Nanovesicles for Nucleic Acid Delivery: Bridging Extracellular Vesicles and Synthetic Lipids for Musculoskeletal Regeneration

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Background:

Therapeutic RNAs, hold potential for tissue regeneration, particularly in skeletal diseases 1. This project aims to develop bio-drugs using extracellular vesicles (EVs) and synthetic lipid vesicles as RNA delivery systems. EVs from mesenchymal stromal cells (MSC) mimic their parent cells' therapeutic effects and are valuable in regenerative medicine due to their ability to deliver therapeutic RNA into tissues efficiently 2–4. In parallel, synthetic lipid vesicles, also called Lipid Nanoparticles (LNP) are effective RNA carriers but still suffer from limitations such as inflammation stimulation, low interaction with target cells and intracellular trafficking 5–7. Here we propose to create hybrid EVs that combine the targeting abilities of natural EVs and efficient RNA delivery with the RNA loading capacity of lipid nanoparticles. These hybrid vesicles will deliver therapeutic RNAs, addressing inflammation, apoptosis, senescence, and promoting bone and joint regeneration.

Method:

Hybrids were formulated using an ethanol injection method, where two phases were mixed by pipetting: an aqueous phase containing PBS, EVs, and siRNA, and an organic phase containing ethanol and lipids (DOTAP/DOPE). The different components of the hybrids—EVs, siRNA, and lipids—were labelled in various combinations (EVs and siRNA, lipids and siRNA and siRNA and lipids). Various labelling combinations for EVs, siRNA, and lipids were tested, revealing that lipophilic dyes were unsuitable. We selected carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) to label primary amines in EVs, minimizing dye diffusion and clarifying hybrid formation. The resulting fluorescence within the hybrids was analysed using a Zetaview®, which also allowed for the characterization of the hybrids' size, concentration, and the proportion and colocalization of the labelled components' fluorescence.

Results:

When fluorescent EVs, fluorescent siRNA and/or fluorescent lipids are used in hybrid formulations, over 40% of the particles show colocalization of either the fluorescent lipid and EV fluorescence, the fluorescent lipid and siRNA or the fluorescent siRNA and EV fluorescence. In addition, the particle size of the hybrids is increased compared to the size of EVs alone and lipoplexes. These preliminary findings suggest that our formulation process effectively induces the formation of hybrids between EVs, siRNA, and lipids although further characterization is ongoing.

Conclusion:

These early observations indicate that our formulation process successfully creates hybrid particles integrating EVs, siRNA, and lipids. The observed colocalization of fluorescent markers and the increased particle size affirm the intended hybridization, presenting a promising strategy for targeted RNA delivery in regenerative medicine. Future work will focus on optimizing hybrid formulations by adjusting lipid compositions, EV sources, and types of nucleic acids (like mRNA). Additional characterization techniques, including super-resolution microscopy and nano flow cytometry, will also be explored. Overall, these results lay the groundwork for further refinement and validation of hybrid EVs, potentially leading to novel therapeutic strategies for skeletal diseases and expanding therapeutic possibilities in tissue regeneration and beyond.

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Keywords:

- **Extracellular Vesicles (EVs)**
- **RNA Delivery**
- **Lipid Nanoparticles (LNP)**
- **Regenerative Medicine**

Shiga toxin B-subunit (STxB) as a synthetic antigen delivery tool to dendritic cells for mucosal vaccination

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Mucosal vaccines are particularly suited to protect entry sites against infection and also for upper airways cancer therapeutic treatment, by triggering tissue resident T-cells and secretory antibodies. Antigen-presenting cells play a key role at mucosal sites to elicit an immune response by initiating adaptive immunity. Thus, targeting dendritic cells by nasal routes has been tailored to elicit potent protective and therapeutic responses. The B-subunit of Shiga toxin (STxB) is a non-toxic homopentameric protein that binds to the glycosylated lipid, globotriaosylceramide (Gb3), which is preferentially expressed by dendritic cells. Chemical synthesis and pentameric assembly of STxB have been developed, obtaining a targeting unit that is endotoxin free and scalable. This straightforward preparation leads to similar biophysical characteristics and highly conserved trafficking and targeting properties as compared with the recombinant protein. The chemical approach allowed the generation of new variants with bioorthogonal handles, enabling chemical coupling to several antigens derived from the mucosal viruses SARS-CoV-2 and type 16 human papillomavirus. These conjugates upon intranasal administration in mice, lead to the recruitment of tissue resident memory CD103+ CD49a+ T cells and IgA antibodies along the respiratory track.

Keywords:

- **Mucosal vaccines**
- **Dendritic cells**
- **STxB (Shiga toxin B-subunit)**
- **Immune response**

STROMAEV Project: Optimization of large-scale clinical-grade production of iPS-derived mesenchymal stromal cell-derived extracellular vesicles

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Extracellular Vesicles (EV) isolated from mesenchymal stromal cells (MSCs) have recently emerged as a new therapeutic product since they exert immunomodulatory activity and promote tissue repair. This tool is of particular interest for osteoarthritis, a degenerative joint disease with few or no effective treatments. The clinical grade production of large batches of such an acellular therapy raises challenges, among which batch to batch consistency in terms of product identity and efficiency is a key issue. In this context, the STROMAEV project aims to produce EVs from MSCs derived from induced pluripotent stem cells (iPSCs), to provide a stable source of well-characterized cells, producing EV with reproducible functionalities. Our objective is to optimize the iMSC production process to generate EVs (iMSC-EVs) with enhanced therapeutic properties and to characterize their functionality.

To this end, a GMP-compatible production process will be established, integrating bioreactor production and optimized purification and concentration methods by tangential flow filtration. iMSC-EVs will be characterized with a wide range of analytical methods, including analysis of particle concentration and size (nanoparticle tracking analysis and interferometric light microscopy technology), structure (Cryo-electron microscopy), and phenotype (flow cytometry). The functionality of iMSC-EVs will be assessed using different potency assays, including the one currently used for clinical batches of MSCs.

This study will allow us to develop scalable and efficient manufacturing of medicinal products for tissue repair in the context of osteoarthritis, with relevant quality controls, potency assays and batch to batch consistency.

Keywords:

- **Extracellular Vesicles (EV)**
- **Mesenchymal stromal cells (MSCs)**
- **Osteoarthritis therapy**
- **Batch consistency and functionality**

Single-hit genome editing optimized for maturation in B cells redirects their specificity toward tumor antigens

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T-cell-based adoptive immunotherapy is a new pillar of cancer care. Tumor-redirected B cells could also contribute to therapy if their manipulation to rewire immunoglobulin (Ig) genes is mastered. We designed a single-chain Ig-encoding cassette (“scFull-Ig”) that redirects antigen specificity when inserted at a single position of the IgH locus. This design, which places combined IgH and IgL variable genes downstream of a pVH promoter, nevertheless preserves all Ig functional domains and the intrinsic mechanisms that regulate expression from the IgM B cell receptor (BCR) expression to Ig secretion, somatic hypermutation and class switching. This single-locus editing provides an efficient and safe strategy to both disrupt endogenous Ig expression and encode a new Ig paratope. As a proof of concept, the functionality of scFull BCR and/or secreted Ig was validated against two different classical human tumor antigens, HER2 and hCD20. Once validated in cell lines, the strategy was extended to primary B cells, confirming the successful engineering of BCR and Ig expression and the ability of scFull-Ig to undergo further class switching. These results further pave the way for future B cell-based adoptive immunotherapy and strategies to express a therapeutic mAb with a variety of switched H-chains that provide complementary functions.

Keywords:

- **Adoptive immunotherapy**
- **Tumor-redirected B cells**
- **Immunoglobulin gene editing**
- **Class switching**

Development of functionalized EV - LNP hybrid vesicles for musculoskeletal regeneration

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Therapeutic RNAs including messenger RNAs (mRNAs) and interfering RNA and are part of the therapeutic arsenal for tissue regeneration. CARN project proposes the development of bio-drugs using EVs and synthetic lipid-based vesicles as RNAs delivery systems for skeleton regeneration. EVs isolated from mesenchymal stromal cells (MSC) reproduce the therapeutic effect of their parental cells and are of real therapeutic interest in regenerative medicine. They contain proteins, lipids, and nucleic acids, in particular miRNAs which are responsible for their biological effect. EVs are also being developed as delivery systems due to their abilities to deliver therapeutic cargo specifically into tissues and their exceptional endosomal escape and intra-cytosolic delivery of RNA. Besides natural EVs, synthetic lipid vesicles (eg, liposomes or lipid nanoparticles) are also used to transport therapeutic biomolecules, including mRNAs (vaccines and therapeutics). While these formulations are particularly suitable for a vaccine strategy, they need to be modified to optimize their impact as regenerative medicine. Moreover, they can be easily targeted with various types of specific ligand.

The CARN's goal is to take the benefit of each system, and for that we plan to associate lipid-based vesicles to EVs, through hybrid formulation. Thus, the tissue targeting and delivery potential of natural EVs will be coupled to the high RNA loading of lipid nanoparticles. MicroRNAs targeting various pathological processes (inflammation, apoptosis, senescence) and mRNAs encoding factors (transcription factors, growth factors, anti-geronic factors) involved in bone and joint regeneration and in the immunosuppressive effects of MSCs will be formulated and encapsulated in these innovative hybrid functionalized vesicles. Various tests to achieve hybridization have been conducted to determine the crucial characteristics and methods that determine the fusibility of the two lipid-based structures. Results gathered during this first period show that the hybridization process is temperature dependent from both sides, for EVs their ratio to LNPs is impactful, and the lipid composition of the LNPs is the main factor governing this hybridization.

Keywords:

- **Therapeutic RNA delivery**
- **Extracellular vesicles (EVs)**
- **Lipid nanoparticles (LNPs)**
- **Tissue regeneration**

Advancing Cancer Immunotherapy: Potent Tumor Growth Inhibition and Enhanced Tumor Immunity via Unmodified mRNA-LNP Vaccines Targeting Dendritic Cells with Ligand-Antigen Fusion.

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Messenger RNA (mRNA) vaccines have shown exceptional efficacy against COVID-19, sparking the exploration of this technology to develop vaccines for various infectious diseases and cancers. Cancer immunotherapy is a transformative approach that harnesses the immune system's ability to target and eradicate cancer cells. In this context, mRNA-LNP vaccines have emerged as versatile tools for delivering mRNA encoding tumor-associated antigens (TAAs) to antigen-presenting cells (APCs), particularly dendritic cells (DCs). This delivery enables efficient TAA presentation on APCs, activating cytotoxic CD8+ T lymphocytes, which play a critical role in the adaptive immune system.

This study focuses on a specific delivery of anti-cancer mRNA vaccines to DCs. We designed an mRNA coding for a fusion protein comprising a tumoral antigen and a specific ligand (X) targeting DCs. Upon intramuscular (IM) injection, LNPs encapsulating mRNA encoding the antigen fused with ligand X transfect both muscle cells and underlying DCs, enhancing the immune response through (i) direct antigen cross-presentation by transfected DCs and (ii) capture and cross-presentation by DCs of antigen-X fusion proteins secreted from transfected muscle cells. The proof of concept was demonstrated using mRNA encoding ovalbumin (OVA) as a model antigen, with our original LNP formulation based on imidazole lipids, showing strong efficacy in delivering mRNA both in vitro and in vivo. Our findings indicate that IM immunizations of mice with mRNAs containing either unmodified nucleosides (UNR) or modified nucleosides (MNR) encoding OVA and OVA fused with ligand X induced OVA-specific CD8+ T cells both systemically (spleen) and locally (lung). A notable increase in the humoral immune response (IgG) was observed in the serum and bronchoalveolar lavage of mice treated with either UNR or MNR OVA fused with ligand X, compared to mice receiving UNR or MNR OVA alone. In a subcutaneous EG7 tumor model, we observed significant tumor progression inhibition and increased survival rates, with ~35% of mice achieving complete tumor regression, particularly when vaccinated with UNR mRNA encoding the OVA-X fusion. Conversely, vaccination with MNR encoding either OVA or OVA-X fusion led to a similar immune response without an additional effect from ligand X. The antitumor effect is attributed to an efficient memory immune response, resulting from the fusion of antigen with ligand X or the modification of OVA mRNAs. Re-challenging mice with EG7 cells resulted in complete protection against tumor recurrence for at least three months.

Overall, our data demonstrate effective cellular and humoral responses, underscoring the robust immunogenicity and therapeutic efficacy of the X-targeted antigen in mRNA-LNP
vaccines. These findings provide compelling evidence for the potential of this approach and support further evaluation in clinical trials.

Keywords:

- **mRNA-LNP vaccines**
- **Cancer immunotherapy**
- **Dendritic cell targeting**
- **Tumor-associated antigens (TAAs)**

Les SNRs (Suspended Nanochannel Resonators): des systèmes microfluidiques oscillants pour la pesée de nanoparticules

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Possédant un rôle essentiel dans la communication intercellulaire, les recherches sur les vésicules extracellulaires connaissent un essor majeur. Elles représentent des biomarqueurs potentiels de certaines maladies et cancers. Etant secrétées par la majorité des cellules, les échantillons obtenus sont très hétérogènes en terme de taille (30 à 150 nm), de caractéristiques physiques et biochimiques (masse, densité, contenu, protéines de surface…) rendant leurs caractérisations difficiles.

La technologie SNR (Suspended Nanochannel Resonator) fabriquée au CEA/LETI permet la pesée individuelle de nanoparticules inorganiques ou biologiques (exosomes, adénovirus), à l'échelle de l'attogramme (10-18 g) [1][2]. Une poutre contenant un canal enterré est mise en oscillation dans une cavité sous vide, à sa fréquence de résonance. Lorsqu'une nanoparticule circule dans le canal enterré, cela induit un décalage fréquentiel proportionnel au changement de masse de la poutre et de son contenu. Différentes méthodes de détection du signal fréquentiel existent, comme la lecture optique ou piézorésistive. Cette technologie unique permet de mesurer la masse flottante d'une nanoparticule et à partir de sa densité et de la densité du fluide porteur, d'en déduire sa masse sèche. Ces capteurs ont prouvé leur efficacité pour distinguer différentes populations de nanoparticules d'or [3]. En utilisant un système de détection optique, il a été possible de peser des exosomes sécrétés de cellules fibroblastiques et hépatocytaires [1].

Dans ce travail, des composants SNRs possédant des jauges piézorésistives implémentées à la base de la poutre sont utilisés pour peser des exosomes et des nanoparticules biologiques. Des premières pesées d'exosomes issus d'une lignée cellulaire PC3 d'adénocarcinome de la prostate ont été réalisées.

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Keywords:

- Vésicules extracellulaires
- Biomarqueurs
- Suspended Nanochannel Resonator (SNR)
- Pesée de nanoparticules

Genetically engineering hASC-derived extracellular vesicles as a biomolecule delivery platform for inflammation therapy

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Background:
Extracellular vesicles (EVs) are a heterogeneous group of lipid membrane vesicles that are important mediators of intercellular communication. They are secreted by almost all cell types and encapsulate various bioactive molecules inherited naturally from their parental cells. EVs can be therapeutically exploited as delivery systems through various bioengineering approaches. However, loading biomolecules of interest within or on the surface of EVs remains a significant challenge and limitation.
Human adipose-derived stem cells (hASCs) are recognized for their potential therapeutic applications in tissue engineering, regenerative medicine, and the regulation of immune responses in damaged tissues. Notably, hASC-derived EVs have demonstrated natural properties for inflammation regulation due to the active molecules, such as RNAs and proteins, that they carry. This makes hASC-derived EVs particularly intriguing for cell-free therapies, as they could avoid the disadvantages of current cell transplantation procedures and the risks of artificial nanoparticles potentially causing immune and inflammatory toxicity during the treatment of inflammation-related diseases. In this study, we aim to genetically modify hASC cells to generate EVs that actively encapsulate inflammatory therapeutic-related molecules, such as mRNA or proteins, while also improving the loading efficiency of these biomolecules.
Methodology:
We utilized gene recombination technology to design a plasmid-based overexpression system that selectively increases the loading of biomolecules of interest into EVs. hASCs were transfected with either Cy5-labeled mRNA encoding luciferase enzyme or a plasmid with GFP tag. The transfection efficiency of mRNA was evaluated through a luciferase activity assay, while the efficiency of the plasmid transfection was assessed by measuring GFP fluorescence using an EVOS cell imaging system (Thermo fisher Scientific) and flow cytometry (cytoflex, Beckman Coulter). We optimized the transfection conditions for both mRNA and plasmid in various immortal hASC clones by comparing different transfection methods: PEI, liposomes, and commercial reagents (Lipofectamine 2000, Lipofectamine 3000, and Lipofectamine Stem). Optimal conditions were identified by screening for the highest transfection efficiency. EVs were harvested from Cy5-mRNA-transfected hASCs following a starvation period and ultracentrifugation. The EVs were characterized using NanoFCM, a highly sensitive flow cytometry platform capable of analyzing nano-sized particles at the single-particle level, to assess EV subtypes and biomolecule loading.
Results:
Two out of the five immortal hASC clones were at least 10 times more sensitive to Lipofectamine Stem reagents for mRNA transfection compared to other commercial transfection reagents. However, there was minimal difference and limited effect on plasmid transfection across the different reagents, and this may be related to the structure of the plasmid and its expression efficiency. Despite the lower efficiency, plasmid transfection was successfully confirmed by the detection of GFP-labeled proteins expression within cells under the EVOS microscope with an approximate transfection efficiency of 10%. Flow cytometry results indicated that, in our designed delivery system, the transfection efficiency of mRNA is 1.3 to 3 times higher than that of plasmid DNA. NanoFCM analysis showed that only around 1% of the total EVs produced from Cy5-labeled mRNA-transfected hASCs were Cy5-positive. Additionally, NanoFCM results indicated that Cy5-labeled mRNAs can be distributed across CD81+, CD63+ or CD9+ EVs subpopulations. This may suggest that a certain biogenesis pathway of EVs is associated with mRNA transport, which could potentially be leveraged for mRNA loading.
Conclusion:
hASCs are difficult to transfect, and transfection efficiency varies due to cellular heterogeneity. mRNA is easier to transfect compared to plasmids, and the transfection efficiency of plasmids decreases as their molecular weight increases. Simply increasing the concentration of mRNAs in the cytoplasm by transfecting hASCs has little effect on enhancing the loading of target mRNA into EVs. This may be due to the random and low efficient transport process of mRNA within the cytoplasm and degradation by cellular enzyme system. We believe that this genetic modification strategy can enhance the clinical application of hASC-derived EVs by combining their natural inflammatory properties with newly created therapeutic functions.

Keywords:

- **Extracellular vesicles (EVs)**
- **Human adipose-derived stem cells (hASCs)**
- **mRNA loading efficiency**
- **Gene recombination technology**

Aggregation of therapeutic antibodies enhances dendritic cell uptake and T-cell responses

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It is now well-accepted that therapeutic antibodies' (Ab)s aggregation is associated with an increased potential for immunogenicity and consequently a loss of clinical response. In this work, we aim to better understand the role of aggregation in dendritic cells (DC)s uptake of Abs and its consequence for the initiation of a specific T-cell response to therapeutic Abs. Thus,we developed a model of nanosized oligomeric therapeutic Abs aggregates by exposing the native Ab to ultraviolet light stress. Using flow cytometry and confocal microscopy, we evaluated the internalization mechanisms of fluorescently labeled native or aggregated infliximab (IFX) and atezolizumab (ATZ) by monocyte-derived DCs (moDC)s. Despite differences in their glycosylation patterns, aggregation of both Abs enhanced their internalization by moDCs. Specifically, for IFX, mannose-dependent endocytosis played a significant role in the increased uptake of IFX aggregates. Moreover, we demonstrated that IFX aggregation enhances its trafficking into the degradative pathway, ultimately leading to peptide generation, as evidenced by its colocalization with lysosomal-associated membrane proteins.

In conclusion, our results indicate that small-sized aggregates with a higher internalization rate in comparison to the native Ab, have a significant role in initiating an immune response. These results emphasize the importance of assessing uptake mechanisms, which are influenced by Abs properties, as a part of the strategy to predict immunogenicity.

Keywords:

- **Antibody aggregation**
- **Dendritic cells (DCs)**
- **Immunogenicity**
- **Internalization mechanisms**

Advancing AAV Purification: A Novel Affinity Chromatography Approach

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Adeno-associated virus (AAV) vectors are highly promising for gene therapy applications, with numerous preclinical and clinical successes demonstrating their potential (1). However, clinical translation requires high doses of AAV, and scaling up production remains a significant bottleneck (2). Chromatographic techniques, particularly affinity chromatography, are the gold standard for large-scale AAV purification (3). The BIOSCALE project aims to develop innovative synthetic ligands designed to enhance AAV purification. These chemically synthesized ligands offer a cost-effective, scalable, and reproducible solution and are well-suited for GMP-compliant manufacturing.

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Keywords:

- **Adeno-associated virus (AAV) vectors**
- **Gene therapy**
- **Affinity chromatography**
- **Synthetic ligands**

PEPR EDITO - Development of a gene therapy based on SMaRT technology for Huntington's disease

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Huntington’s disease is a genetic neurodegenerative disorder characterized by motor, cognitive, and psychiatric symptoms that slowly but inevitably lead to the patient’s death. It is caused by an abnormal expansion of CAG triplet repeats in the huntingtin gene (HTT), which leads to the production of a toxic protein responsible for the degeneration of striatal neurons. There is currently no cure for Huntington’s disease. However, gene therapy has emerged as a promising option for the treatment of genetic disorders in the recent years.

As part of PEPR EDITO, we are exploring SMaRT (Spliceosome-mediated RNA trans-splicing) technology, which is based on the mechanism of trans-splicing. This process enables the generation of a functional messenger RNA (mRNA) by joining exons from different mRNA precursors (pre-mRNA). SMaRT technology uses an artificial RNA called PTM (pre-RNA Trans-splicing Molecule), that can replace exons in an endogenous pre-mRNA during splicing, resulting in a hybrid mRNA without mutations. During the splicing of the pre-mRNA, a competition arises between the target splice site of the endogenous RNA and the one of the PTM, resulting in the production of either the endogenous mRNA (cis-splicing) or the hybrid version (trans-splicing). The main challenge with this technology is the development of a PTM that can promote trans-splicing at a sufficiently high rate to reach therapeutic efficiency. A key factor in achieving this high rate of trans-splicing is the sequence of the binding domain (BD), which allows the PTM to target the intronic sequence of the pre-mRNA.

To overcome this challenge, we are developing an unbiased, high-throughput strategy for BD selection. First, a screening process is implemented to select effective BDs for trans-splicing from BD libraries. For this purpose, cell lines are generated by transduction enabling the quantification of trans-splicing events with fluorescent reporter genes. Screening of the BD library is then performed by the transfection of BD candidates into reporter cell lines. Trans-splicing events are sorted by FACS and analyzed by high-throughput sequencing (NGS) to identify the most promising BD candidates. The candidate sequences will then be cloned into vectors to evaluate and select the optimal PTMs that may provide therapeutic benefit for Huntington’s disease.

SMaRT technology using PTMs is a promising gene therapy tool. Its potential has been demonstrated with encouraging results for other inherited disorders, such as retinitis pigmentosa and epidermolysis bullosa. This technology could offer new therapeutic perspectives for Huntington’s disease

Keywords :

- **Huntington’s disease**
- **Gene therapy**
- **SMaRT (Spliceosome-mediated RNA trans-splicing)**
- **Trans-splicing**

Development of an AF4 analytical approach to understand the complexity and evolution of cell secretomes, and to identify the effect of purification techniques

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Background

More refined and complete characterization of cell secretomes are needed to accelerate the development of extracellular vesicles (EV) derived medicine towards clinical applications. The AF4 separation technique (Asymmetric Flow Field-Flow Fractionation) is well suited to fractionate complex media into subpopulations, in order to characterize each of their individual components. It can be used for quality control, or to identify potent fractions. Currently, most EV studies focus on exosome isolation with little interest in the other elements produced by the cells. AF4 can be developed in a dedicated fashion to address EV issues. The goal of this work is to establish a polyvalent separative method to use AF4 at its full potential for extended secretome characterization.

Material & Methods

Conditioned supernatants recovered from 2D HeLa-cultures placed in complete medium or serum-deprived medium for 48h were used to model 2 types of secretomes. They served to fine-tune the AF4 separative and detection parameters. Isolated subpopulations were identified using inline AF4 detectors (MALS/DLS/UV-Vis/dRI), dot blotting and TEM. The purification of HeLa conditioned media were performed by ultracentrifugation (UC), tangential flow filtration (TFF) and ultrafiltration (UF) and analysed by AF4.

Results

The AF4 separation method developed allows to track the evolution of a cell secretome composition over time. The coupling with dot blotting and electronic microscopy is a powerful tool to get access to the identity of each subpopulation. The comparison of three EV purification techniques by AF4 shows that UF and TFF can conserve the full sample with little changes regarding subpopulation proportions while increasing concentration. UC selects a range of sizes depending on the centrifugation parameters and can isolate subpopulations.

Conclusion

AF4 coupled to MALS/DLS/UV-Vis/dRI analyses is effective to investigate fine variations in biological samples, spot effects related to production and isolation protocols and identify the composition of a mix. This new approach paves the way for further multimodal analyses, and the use of AF4 as a quality control in the field of nanotherapeutics and bioproduction.

Keywords:

- **Extracellular vesicles (EVs)**
- **AF4 (Asymmetric Flow Field-Flow Fractionation)**
- **Secretome characterization**
- **Purification techniques**

Bio'Occ: Biothérapie & Bioproduction en Occitanie

Valérie Planat

Le projet Bio’Occ a pour objectif d’attirer et former les acteurs de demain pour accompagner l’innovation en réponse aux besoins du secteur d’activité.
Mené par un consortium réunissant 4 établissements de formation, l'éducation nationale, des partenaires industriels avec le soutien du pôle de compétitivité Eurobiomed, de la région Occitanie et de l’Inserm, le projet s’organise en 4 axes :
Adapter les formations existantes pour améliorer leur adéquation aux besoins en renforçant les partenariats avec l’industrie, la mise en situation professionnelle et la pluridisciplinarité
Compléter l’offre de formation existante avec la création de nouvelles formations, notamment au niveau Bac +3
Développer des outils de prospective afin de mieux cibler l’évolution des besoins et mieux adapter en fonction les formations
Déployer des actions de communication, pour améliorer l’attractivité des métiers auprès des élèves dès le lycée, mais également l’acceptabilité sociale de ces thérapies innovantes.
Le projet Bio’Occ cible ainsi tous les niveaux ; du Bac à Bac+8 en FI comme FC, et au terme de ses 5 ans, devrait permettre de former plus de 2000 diplômés en proposant une offre de formation plus complète, plus attractive, plus lisible, et de sensibiliser plus de 200 enseignants de lycées et leurs élèves aux enjeux des biothérapies.

Keywords :

- **Projet Bio'Occ**
- **Formation et éducation**
- **Innovation en biothérapies**
- **Collaboration industrie-académique**

Generation of bispecific antibodies

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The term of bispecific antibodies (bsAbs) is used to describe immunoglobulins that can bind to two different antigens or two different epitopes of the same antigen. Thanks to their dual specificity, bsAbs are important therapeutics tools that widen therapeutics possibilities. The first bsAb, Catumaxomab, was designed in 2009 as a bridge for retargetting T cells to tumoral cells1, therefore resulting in the destruction of the latter. BsAbs diversity is composed of more than 100 different structures2, but the main problem is that most of them diverge significantly from the structure of human immunoglobulin, with expected changes in immunogenicity and pharmacological properties due to the lack of Fc fragment. To counteract undesirable outcomes, a few different engineering strategies were developed to increase heterodimer yield of bsAbs while keeping native-like the Fc region : strand-exchange engineered domain (SEED)3, electrostatic steering technologies4 or the CH3-focused method called knob-into-hole method (KiH)5. Here we decided to improve heavy chain heterodimerization vs homodimerization building from original KiH approach. Thanks to Twist BioScience “Multiplexed Gene Fragments” technology and a dedicated python script we designed around 9998 CH3 KiH DNA sequences of 400 bp. These variants were combined in 3 libraries and cloned into vectors of the quantitative bacterial two-hybrid system (qB2H) build by our team. Interaction strength of several hundred thousands pairs of CH3 variants have been evaluated using this system coupled to Illumina sequencing and bioinformatics. Results should highlight asymmetric mutations that favor heterodimer formation over homodimer to improve the yield of bispecific antibodies production. This innovative technology can be applied to other immunoglobulin domains interactions and should ultimately be useful for bioproduction of bsAbs applied to different demands such as cancer treatment.

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Keywords:
- **Bispecific antibody**
- **Quantitative bacterial two-hybrid system**
- **Knob-into-Hole**
- **Synthetic biology**

Analysis of therapeutic monoclonal antibodies exposed to oxidative stress

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Monoclonal antibodies (mAbs) are increasingly developed for the treatment of a variety of autoimmune and inflammatory diseases, as well as cancer. mAbs are often administered intravenously and require high doses to achieve a therapeutic response, which can increase treatment costs and expose patients to potential toxicity. In contrast, oral inhalation accelerates the onset of action and could improve the therapeutic index by targeting the pulmonary areas [1,2]. However, mAbs during aerosolization may undergo different stresses [1], and oxidation may occur during the nebulization process. This can lead to mAbs unfolding and renders them prone to form oligomers. Therefore, to better understand the impact of oxidation on mAbs conformation, mAbs have been submitted to UV light and H2O2. Infliximab samples at a concentration of 1 mg/mL were exposed to UV light at 365 nm for 20 hours. In parallel, varying amounts of H_2O_2 were added to Infliximab samples, followed by incubation at 37°C. After exposure to these oxidative stresses, samples were analyzed using various chromatographic (e.g., size exclusion chromatography) and spectroscopic techniques (e.g., fluorescence spectroscopy). The first results show that UV light promotes aggregation by around 9%, whereas H2O2 (1%) promotes rather a fragmentation of mAbs (2.2%) (Figure 1).

Mass spectrometry experiments will be subsequently conducted to determine the level ofmAb oxidation.

Acknowledgment

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Keywords:

- **Monoclonal antibodies**
- **Aggregates analysis**
- **Size exclusion chromatography**
- **Spectroscopic techniques**

Immune-Evasive Strategies for Allogeneic Neural Grafts in Non-Human Primate Models of Huntington's Disease

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Cell replacement therapy using human pluripotent stem cell-derived neural grafts holds promise for treating neurodegenerative diseases like Parkinson's and Huntington's disease. While autologous grafts derived from patient-specific iPSCs are ideal, their logistical and economic limitations necessitate the development of large-scale, GMP-compliant manufacturing of off-the-shelf allogeneic cellular products suitable for large patient populations. However, overcoming immune rejection of such allogeneic grafts remains a critical hurdle currently managed only by pharmacological induction of graft immune tolerance, which can have potentially severe adverse effects.

Several "immune-evasive" approaches based on genetic editing/engineering of donor stem cell lines to induce tolerance have been proposed and tested in peripheral organs in humanized mice or non-human primates. We propose to explore and compare some of these strategies for neural transplantation in a fully immunocompetent excitotoxic lesion model of striatal dysfunction in non-human primates.

We have produced striatal neuronal and glial grafts from Macaca fascicularis (MaFa) iPSCs for allogeneic transplantation in MaFA recipient. These grafts will be engineered to express immune-modulatory molecules and/or undergo genetic modifications targeting B2M or CIITA genes. We will assess the immune-evasive properties of these grafts in vitro, ex vivo, and in vivo in rodents. In parallel, we will test the impact of the immune-evasive strategies on neuronal maturation and synaptic homeostasis in vitro. Ultimately, we aim to produce optimized, immune-cloaked grafts for transplantation into our fully immunocompetent non-human primate model of striatal dysfunction.

Keywords:

- **iPSC**
- **Huntington**
- **Non-human primate**
- **Immune evasion**
- **Neural grafts**

Evaluating LNP-Mediated mRNA Delivery in Primary Cells as a Precursor to Cancer Vaccine Development

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Cancer immunotherapy has emerged as a transformative approach in oncology by harnessing the body's immune system to target and eliminate tumours. One key strategy involves activating innate immune pathways, such as the STING (stimulator of interferon genes) pathway, which triggers robust anti-tumour immunity. Lipid nanoparticles (LNPs) encapsulating mRNA represent an innovative platform in immunotherapy, enabling rapid antigen expression and immune activation. However, understanding the immune responses triggered by LNPs in primary cells is critical before progressing to more complex in vivo cancer models.

This study aims to evaluate the immunological profile and transfection efficiency of LNPs encapsulating mRNA encoding GFP in primary cells, serving as a baseline for future cancer vaccine formulations. By ensuring minimal immune activation at this early stage, we aim to establish a safe delivery platform for future experiments involving tumour antigens and STING pathway activation.

Bone marrow-derived macrophages and dendritic cells were transfected with LNPs encapsulating GFP mRNA. Immune responses were assessed by quantifying the expression of pro-inflammatory cytokines, while transfection efficiency and cell-specific immune activation were measured using flow cytometry and ELISA.

Initial findings show that LNP-mediated transfection of GFP mRNA in primary cells was efficient and did not induce measurable immune responses, as indicated by the lack of upregulated cytokine production. These results suggest that the LNP formulation is well-tolerated in this cellular context, paving the way for future research involving STING activation and tumour antigens in mouse models of colon cancer. Future work will focus on incorporating tumour-specific antigens and evaluating therapeutic efficacy in vivo.

Keywords:

- **Cancer immunotherapy**
- **Lipid nanoparticles (LNPs)**
- **STING pathway**
- **mRNA transfection**