jpps Journal of Pharmacy and Pharmaceutical Sciences

CSPS

Abstracts of Poster Presentations at the Canadian Society for Pharmaceutical Sciences (CSPS) Symposium

May 27-29, 2025 – Montreal, QC, Canada

jpps Journal of Pharmacy and Pharmaceutical Sciences

2025 Poster Abstracts

The annual meeting and symposium of the Canadian Society for Pharmaceutical sciences was held on the Campus of the University of Montreal, Montreal, QC, Canada, May 27-29. The Symposium was held jointly with the Canadian Branch of the Controlled Release Society and with participation of the Pharmaceutical Society of Japan.

Pharmaceutical Sciences Beyond Borders: Advancing Global Health Through Innovation and Collaboration

In addition to the scientific sessions and poster presentations, the highlights included Young Scientist Network (Future Proofing Supply Chains for Pharma: Opportunities for Early-Career Scientists) and Industry Day (Resilience in Essential Medicines Manufacturing and Supply Chain: Challenges and Opportunities for the Pharmaceutical Scientist)

Sessions Titles

Sports Medicine, Breakthrough Technologies for Skin Diagnostics & Therapeutics (French Session), Advanced Technologies for Improving Women's Health, Global Health Access and Equity- Essential Medicines for Neglected Tropical Diseases, Unlocking the Future of the Self-Emulsifying Drug Delivery Systems; Advanced tissue and disease-specific drug therapy: The intersection of Traditional Chinese Medicine, Kampo and pharmacognosy, Interactions between Biomaterials, Cells and Tissues, Emerging Trends in Pharmaceutical Science

Abstracts of the Poster Presentations

Combining Fragment-Based Drug Discovery and Phenotypic Cell-Based Screening Approaches to Target Triple Negative Breast Cancer

Fatma Shahout[1], Steven LaPlante[1], Yann Ayotte[2], Eleonore Delaire[1], Salim Timo Islam[1], Stephanie Gallant[2], Yarelys Elena Jimenez[3]

Institut National de la Recherche Scientifique, Québec City, QC, Canada , [2]NMX, Laval, Québec, Canada [3]Paraza, Montreal, Quebec, Canada

Purpose: Is to combining fragment-Based drug discovery and phenotypic cell-Based Screening Approaches to Target Triple Negative Breast Cancer.

Methods: We used FBDD (fragment-based drug discovery) as a target-based method which can identify relatively low-affinity hits that can then be optimized. Another technique is phenotypic assays that focuses on screening drug-like compounds, which are practical for disease areas that have relatively few validated molecular targets. In our study, we combined both techniques to identify fragment hits capable of inhibiting cellular activity of triple-negative breast cancer (TNBC). We used XCELLigence and MTT proliferation assays to assess the inhibitory effect of our compounds. The identified hit will then used as starting points for the synthesis and design of more potential active drug-like compounds via the systematic modification and exploration of structure-activity relationships (SAR) around the core of the original hit. For monitoring the free-state behaviors of our compounds, a variety of NMR experiments such as 1D NMR and T2-CPMG are used.

Results: From Imidazole pyridine core (Hit-PS-5399), we modified first the location of the alkyl group, then the length of the alkyl group - we found that R3 and R4 seem to be the best position, and Heptanoyl group seems to be the most optimal length of the alkyl chain and showed the best activity against MDA-MB-231. Ester in position R gave the better activity.

Conclusion: This current workflow offers examples of the strategies implemented by our research group that includes effective hit optimization follow-up via medicinal chemistry.

Development and Validation of a HPLC-MS/MS Method for the Quantification of the Senolytic Agent ML-210 and its Applications

Maxime Murphy[1, 2], Louiza Mahrouche[1], Martin Jutras[1], Mihaela Friciu[1], François Yu[2], Valérie Gaelle Roullin[1], Grégoire Leclair[1]

[1]Plateforme de biopharmacie, Université de Montréal, Montréal, Canada, [2]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CR-CHUM), Montréal, Canada

Purpose. Cancer treatment, particularly for pancreatic cancer, remains a major challenge. Chemotherapy and radiotherapy can induce a state of cellular dormancy known as cellular senescence, leading to treatment resistance and tumor recurrence. To address this, senolytic drugs like ML-210 are being investigated as second-line treatments to eliminate senescent cells. ML-210 is a covalent GPX4 inhibitor that induces ferroptosis in this cell type. However, its clinical application is limited by poor bioavailability and rapid *in vivo* clearance. Nanoencapsulation is explored as a strategy to enhance ML-210's stability and delivery. This phase of the project focuses on developing and validating analytical and bioanalytical methods for quantifying ML-210 in different matrices, including nanoparticle formulations, to support *in vitro* and *in vivo* studies.

Methods. High-performance liquid chromatography with ultraviolet detection (HPLC-UV) was employed to quantify ML-210 encapsulated in nanoparticles, while liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was developed for its quantification in biological matrices, including culture media and mouse plasma. Method validation followed ICH Q2(R1) guidelines for analytical methods[1] and ICH M10 guidelines for bioanalytical methods[2]. The validated techniques were subsequently applied to quantify ML-210 in preliminary formulations, as well as in mouse plasma during plasma stability assays and pharmacokinetic studies.

Results. The validated HPLC-UV method demonstrated specificity for ML-210, confirmed by degradation studies. Accuracy ranged from 98.4% to 108.4%, with intra-day precision (RSD ≤ 0.9%) and inter-day precision (RSD ≤ 3.3%). The LC-MS/MS method met ICH criteria for sensitivity, carry-over, and selectivity. Accuracy ranged from 89.6% to 106.7%, with good precision (RSD ≤ 9.6%) and strong linearity ($R^2 \geq 0.9994$). ML-210 remained stable in both biological matrices for 90 days at -20°C. Plasma stability assays showed 27% degradation after 4 hours at 37°C. Free ML-210 exhibited a plasma elimination half-life of ~310 minutes in healthy mice.

Conclusion. Given ML-210's therapeutic potential and the increasing interest in senolytics, these validated quantification methods will be valuable for future studies. The next phase of research will explore combining frontline therapies with nanoencapsulated senolytic drugs to mitigate cancer recurrence associated with therapy-induced senescence.

Development and validation of a SPR method to evaluate anti-PEG antibodies

Suzanna Saadat. Nicolas Bertrand

Universite Laval, QC Canada

Scientific Challenge: Polyethylene glycol (PEG) is a widely used polymer in pharmaceutical sciences, known for enhancing the circulation time, stability, and solubility of drugs. Although PEG is generally well-tolerated, several reports from patients and animals indicate that its use in drug products can trigger the production of antibodies that recognize the polymer (i.e., anti-PEG antibodies). Our overarching goal is to characterize this immune response. In the present work, we developed a surface plasmon resonance (SPR) method to evaluate the affinity, specificity, and kinetics of antibody-PEG binding for these antibodies. [1,2]

Objectives: MonoPEGylated BSA was synthesized and purified using SEC chromatography. The PEGylated BSA was then immobilized on the SPR chip using EDC/NHS conjugation. SPR experiments were conducted in parallel across two channels: the test channel containing PEGylated BSA and the control channel containing BSA. The subtraction of these two signals allowed for the evaluation of the binding affinity of anti-PEG antibodies.

Results: The findings indicate that conjugation of PEG to BSA is feasible, and PEGylated sensors can be successfully prepared, enabling the characterization of anti-PEG antibodies.

Conclusions: This SPR method is effective in characterizing the immune response against PEG in both mice and humans.

Generation of Combinatorial Oligonucleotide Libraries on Magnetic Nanoparticles for Aptamer Discovery

John V. L. Nguyen[1], Lidija Malic[1, 2], Christina Nassif[2], Dillon Da Fonte[2], Maryam Tabrizian[1, 3]

[1]Department of Biomedical Engineering, McGill University, 3775 University Street, Montreal, QC H3A 2B4, Canada, [2]Life Sciences Division, National Research Council of Canada, 75 de Mortagne Boulevard, Boucherville, QC J4B 6Y4, Canada, [3]Faculty of Dental Medicine and Oral Health Sciences, McGill University, 2001 McGill College Avenue, Montreal, QC H3A 1G1, Canada

Purpose: DNA aptamers have exceptional potential for diagnostic and therapeutic applications given their numerous advantages over antibodies, such as their superior stability, faster production time, lower production cost, and the ability to find aptamers against non-immunogenic targets. However, conventional library construction poses challenges in detection and analysis, as each unique sequence occurs infrequently, necessitating multiple time-consuming amplification steps during the aptamer discovery process. To aid in the detection and analysis of candidate aptamers, we sought to develop a novel ssDNA library synthesis method that produced multiple copies of the same sequence bound to a magnetic nanoparticle.

Methods: We sequentially ligated DNA onto magnetic nanoparticles using a set of pre-synthesized DNA fragments. The process involved biotin-streptavidin conjugation of an initial fragment, followed by split-and-pool synthesis to randomly build the growing strand using fragments containing variable regions. A trio of digested restriction sites facilitated attachment by complementary base pairing, with all intermediate fragments double-digested, and the initial and terminal fragments single-digested. PCR amplification produced unbound strands for sequencing, while the original library was treated to remove the complementary strand, forming ssDNA attached to beads ready for aptamer discovery. Bioinformatics was applied to the sequencing data to characterize the produced DNA oligonucleotides.

Results: As expected, 99.9% of library synthesis products were within 40 to 118 bp, and 77.4% were within 40 to 58 bp. Every possible two-nucleotide combination was represented, with CCTACC and its reverse complement GGTAGG represented the least, and CCGTCC and its reverse complement GGACGG represented the most, at 1.76% and 15.16%, respectively, when looking at only the unique sequences and ignoring repeated sequences.

Conclusion: This novel oligonucleotide library generation method successfully conjugated ssDNA onto nanoparticles, with diverse sequence representation and nearly all sequences within the expected size range.

Investigating the cryptic behavior of the SI/II Pocket in the Ras family of GTPases

Tanos Celmar Costa Franca[1], Salim Timo Islam[1], Steven R. LaPlante[2]

Institut National de la Recherche Scientifique, Québec City, QC, Canada , [2]NMX Research and Solutions Inc. Laval, QC, Canada

Purpose. Mutants of the Ras-family of small GTPases are among the most common molecular oncogenic drivers, being associated to several types of cancers.[1] Although once considered "undruggable," recent efforts have identified a structurally conserved surface pocket in the Ras family, designated the SI/II pocket, situated near the binding site of the guanidine exchange factor (GEF) SOS1.[1] Since this pocket may represent a potential target site for a pan-Ras drug our research group have being engaged in a theoretical investigation of its dynamical behavior with the goal of characterizing its topology. Our *in house* crystal structure representing the native state of GDP-bound $HRas^{G12V}$ (PDB ID: 7TAM), and the GppNHp-bound $HRas^{G12V}$ structure in state 1 (PDB ID: 4EFM), were submitted to rounds of molecular dynamics simulations exploring the conformational dynamics of the SI/II pocket. The results suggests that the SI/II pocket might show a cryptic behavior being natively inaccessible in GDP-bound HRas yet becoming accessible in state 1 GppNHp-bound HRas systems.[2] Occlusion of the SI/II pocket is dictated by the spatial position of the α2 α helix in relation to the protein core, with α2 residue Y71 acting as a "tyrosine toggle" capable of restricting the pocket access (Figure 1).

Methods. Here we further extend this investigation by applying the same approach to the most revelant oncogenic mutated forms of Ras: $KRas^{G12C}$, $KRas^{G12D}$, $NRas^{Q61R}$.

Results and Conclusions. Our preliminary results show that the dynamical behavior of the SI/II pocket in these mutants mirror the findings for the $HRas^{G12V}$ mutants corroborating the trend of cryptic behavior.

NMR Fragment-Based Drug Discovery as a Tool for Efficient Hit-to-Lead Identification in Medicinal Chemistry

Kathleen Amiens[1], Yann Ayotte[2], Simon Woo[2], Luc Farmer[2], Fatma Shahout[1], Eleonore Delaire[1], Tanos Franca[1], Shima Rezaeian[1], Salim Timo Islam[1], Steven R. LaPlante[1]

[1]Institut National de la Recherche Scientifique (INRS-AFSB), [2]NMX Research and Solutions

Purpose: The fragment-based drug discovery (FBDD) approach using NMR is a powerful method for studying the inherently weak interactions (Kd) between protein targets and low-molecular-weight fragments (MW < 250). One key advantage of FBDD is that these small molecular fragments have a greater chance of fitting into target binding pockets. Medicinal chemistry can then optimize these interactions to fully exploit the potential of the binding site. This approach contrasts with conventional high-throughput screening, which relies on larger drug-like compounds (MW > 350) and requires extensive deconstruction and rebuilding efforts. However, FBDD starts with weak binders, necessitating high compound concentrations that can lead to artifacts such as self-aggregation and misleading structure-activity relationships (SAR)—a crucial tool for guiding medicinal chemistry.

Methods: To address these challenges, our team has developed the "NMR for SAR" strategy, integrating NMR at every stage of the FBDD process.

Results: This strategy is essential for assessing the free-state behavior of compounds, ensuring that only well-behaved (soluble and non-aggregating) molecules contribute to reliable SAR data. Furthermore, this approach provides insights into the impact of compounds on protein fingerprints and binding pockets.

Conclusion: Through a collaborative effort involving medicinal chemists, biophysicists, and biochemists, we applied the NMR for SAR methodology to enhance the binding affinity (Kd from ~7-10 mM to low µM) of compounds targeting HRAS, a protein historically deemed undruggable.

Overview of biological screening assays used to evaluate PROTACs for cancer treatment

Raghd Obidat[1], Jazmin Reyes Martinez[1], Carlos Velazquez-Martinez[1]

[1]Faculty of Pharmacy and Pharmaceutical Science, University of Alberta, Edmonton, Alberta, Canada

Purpose. Proteolysis targeting chimeras (PROTACs), are heterobifunctional compounds that make use of the proteasome degradation system in cells to selectively degrade a protein of interest (POI); Protein degradation using PROTACs involves binding to the POI and the E3 ligase, bringing them into proximity, followed by ubiquitination of POI and subsequent degradation by proteosomes. This work aims to comprehensively investigate the most commonly used biological screening assays to assess PROTACs designed to induce degradation of proteins involved in cancer. We were interested in listing, classifying, and ranking frequently employed experimental approaches in preclinical assessment of PROTACS.

Methods. Literature review: retrieve references from Web of Science published in the last five years. Reference classification: according to the assays included in each article, the reference will be assigned to binding affinity, ternary complex formation (proximity), ubiquitination, degradation, in silico, and/ or in vivo assays.

Results. Binding affinity and proximity assays were used to evaluate binary binding and ternary complex formation, respectively. Binding affinity screening assays include fluorescence polarization binding assay (FP), isothermal titration calorimetry, Cellular thermal shift assay, time-resolved fluorescence resonance energy transfer (TR-FRET) assay, and Others; the FP assay was the most frequently employed test. Some of these assays were used to evaluate (POI-PROTAC-E3 ligase) complex formation. The majority of studies used a degradation assay to monitor protein degradation, and Western blotting was the most commonly used test for this purpose. Some articles have tested the ubiquitination level through various assays, including the TUBE assay, Immunoprecipitation, and most commonly using proteosome inhibitors. In silico modeling was also considered in some articles to study ligand-protein binding. Finally, in vivo assays were reported to provide more predictive results about anti-cancer PROTACs activity.

Conclusion. In total, 70 articles were retrieved from the literature, and they include several assays to evaluate the biological activity of anticancer PROTACs over different types of cancer. These screening assays fall under six main screening categories. Over 97% of the articles utilized western blotting to assess protein degradation.

Quantitative Chemical Titration for Assessing the Degree of PEGylation

Kevin Coutu[1], Amatus Ngabonziza Sangwa[1], Nicolas Gaudrault[2], Sayed Said Tayebi[3], Todd Hoare[3], Prashant Mhaskar[3], Nicolas Bertrand[2], Andrea A greschner[1], Marc A Gauthier[1]

Institut National de la Recherche Scientifique, Québec City, QC, Canada. [2]CHUL-Quebec, [3]McMaster University, Hamilton, ON, Canada

Purpose : Several methods are available to determine the average number of methoxy poly(ethylene glycol) (mPEG) chains grafted to a protein or peptide, referred to as the degree of PEGylation. However, many of these approaches require sophisticated instrumentation, are time-intensive to implement and can - more generally - be inapplicable to intact PEGylated nanoparticles of interest in nanomedicine. The development of simple, versatile, and multiplexable methods for determining PEGylation is thus highly desirable.

Methods : The foundational works of Childs in 1975 and Kurfürst in 1992 respectfully report the quantitative and qualitative use of a colored 'barium-iodide-PEG' complex (inspired by the original work of Monacelli and Doretti) for the titration of PEG in solutions and to stain PEG-containing bands on electrophoresis gels. Remarkably, this colorimetric assay for mPEG has not been employed to directly determine the degree of PEGylation of PEGylated proteins or to quantify the surface PEGylation of intact nanoparticles. This study validates this assay for these purposes, via libraries of 54 mPEG-protein conjugates and 10 polymeric nanoparticles. The effect of mPEG molecular weight, terminal functional groups, and architecture were analyzed, amongst other parameters.

Results : The correlations of PEGylation values obtained using the barium/iodide assay and ^{1}H NMR spectroscopy are excellent ($R^2 = 0.91$) when using the corresponding mPEG-matched calibration curves. Several practical considerations are reported to increase the reliability of the data, thus making this assay more broadly applicable to other nanosystems.

Conclusion : Overall, this assay is simple, multiplexable, reliable, and yields values that are highly comparable to established methods.

Rational Design of A New Class of Versatile Enzyme-Based Biosensor

Shihao Pei[1], Davide Brambilla[1]

[1]Université de Montréal, QC, Canada

Purpose: While electrochemical systems dominate enzyme-based sensing (e.g., commercial glucometers), their requirement for direct electrical contact limits non-invasive applications. The goal of this study is to develop contactless alternatives through photon-mediated detection.

Methods: We engineered enzyme-fluorophore conjugates using pH-sensitive Cy7 derivatives[1] paired with acid/base-producing enzymes (glucose oxidase, phenylalanine ammonia-lyase, β-lactamase). The produced acid/base can be quantitatively monitored by the conjugated pH-sensitive Cy7 derivatives, which can be further utilized for the quantification of the substrate (glucose, phenylalanine, and penicillin). Moreover, we investigated this biosensor's potential by tuning linkers' properties to modulate the distance between the enzyme and fluorophore, as well as the hydrophilicity of the linker. Conjugation chemistry enabled real-time monitoring of enzymatic pH changes for substrate quantification.

Results: The experimental data confirmed that the fluorophore-conjugated enzymes exhibit substrate-dependent fluorescence responses under physiologically relevant buffered conditions (with/out albumin), enabling quantitative analysis of substrate concentrations through fluorescent signal detection. This approach is proved effective at least for GOx, PAL and beta-lactamase, and could be extended to other enzymes that produce acid or base. Moreover, the conjugation of enzymes with pH-sensitive fluorophores enabled the detection of the microenvironment's pH, thereby providing insights into the local pH of the enzymes that cannot be captured by measuring the bulk pH of the surrounding buffer.

Conclusion: The distance between the enzyme and the fluorophore plays a crucial role in the sensor's performance; shorter distances are proved to be able to enhance the enzyme's affinity for its substrate and potentially reduce the unwanted adsorption onto the enzyme's surface, particularly in hydrophobic carbon chain groups. Hydrophilic linkers allow the fluorophores to diffuse into the buffered environment, further reducing the risk of adsorption and improving microenvironment pH sensing. The platform's compatibility with diverse detection modalities — from fluorescence microscopy to spectrofluorometers — positions it as a transformative tool for closed-loop biochemical monitoring. By bridging nanoscale enzymatic activity with macroscopic optical readouts, this work establishes a new paradigm for real-time metabolite tracking in both clinical diagnostics and fundamental enzymology studies[2].

Targeting Cryptic Pocket: A Fragment-Based Screening Approach

Shima Rezaeian[1], Tanos Celmar Costa Franca[1], Yann Ayotte[2], Simon woo[2], Gregory R. Bowman[3], Prajna Mishra[3], Salim Timo Islam[1], Steven R. Laplante[1]

Institut National de la Recherche Scientifique, Québec City, QC, Canada , [2]NMX, [3]University of Pennsylvania, Philadelphia, USA

Purpose: VP35, a key Ebola virus protein, plays a critical role in immune evasion and viral replication, making it an attractive target for antiviral drug discovery. Recent studies have identified a cryptic pocket within VP35 [1], which could serve as a potential binding site for small-molecule inhibitors. However, targeting cryptic pockets requires advanced screening and validation techniques due to their transient nature.

Method: Our research team applied a combination of biophysical methods to screen a fragment library and identify initial hit compounds targeting this cryptic pocket. Using ^{15}N HSQC NMR spectroscopy, we monitored fragment binding and characterized the conformational dynamics of VP35 upon ligand interaction. Additionally, T2-CPMG, Differential Line Broadening (DLB), and Differential Line Broadening and Shifting (DLBS) experiments provided further insights into the structural flexibility and allosteric regulation of VP35 [2]. To complement experimental findings, molecular docking was employed throughout the screening process to predict fragment binding modes and guide hit optimization. This computational approach allowed us to prioritize fragments with the highest binding potential and refine their interactions within the cryptic pocket.

Results: Our results confirm that selected fragments can bind to the identified cryptic pocket and modulate VP35's structural dynamics, providing a foundation for future structure-based drug design efforts.

Conclusion: This study highlights the power of integrating NMR spectroscopy, fragment-based screening, and computational docking to uncover novel druggable sites in viral proteins. The insights gained from this work contribute to the ongoing development of antiviral therapeutics targeting VP35 and, more broadly, strategies for drug discovery against other challenging viral proteins.

Advancing Melanoma Treatment: Integrating Immunoinformatics and Biophysics in Multi-Neoantigen Vaccine Design

Saba Ismail, Khaled Barakat

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: Melanoma, an exceedingly aggressive form of skin cancer, necessitates enhanced therapeutic approaches. Neoantigen-based vaccinations provide a precise immunotherapeutic strategy by activating the immune system to identify tumor-specific antigens. This research develops a multi-neoepitope vaccine construct (MNVC) for melanoma utilising immunoinformatics, molecular docking, molecular dynamics (MD) simulations, and immunological simulations to guarantee antigenicity, stability, and immunogenicity.

Methods: A dataset of 700 neoantigens from the Cancer Epitope Database and Analysis Resource (CEDAR) was evaluated for MHC-I and MHC-II binding affinity, allergenicity, antigenicity, toxicity, solubility, and immunogenicity. Eight neoantigens were selected using strict immunoinformatics criteria and combined into a multi-epitope vaccine construct with GPGPG linkers and a β-defensin adjuvant linked by EAAAK to improve immune response. Molecular docking and normal mode analysis (NMA) were conducted to evaluate binding interactions with MHC-I, MHC-II, and TLR-9 receptors. The stability of the MNVC was confirmed through 1000 ns molecular dynamics (MD) simulations, utilising root mean square deviation (RMSD) and hydrogen bonding analysis.

Findings: The MNVC demonstrated high antigenicity (score: 0.8335), non-allergenicity, and stability. Molecular docking demonstrated significant binding affinities with MHC-I (-1045.5 kcal/mol), MHC-II (-1517.9 kcal/mol), and TLR-9 (-1020.1 kcal/mol). Normal mode analysis revealed stable structural flexibility. Molecular dynamics simulations validated the structural integrity of vaccine-receptor complexes, demonstrating stable RMSD and RoG values. The SASA analysis indicated that the vaccine-receptor interaction preserved solvent exposure levels conducive to immune activation. Hydrogen bond analysis indicated stable binding interactions throughout the simulation period. Simulations of the computational immune response indicated a significant immune reaction characterised by elevated levels of IgG, IgM, and cytokines (IFN-γ, IL-4), affirming strong adaptive immune activation.

Conclusion: The computationally engineered multi-neoepitope-vaccine-construct (MNVC) has significant antigenicity, stability, and immune receptor binding, making it a promising melanoma immunotherapy option. Additional in vitro and in vivo validation is necessary to verify its efficacy and clinical relevance.

A Hybrid TriTE Platform Targeting Malignant B-Cell Tumors

Ahlem Ben Mansour[1], Marc Andre Gauthier[2], Andrea A Greschner[3], Maya Saleh[4], Melanie Duchesne[5], Laurence Delafosse[6]

[1]Ben Mansour, INRS-EMT, Varennes, Canada, [2]Andre.Gauthier, INRS-EMT,Varennes, Canada, [3]Greschner, INRS-EMT, Varennes, Canada, [4]Saleh, INRS-AFSB, Laval, Canada, [5]Duchesne, CNRC, Montréal, Canada, [6]Delafosse, CNRC, Montréal, Canada

Introduction : Immunotherapy is a therapeutic approach uses antibodies as therapeutic agents. Bispecific antibodies have emerged as a new generation of therapeutic antibody, designed to bind simultaneously to two distinct targets. Nine bispecific antibodies have been for clinical use. And over 200 are in ongoing trials. To reduce the risk of tumor resistance, multispecific antibodies are being developed to target multiple antigens (i.e., more than two). This study explores the development and screening of trispecific antibodies targeting Cluster of Differentiation 19 (CD19), Cluster of Differentiation 3 (CD3) and either Programmed Cell Death Protein 1 (PD-1) or Programmed Death-Ligand 1 (PD-L1) via a hybrid deoxyribonucleic acid-protein (DNA-protein) multi-specific antibody platform developed in the Gauthier group.

Methods : Four single-chain variable fragment (scFv) antibodies specific to CD3, CD19, PD-1 and PD-L1, along with Blinatumomab (control bispecific antibody targeting CD19 and CD3), were produced in the laboratory of Yves Durocher at the National Research Council of Canada (NRC) using Chinese hamster ovary cells from the CHO3E7 cell line as the host system for recombinant protein production. A programming algorithm was used to identify suitable DNA sequences for constructing triangular and tetrahedral structures to be used as scaffolds for appending the scFvs, to create and adjust the structural properties of the trispecific antibodies.

Results : The production of scFv fragments in CHO3E7 cells yielded varying concentrations for each protein, with purity confirmed by by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Triangular and tetrahedral nanostructures were designed and computationally optimized to limit unintended base pairing interactions.

Conclusion : At this stage of the project, scFv fragments have been successfully produced, and the necessary DNA sequences for the design of triangular and tetrahedral structures have been identified. The next steps will involve assembling the different DNA nanostructures leading to the creation of a diverse library of DNA-scFv hybrids, which will then be evaluated in *vitro* for T-cell engagement, to identify the most efficient combinations.

A sEH/COX-2 Dual Inhibitor PTUPB Reduces Endothelium-to-Mesenchymal Transition and Improves Doxorubicin-induced Vascular and Cardiac Toxicity

Zaid Almaayah[1], Hevna Dhulkifle[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, Doha 2713, Qatar.

Purpose: Doxorubicin (DOX) is an effective anthracycline agent used to combat many neoplastic diseases. However, DOX causes cardiovascular toxicity in juvenile and young adult cancer survivors that can lead to future cardiomyopathy. Thus, it is essential to address the cardiovascular toxicity caused by DOX to improve the long-term health of cancer patients. Several studies have suggested that soluble epoxide hydrolase (sEH) and cyclooxygenase-2 (COX-2) are implicated in cardiovascular diseases by impairing vascular health and promoting the transition of Endothelial cells to Mesenchymal cells (EndMT). Given the role of sEH and COX-2 in EndMT cardiovascular toxicity, we aimed to investigate the effect of a dual sEH/COX-2 inhibitor, PTUPB, on DOX-induced EndMT, vascular and cardiac toxicity. **Methods:** We tested the beneficial effect of PTUPB on DOX-induced cardiovascular toxicity in zebrafish. The cardiovascular parameters were measured via the Viewpoints MicroZebralab. We also assessed the effect of PTUPB on DOX-induced EndMT in human endothelial cells. **Results:** Our data indicate that inhibition of sEH and COX-2 using PTUPB reduces DOX-induced EndMT and vascular toxicity. We also show that PTUPB improves cardiac function and morphology in zebrafish incubated with DOX. Importantly, our results demonstrate that PTUPB downregulates inflammation and oxidative stress markers to contribute to the improvement observed in DOX-induced cardiovascular toxicity. **Conclusion:** Our findings indicate that suppressing sEH/COX-2 using PTUPB reduces DOX-induced EndMT, vascular, and cardiac toxicity.

Building Beating Heart Tissue: Three-Dimensional Printing Vascularized and Contractile Constructs

Farinaz Ketabat[1], Andy Kim[2], Michelle Collins[2], Xiongbiao Chen[1], Michael E. Kelly[3], Ildiko Badea[4]

[1]Division of Biomedical Engineering, University of Saskatchewan, SK, Canada, [2]Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan, Saskatoon, Canada, [3]Department of Surgery, College of Medicine, University of Saskatchewan, Saskatoon, Canada, [4]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Canada

Purpose: Cardiac tissue engineering has the potential to become standard for treating cardiovascular disease, offering a solution to the limitations of heart donor availability (1). A key objective is to regenerate infarcted tissue after myocardial infarction, thereby preventing heart failure and post-attack complications. However, several challenges persist, including replicating the complex structure of the myocardium, ensuring vascularization to prevent tissue necrosis, and achieving functional contractility. Three-dimensional (3D) bioprinting has emerged as a technique for constructing precise, biomimetic structures that support vascularization and improve tissue integration. This study explores these critical factors using 3D bioprinting to develop functional cardiac tissue.

Methods: A 3D-bioprinted construct mimicking myocardium fiber orientation (-70° to +80°) was created using an alginate-gelatin bioink containing Human Umbilical Vein Endothelial Cells (HUVECs) at 6 million cells/mL. Constructs were cultured in complete media with growth factors in a static incubator for 10 days. Once endothelialization began, they were transferred to a bioreactor (days 10-12) to simulate mechanical compression and diffusion, promoting vascularization. On days 15-16, cardiac cells (H9c2 or Neonatal Rat Ventricular Myocytes, NRVM) were seeded. Live/dead and immunofluorescence assays were performed after 21 days, and calcium transients were analyzed using the Fluo-8 assay.

Results: Constructs placed in the bioreactor exhibited high cell viability and formed tubular-shaped vessels, whereas those outside the bioreactor, showed limited vascularization. HUVECs expressed CD31 and Connexin 43. NRVMs expressed cardiac troponin T (CTnT) and exhibited calcium transients from day 17. NRVMs on vascularized constructs displayed regular contractility and spreading, compared to non-vascularized controls, which lacked consistent signals. By day 21, NRVMs on vascularized constructs showed strong calcium signals and contractility, attaching preferentially to the vascularized bed atop endothelial cells.

Conclusion: Our scaffolds demonstrate strong potential as cardiac patches or in vitro cardiac tissue models, effectively replicating myocardium fiber orientation. Their vascularization and contractility are evidence of vessel integration into the heart's vascular network and synchronized contractility with the heartbeat.

Coated Gold Nanoparticles for Improving Radiofrequency Ablation in Hepatocellular Carcinoma

Michelle Fernández-Schmitz[1], Dayana Fausto[2], Eiko Kawamura[3], Michael A. J. Moser[4], Wenjun Zhang[1], Ildiko Badea[2]

[1]College of Engineering, University of Saskatchewan, [2]College of Pharmacy and Nutrition, University of Saskatchewan, [3]CVM Imaging Centre, Western College of Veterinary Medicine, University of Saskatchewan, [4]College of Medicine, University of Saskatchewan

Purpose: Liver cancer is the third leading cause of cancer-related deaths worldwide **[1]**, and is a silent cancer often detected in advanced stages. Among available treatments for inoperable tumours, radiofrequency ablation (RFA) has shown positive results for small ablation areas (<3cm) **[2]**. Improved approaches are needed to induce a more uniform cell death and avoid relapses. This study aims to enhance RFA therapy using gold nanoparticles (AuNPs) due to their outstanding conductivity properties and bioavailability. AuNPs coated with positively charged molecules poly-L-lysine (PLL) and three different gemini surfactants were tested on HepG2 cellular models.

Methods: Effects on cell viability of AuNPs, AuNPs-PLL (Poly-L-Lysine), AuNPs-GS12 (12-7N(GK)-12), AuNPs-GS16 (16-7N(GK)-16), and AuNPs-GS18 (18:1-7N(GK)-18:1) were evaluated using the ATP assay. HepG2 spheroids were grown for 11 days in 96-well spheroid culture plates and treated with coated and uncoated AuNPs for 24 hours. Cell viability was measured post-treatment using a Live/Dead assay kit and imaged with a confocal microscope (Nikon ECLIPSE Ti2). Spheroid samples were stained with hematoxylin and eosin (H&E) for analysis. The presence of AuNPs within the spheroids was observed by transmission electron microscopy.

Results: AuNPs maintained viability above 80% across all concentrations. Similar results were noted for AuNPs-GS12 and AuNPs-GS18, with both coatings being the most effective for HepG2 cells. AuNPs-GS16 at its highest concentration (1.58x10^10 particles/ml) showed a significant reduction in cell viability. AuNPs-PLL exhibited a decrease in cell viability of 50% at the highest concentration. Confocal microscopy did not indicate adverse effects of AuNPs-GS in HepG2 spheroids. TEM images demonstrated endocytic uptake, with a predominant concentration in the proliferative layer.

Conclusions: AuNPs-GS16 and PLL possess a dose-dependent cytotoxic effect in HepG2 cells. AuNPs-GS12 and AuNPs-18 showed a high safety profile confirmed in 2D and 3D cultures. Cellular internalization, especially in the exterior of the spheroids indicates the potential for augmenting RFA in future studies.

Development of a Structure-Based Pharmacophore Model for Identifying Novel Allosteric Inhibitors of Protein Tyrosine Phosphatase 1B (PTP1B)

Raed M. Al-Zoubi[1], Mona A. Sawali[2], Muhammad Ammar Zahid[2], Abbas Khan[3, 4], Mohanad Shkoor[5], Abdelali Agouni[2]

[1][1] Surgical Research Section, Department of Surgery, Hamad Medical Corporation, 3050, Doha, Qatar [2] Department of Chemistry, Jordan University of Science and Technology, P.O.Box 3030, Irbid, 22110, Jordan [3] Department of Biomedical Sciences, College of Health Sciences, QU-Health, Qatar University, Doha, 2713, Qatar, [2]Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, P.O. Box 2713, Doha, Qatar., [3]Division of Bioinformatics, Department of Biomedical Sciences, School of Medical and Life Sciences, Sunway University, 5, Jalan Universiti, Bandar Sunway, 47500 Petaling Jaya, Selangor, Malaysia., [4]Qatar UniversDepartment of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, 2713, Doha, Qatar, [5]Department of Chemistry and Earth Sciences, Qatar University, P.O. Box 2713, Doha, Qatar

Purpose. Protein Tyrosine Phosphatase 1B (PTP1B) is a key contributor to type 2 diabetes and obesity, acting as a negative regulator of insulin signaling. Despite numerous molecules showing inhibitory activity, none have advanced to late-stage clinical trials due to low selectivity and side effects. This study develops a pharmacophore model to screen large compound libraries for novel, selective PTP1B inhibitors targeting its allosteric site.

Methods. The 3D crystal structure of PTP1B in complex with an allosteric inhibitor (PDB entry: 1T48) was obtained from the RCSB Protein Data Bank. Using Schrödinger's Phase module, a pharmacophore model was generated based on receptor-ligand interactions at the allosteric site. The model was validated against 42 active compounds and 1526 inactives and decoys, with enrichment metrics calculated to assess its predictive power.

Results. The pharmacophore model consisted of five key features: two aromatic ring systems, one hydrophobic core, and two hydrogen bond acceptors, capturing essential interactions for PTP1B binding. Validation showed the model ranked 31 out of 42 actives among inactives and decoys, with an average of 41 outranking decoys. It achieved 14.3% and 54.8% of actives in the top 1% and 5% of results, respectively. The area under the accumulation curve (AUC) was 0.83, with robust initial enhancement (RIE) and receiver operating characteristic (ROC) values of 8.17 and 0.72, confirming its reliability.

Conclusion. This study developed a structure-based pharmacophore model using in silico techniques, enabling efficient screening of large compound libraries for novel PTP1B inhibitors. The model's strong validation metrics highlight its potential for identifying selective allosteric inhibitors, offering a promising starting point for future drug development.

Acknowledgment. This work was supported by the Qatar National Research Fund [grant No. ARG01-0601-230451]. The statements made herein are solely the responsibility of the authors.

Development of bispecific Nanobodies targeting human Galectin-7 and functional analogs using DNA nanostructures

Mighel Bernier, Andrea A. Greschner, Nicolas Doucet, Marc-André Gauthier

Institut national de la recherche scientifique, Québec City, QC, Canada

Purpose. Selective targeting of protein-protein interactions plays a crucial role in modulating and regulating cell apoptosis. Human galectin-7 (hGAL-7), a prototypic galectin, functions as a homodimer that mediates glycan-dependent interactions between pro- and anti-apoptotic partners, ultimately influencing cell fate. Structural modifications of GAL-7 can disrupt these interactions, leading to apoptosis resistance in various human cancer cells. This highlights GAL-7's critical role in cell survival and underscores its potential as a therapeutic target. Our group has evolved selected camelid antibody fragments (Nanobodies, Nbs) that can specifically bind and modulate the function of hGAL-7. These Nbs presumably bind to different epitopes on hGAL-7. It is hypothesized that multiple Nbs can act cooperatively, for example, in the form of bispecific antibodies, to enhance hGAL-7 inhibition. To verify this hypothesis, we will use DNA nanotechnology to build and randomize artificial bispecific nanobody architectures and test their functional effect in vitro and in vivo.

Methods. His-tagged humanized recombinant Nbs were expressed in E. coli BL21(DE3) and purified by nickel affinity chromatography. Single-stranded DNA (ssDNA) oligonucleotides with a maleimide group at one terminus will be covalently coupled

to an engineered cysteine residue near the C-terminus of selected Nbs. These ssDNA oligos will be hybridized to DNA nanostructures to prepare hybrid bispecific Nbs. The concepts of DNA nanotechnology will be applied to explore various bispecific nanobody geometries and architectures. Affinity and specificity will be assessed via ELISA, MST and fluorescence assays, while structural and biophysical properties will be analysed via NMR and X-ray crystallography. The modulatory activity of the hybrid bispecific Nbs will be tested via apoptosis assays in Jurkat T cells.

Results and Conclusion. The project is still in its initial stages, so meaningful results have not yet been obtained. It is anticipated that modifications made to the DNA nanostructure used as scaffold to connect the Nbs will influence activity, yielding useful structure-activity relationships. Furthermore, it is anticipated that testing different combinations of Nbs within our library via a mix-and-match approach will yield information into the optimal bispecific structure for future development.

Exploring the Cardioprotective Mechanisms of Dapagliflozin and Ertugliflozin with Nav1.5 from a Computational Aspect

Arkapravo Chattopadhyay[1], Peter Light[2], Khaled Barakat[1]

[1]Faculty of Pharmacy and Pharmaceutical Science, University of Alberta, Edmonton, Alberta, Canada, [2]Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada

Purpose: The voltage-gated sodium ion channel Nav1.5 is essential for cardiac electrical signaling, and its dysfunction is linked to arrhythmias. Sodium-glucose co-transporter 2 (SGLT2) inhibitors, such as dapagliflozin (Dapa) and ertugliflozin (Ertu), may provide cardio protection beyond their glucose-lowering effects. However, while Dapa has shown benefits, Ertu lacks similar protective properties[1]. The structural basis for this difference remains unclear. This study investigates how Dapa and Ertu interact with Nav1.5 at two binding sites and their influence on protein dynamics, particularly the allosteric regulation of phosphorylation site Serine 571 (S571) and IFMT motif in Domain 3-4 loop which is critical for channel function.

Methods: The full-length Nav1.5 structure was generated using AlphaFold and RoseTTAFold deep learning model, referencing PDB ID 7DTC. Molecular dynamics (MD) simulations were performed on seven systems, including Nav1.5 bound to Dapa and Ertu at different sites, both ligands together and a ligand-free control. Each system was simulated for 210 ns, repeated thrice for statistical accuracy. Root mean square deviation(RMSD) and B-factor analyses were conducted to observe the key residue fluctuation. MMGBSA(Molecular Mechanics/Generalized Born Surface Area) binding free energy, energy decomposition, H-bond analysis, and correlation heat maps performed to observe ligand binding effects.

Results: B-factor analysis revealed that Dapa binding at both sites increased rigidity at S571, whereas Ertu binding increased flexibility. H-bond analysis showed that Dapa interacted with S1710 and TRP 1345, stabilizing S571, while Ertu interacted with THR 1708, promoting flexibility. MMGBSA results indicated that site 2 had stronger ligand interactions than site 1, with Dapa showing higher binding affinity than Ertu. Correlation heat maps highlighted dynamic relationships between binding residues, S571, and key protein regions such as the DIII-IV loop.

Conclusion: Dapa stabilizes Nav1.5 by increasing S571 rigidity, while Ertu promotes flexibility, potentially explaining Dapa's cardioprotective effects. Future work will use coarse-grained simulations to further explore allosteric regulation and ligand-induced effects.

Investigating the Non-Thermal Effects of Radiofrequency Radiation on Staphylococcus aureus

Aiman Ali Alhadi Elkilani

Institut National de la Recherche Scientifique, Québec City, QC, Canada

Introduction: Exposure to radiofrequency electromagnetic fields (RFE) is an emerging area of interest due to its potential biological effects on bacterial physiology. This pilot study will investigate the non-thermal impacts of 5G and 6G microwave radiation on the human skin microbiome, starting with *Staphylococcus aureus*. The focus will be on bacterial growth, biofilm formation, antibiotic susceptibility, and molecular adaptations.

Methods: A pure culture of *S. aureus* will be exposed to controlled microwave radiation (5-6 GHz) at non-thermal power levels. To assess the effect of RFE on the proliferation and viability of *S. aureus*, the agar plates will be exposed to RFE during a 24-h incubation period. Colonies will be counted after the 24 h-incubation at 37 ºC to calculate the bacterial concentrations (colony-forming units (CFU) per mL). Morphological alterations will be analyzed through scanning electron microscopy (SEM), while

molecular changes will be examined using PCR, RNA sequencing (RNA-seq), and proteomics to evaluate gene and protein expression. Additionally, the impact on biofilm formation and antibiotic resistance will be studied to determine adaptive bacterial responses.

Prospective Results: We anticipate that non-thermal microwave exposure will induce structural and functional modifications in *S. aureus*, impacting cell wall integrity and biofilm formation. RNA-seq and proteomic analyses are expected to reveal alterations in stress response pathways, probably involving heat-shock proteins and DNA repair enzymes. Additionally, changes in antibiotic sensitivity may be measured, suggesting a potential link between RF exposure and bacterial adaptability.

Conclusion: This study provides novel insights into the biological responses of *S. aureus* to non-thermal microwave radiation. Understanding these effects can contribute to advancements in antimicrobial strategies and sterilization techniques while raising concerns regarding bacterial adaptation in RF-exposed environments.

Investigation of commercial LNP compositions for mRNA delivery across respiratory mucosa

Dhruv Patel[1], Aneesh Thakur[1]

[1]Vaccines and Infectious Disease Organization (VIDO), University of Saskatchewan, [2]Department of Biochemistry Microbiology and Immunology, University of Saskatchewan

Purpose: Parenteral messenger RNA (mRNA)-lipid nanoparticle (LNP)-based vaccines *e.g.*, mRNA-LNP vaccines against COVID-19 effectively induce systemic immunity but suboptimal mucosal immunity in the lungs. Mucosal immunity, particularly through IgA and resident memory T cells, is essential for protection against respiratory pathogens. It is not known if commercial LNPs used in parenteral mRNA therapeutics or vaccines can be used for mRNA delivery in the lungs. Here, we evaluated commercial mRNA-LNP compositions for their safety and RNA delivery following respiratory mucosal immunization.

Methods: Lipid nanoparticles (LNPs) encapsulating firefly luciferase (Fluc) protein-encoding mRNA was prepared by microfluidic mixing. SM-102 (SpikeVax®, Moderna), ALC-0315 (Comirnaty®, Pfizer-BionTech), MC3 (Onpattro®, Alnylam), and C12-200 were selected as ionizable lipids to formulate LNPs. *In vitro* protein expression was evaluated in cell lines such as A549, BHK21, RAW 264.7, and Calu3. *In vivo* protein expression was assessed in BALB/c mice via intranasal (i.n.) (by micropipette) and intrapulmonary (i.pulmon.) (by PennCentury™ Microsprayer® aerosolizer) routes.

Results: *In vitro* FLuc protein expression levels in cell lines were found to be highest ($p<0.0001$) for C12-200 LNPs across all the cell lines. *In vivo* protein expression upon i.n. immunization showed no significant difference among SM-102, ALC-0315, and C12-200 LNPs, whereas MC3 LNPs showed lowest ($p<0.05$) expression. *In vivo* protein expression in the lungs upon i.pulmon. delivery demonstrated non-comparable FLuc expression levels between SM-102 and C12-200 LNPs, whereas the expression in ALC-0315 and MC3 LNPs was significantly lower ($p<0.05$). Among LNPs, C12-200 LNPs induced the highest inflammatory cytokine IL-1β production in the lungs.

Conclusion: Overall, FLuc mRNA-loaded SM-102 LNPs generated the highest protein expression in cells and mice upon i.n. and i.pulmon. administration, and induced the least inflammatory cytokine response in the lungs. Further *in vivo* immunogenicity studies will assess if this high protein expression by SM-102 LNPs promotes antigen-specific T cells and antibody responses following respiratory mucosal immunization.

miR-486-5p-Carrying Nanoparticles to Target Cultured Endothelial and Kidney Tubular Epithelial Cells

Äli Maadelat[1,2], Savindi Wehella[1,3], Adrianna Douvris[1,4,5], Shireesha Manturthi[1,3], Kevin Burns[1,3,4,5], Suresh Gadde[1,3,4]

[1]Kidney Resarch Center, the Ottawa Hospital Research Institute, [2]University of Ottawa, Faculty of Science, [3]University of Ottawa, Cellular and Molecular Medicine, [4]University of Ottawa, Faculty of Medicine, [5]The Division of Nephrology, the Ottawa Hospital

Acute kidney injury (AKI) is a serious condition characterized by a sudden decline in kidney function, often leading to chronic kidney disease (CKD). Current treatment options are limited, necessitating novel therapeutic strategies. We previously showed that microRNA-486-5p (miR-486-5p) is a promising therapeutic candidate due to its ability to regulate apoptosis (1,2). However, efficient and targeted delivery remains a major challenge. In this study, we designed four types of nanoparticles (NPs)—polymeric (PNP),

hybrid (HNP), and poloxamer-based (Pl-NP1, Pl-NP2)—to encapsulate and transfer miR-486-5p to cultured endothelial and kidney tubular epithelial cells.

The NPs were characterized for size, polydispersity index (PDI), and charge via dynamic light scattering (DLS). Stability was assessed by measuring size after long-term storage at 4°C or incubation in fetal bovine serum (FBS). Cell lines investigated were human umbilical vein endothelial cells (HUVECs), human kidney tubular epithelial (HK2) cells, and human proximal tubular epithelial cells (hPTECs). Cytotoxicity was assessed by lactate dehydrogenase (LDH) assay. Fluorescently labelled microRNAs were used to measure the encapsulation efficiencies (EE%s) and, through RT-qPCR and microscopy, cellular uptake. Functional assessments included immunoblots, flowcytometry, and cleaved caspase3 enzyme activity assay. Cellular injury mimicking the effects of AKI was induced by hydrogen peroxide (H2O2) treatment.

All NPs demonstrated ideal sizes, PDIs, charges, EE%s, and stability in FBS and after long-term storage. LDH assay showed no toxicity in NP-treated HUVECs (n=3). RT-qPCR and microscopy data suggested effective NP uptake by HUVECs (n=3). Immunoblots confirmed that miR-486-5p-loaded HNPs reduced Forkhead Box Protein O1 (FOXO1) expression, a validated target of miR-486-5p, in HUVECs (n=4, $p<0.05$) and HK2 cells (n=3, $p<0.05$). Apoptosis assessments indicated that HUVECs (n=2) and hPTECs (n=3, $p<0.05$) transfected with miR-486-5p-loaded HNPs prior to H2O2 addition had significantly lower cleaved caspase3 enzyme activity than controls. Flowcytometry revealed less apoptotic and necrotic HUVECs and hPTECs in H2O2-exposed samples pretreated with miR-486-5p-loaded HNPs compared to controls.

These results illustrate favorable properties for miR-486-5p-HNP systems, highlighting their potential as a targeted nano-therapy for AKI and paving the way for future *in-vivo* studies.

pH-sensing hydrogel for point-of-care wound diagnostics : a novel tool for diagnosis, prognosis and therapeutics

Katia Cherifi[1], Marie-Lynn Al-Hawat[1], Farnoush Toupchinejad[2], Katerina Christodoulopoulos[1], Simon Matoori[1]

[1]Université de Montréal, Faculté de Pharmacie, [2]McGill University, Department of Pharmacology and Therapeutics

Chronic wounds like diabetic foot ulcers (DFUs) are a serious complication of diabetes and the leading cause of lower limb amputations worldwide. Current diagnostic options and standard wound care rely on macroscopic evaluation of the wound for its size, depth, implication of other tissues and signs of infection like redness and swelling. However, the long healing time associated with multiple in-clinic visits, associated with a high recurrence rate, contribute to a reduced quality of life and high financial burden for those affected. In spite of this, there is motivation for the development of a novel molecular-based diagnostic tool allowing for specific point-of-care management of chronic wounds. With multiple studies having highlighted the role of pH in wound healing, with chronic wounds exhibiting more alkaline values compared to acute wounds, wound pH appears to be a promising biomarker for the diagnosis and monitoring of chronic wounds. In this study, we developed a rapid and easy-to-use pH-sensing bandage and demonstrated multiple analytical methods to enable point-of-care use by both patients and clinicians. This bandage, when applied onto the wound, absorbs exudate which interacts with a pH-sensitive dye (pyranine) loaded onto microparticles encapsulated in a hydrogel matrix. This bandage exhibits linear fluorescence signals *ex vivo* ($R^2 = 0.9909$) upon clinically relevant pH variations (6.0-9.0). By leveraging pyranine's ability to change color intensity as pH changes, this bandage has also been validated with two colorimetric detection methods: using a smartphone camera app and a home-built RGB detectors. Both methods showed a consistent decrease of Blue in RGB values as pH increased, with Blue values similar to Red and Green at low pH (pH 6.0: B ≈ R ≈ G) but significantly lower at higher pH, even reaching 0 at pH 9.0 (pH 9.0: B = 0, B <<< R & G). These findings indicate the potential of this system to improve DFU diagnosis and treatment management by enabling point-of-care detection of wound pH with various point-of-care methods (portable fluorometer, smartphone camera, homebuilt detector) accessible to patients and clinicians no matter how remote.

Polymersome-Based Assay for Point-of-Care Quantification of Blood Ammonia

Marie-Lynn AL-HAWAT[1], Justine Caron[1], Sarah Djebbar[1], Simon Matoori[1]

[1]Faculty of Pharmacy, Université de Montréal

PURPOSE: Ammonia is an endogenous metabolite that has crucial roles in pH-homeostasis and protein metabolism but is neurotoxic at high concentrations[1,2]. Its levels are increased in liver diseases such as urea cycle disorders and cirrhosis, and it serves as a key biomarker in liver diseases. Rapid detection of hyperammonemia is critical for timely clinical intervention but point-of-care blood

ammonia diagnostics remain unavailable[3]. Polymersomes are vesicular structures composed of block copolymers. Polymersomes loaded with a pH-sensitive-dye, have been shown to selectively sequester ammonia in plasma, providing a diagnostic for hyperammonemia[4]. We hypothesized that transmembrane pH-gradient-polymersomes loaded with a near-infrared-fluorophore (NIRF) can detect and quantify ammonia in whole blood. Specific objectives were to develop polymersomes encapsulating a pH-sensitive near-infrared-fluorophore for ammonia sensing, and evaluate in fresh whole-blood using a plate reader then a portable fluorometer.

METHODS:Polymersomes were prepared by dissolving mPEG(2000)-b-PS(3000)polymers, followed by emulsification in isotonic citric acid containing pH-sensitive NIRF (IRDYE680RD). Fluorescence of IRDYE680RD at different pH-values using a plate-reader and a portable-fluorometer were measured. Mouse blood, obtained from cardiac puncture, was spiked with ammonium-chloride standards up to 0.5mM. 3µL of fresh murine whole blood, mimicking capillary blood assays, was used. Measurements were conducted using both a plate-reader and a portable fluorometer to evaluate potential point-of-care use.

RESULTS:The polymersome-based ammonia-assay demonstrated a highly linear fluorescence response (R^2=0.9948) within 5minutes using a plate-reader, covering clinically relevant ammonia concentrations up to 500µM. It maintained excellent sensitivity with a 31µM quantification limit after just 2minutes. A portable-fluorometer confirmed a concentration-dependent signals in buffer and blood. The assay exhibited high specificity, with minimal interference from common blood metabolites. The transmembrane pH-gradient remained stable and the intra- and inter-day variability remained low, underscoring the assay's reproducibility and robustness for clinical settings.

CONCLUSION: The portable fluorometer successfully detected a pH-dependent fluorescence increase of the pH-sensitive NIRF in the polymersome-based ammonia assay, which required only 3µL of blood. This assay offers a portable, sensitive solution for point-of-care blood-ammonia diagnostics, with high-specificity and linear responses. These results encourage further validation in human samples to fully realize its potential for clinical use.

Saturated vs Unsaturated fat-based ketogenic diets : Effects on Weight Loss and Atherosclerosis in Mice

Xiaobei Li[1], abdualrahman mabdualkader[1], Jiaheng Guan[1], Yiming Yin[1], Parisa Pourfarziani[1]

[1]University of Montreal

Purpose : Ketogenic diets (KDs), which are high in fat and very low in carbohydrates, have gained popularity for their weight loss benefits. However, the high fat content, particularly saturated fats, has raised concerns about their potential to promote atherosclerosis and adversely impact cardiovascular health. Clinical studies on this topic have produced inconsistent results, largely due to variations in diet composition, study duration, and outcome measures [1]. Adding to the complexity, several murine studies suggested that unsaturated fats, despite their known cardioprotective properties, may paradoxically promote weight gain [2]. These uncertainties highlight the need to study how different fats in KDs affect weight and cardiovascular health.

Methods : $LDLr^{-/-}$ mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 10 weeks to induce obesity. After this period, obese mice were either maintained on the HFD or switched to a saturated fat (SFA-KD) or an unsaturated (UFA-KD) fat-based KD for 18 weeks, while LFD-fed mice continued their original diet.

Results : After switching from HFD to KDs, mice experienced a rapid decrease in body weight. After two weeks, UFA-KD mice started to regain weight, ultimately reaching levels comparable to the HFD group. In contrast, SFA-KD mice sustained their weight loss, maintaining levels slightly higher than those of the LFD group. In situ imaging of the aortic arch revealed severe atherosclerosis in the HFD group, while both KD groups displayed moderate and comparable plaque levels. Despite these differences, both KDs significantly reduced plasma triglyceride and cholesterol levels compared to the HFD group, though these levels remained higher than those observed in the LFD group. Interestingly, plasma inflammatory markers (MCP1, IL6, VCAM-1, ICAM-1, and TNF-α) were comparable between KD and LFD groups but were significantly elevated in the HFD group. However, IL1β and IL18 levels were elevated in both KD and HFD groups, suggesting activation of the NLRP3 inflammatory pathway.

Conclusion : Regardless of fat type, both ketogenic diets were atherogenic but reduced fat-induced inflammation and slowed atherosclerosis progression compared to the HFD. However, SFA-KD was more effective in sustaining weight loss.

Sex Differences in ISO-Induced Kidney Injury: Role of CYP Enzymes and AA Metabolism

Sara Helal[1], Samar Gerges[1], Ayman El-Kadi[1]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

Purpose: Kidney injury can lead to chronic kidney disease and end-stage renal diseases if left untreated. Isoproterenol (ISO)-induced kidney injury is mediated by the activation of the renin-angiotensin-aldosterone system (1). The expression and activity of cytochrome P450 (CYP) enzymes are strongly influenced by sex differences, and their arachidonic acid (AA)-mediated metabolites play crucial roles in both physiological and pathological conditions (2). This study aims to investigate sex-related differences in CYP enzyme expression and activity following ISO-induced kidney injury.

Methods: Male and female rats were injected with ISO (1 mg/kg, i.p.) for seven days. Kidney tissues were analyzed for injury markers. The gene and protein expression of CYP enzymes were measured using real-time PCR and Western blot. Kidney microsomes were incubated with AA, and the metabolite formation rate was analyzed. Additionally, the soluble epoxide hydrolase (sEH) level was evaluated.

Results: Our results indicated that ISO-treated female rats exhibited higher kidney injury markers, such as kidney injury molecule-1 and transforming growth factor-β. Gene expression of CYP1A1, 4A1, and 4A11 was significantly higher in ISO-treated female kidneys, whereas CYP2E1, 4F6, and 4A2 were significantly elevated in the treated males. The protein level of CYP4A1 and 2E1 increased in both sexes, while CYP1B1 increased only in male-treated rats. The formation rate of 14S(15R)-EET significantly decreased in females, likely due to having higher gene expression and activity of sEH.

Conclusion: These findings revealed significant sex differences in kidney injury severity and CYP expression, with female rats experiencing greater injury severity. These results highlight the potential relation between kidney injury progression and CYP enzymes level and activity, which may have implications for sex-specific therapeutic strategies.

This work was supported by a grant from the Canadian Institutes of Health Research [CIHR PS 168846] to A.O.S.E-K. S.A.H. is the recipient of the Egyptian Government Scholarship.

Study on the structure-activity relationship of interfacial lubrication in proton solutions

hui guo[1], Chang-sheng Wang[1], Nahid Hassanpour[1], Duy Anh Pham[1], Xavier Banquy[1]

[1]Montreal university, Montreal, CA

Purpose: The protic solution refers to a solvent system containing exchangeable protons (H^+), typically involving hydrogen-bonding molecules like water and alcohols, play a pivotal role in biolubrication. Their ability to modulate interfacial friction through proton dynamics and hydration layers is crucial. However, to regulate the hydration layer precisely and utilize the hydration mechanism, a deeper understanding of hydration mechanisms is needed.

Methods: To address these challenges, we utilize the Surface Force Apparatus (SFA) to investigate the influence of interfacial structure on lubrication performance under different protic solutions, establishing the correlation between interfacial structure and lubrication properties. Based on these findings, we modulate the composition and structure of hydration layers to study their lubrication behavior.

Results: At the nanoscale, different interfacial structures influence the lubricating ability of solutions. By adjusting the type and concentration of protonic solutions, the interfacial structure can be modulated to finely control interfacial lubrication. Protonic solutions with different concentrations exhibit variations in dielectric constants, affecting their Debye screening length and thereby altering the interfacial structure. Different types of protonic solutions (methanol, ethanol, and heavy water) possess distinct molecular structures, which influence the mica surface structure and lead to differences in interfacial interactions. Additionally, different protonic solutions affect the interaction between salt ions and water molecules.

Conclusion: The human body, composed of over 70% water, possesses the most complex and extensive water-based lubrication system known. Extensive experimental evidence highlighting the superiority of hydration layers in water-based lubrication. This study aims to provide novel insights for treating diseases associated with lubrication deficiency (including osteoarthritis, dry eye syndrome, esophagitis, and xerostomia)

Targeted mesenchymal stem cell-derived bioactive nanoparticles for osteoarthritis therapy

Antoine Karoichan[1], Ling Li[2], Maryam Tabrizian[1, 3]

[1]Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, QC, [2]Department of Anatomy and Cell Biology, McGill University, Montreal, QC, [3]Department of Biomedical Engineering, McGill University, Montreal, QC

Purpose: Osteoarthritis (OA) is a progressive disease of the joints typically characterized by cartilage degradation, abnormal subchondral bone remodelling, inflammation, and disabling pain. Despite being one of the most prevalent chronic disorders globally, a treatment for OA remains lacking. Current research strategies have predominantly focused on cartilage, neglecting the critical role of other joint tissues, such as the subchondral bone, in disease progression. Emerging evidence suggests that mesenchymal stem cell-derived nanoghosts (MSC-NGs) can enhance tissue repair, but their efficacy in OA remains limited by suboptimal localization to key joint compartments.[1] This study aims to enhance the therapeutic potential of MSC-NGs by engineering them for targeted delivery to the subchondral bone for more effective attenuation of OA pathology.

Methods: MSC-NGs were generated by sonication-mediated disruption of ghost mesenchymal stem cells while preserving bioactive membrane components. To improve targeting efficiency, NGs were functionalized with an aptamer specific to the bone marrow environment before being administered intra-articularly into mouse models of OA induced through the surgical destabilization of the medial meniscus.[2] Nanoghost biodistribution was evaluated using fluorescence imaging and flow cytometry to quantify their retention and localization within the joint. Disease progression and therapeutic outcomes were assessed through micro-computed tomography (microCT), histological analyses, and pain sensitivity assessments.

Results: Functionalization with the bone marrow-targeting aptamer improved MSC-NG retention in the joint and enhanced delivery to the intended subchondral bone target. Flow cytometry analysis showed that only 0.047% of the intended target cell population had a detectable signal for unmodified NGs, whereas 0.961% had a signal for aptamer-functionalized NGs, indicating superior targeting efficiency. MicroCT and histological evaluations revealed that subchondral bone-targeted NGs preserved joint morphology more effectively than non-targeted NGs. Pain sensitivity assessments further demonstrated improved gait outcomes, suggesting enhanced disease attenuation with targeted therapy.

Conclusion: These findings highlight the potential of MSC-NGs as precision therapeutics for OA by optimizing their delivery to subchondral bone. Targeted NGs not only improved retention and biodistribution within the joint but also yielded superior structural and functional outcomes, underscoring their promise as a clinically translatable strategy for OA treatment.

The effect of the rs2072446 variant of the gene encoding the pan-neurotrophic receptor p75NTR on the platelet response to BDNF

Anne-Sophie Marceau[1, 2], samuel fleury[1, 2], jessica blais[1, 2], florence lipp[1, 2], imane Boukhatem[1, 2], mélanie welman[1], georges jourdi[1, 2], marie lordkipanidze[1, 2]

[1]Montreal Heart Institute, Montreal, Qc, Canada, [2]Faculty of Pharmacy, University of Montreal, Montreal, Qc, Canada

Purpose : Two receptors for the Brain-Derived Neurotrophic Factor (BDNF), the high-affinity TrkB receptor and the low-affinity $p75^{NTR}$ receptor, are present in platelets. While TrkB mediates BDNF-induced platelet aggregation, the role of $p75^{NTR}$ remains unknown. The rs2072446 (17:49510457C>T) polymorphism in the gene encoding $p75^{NTR}$ results in the substitution of serine for leucine, in a region important for receptor glycosylation. In this study, we sought to study the effect of the variant on the structure and levels of $p75^{NTR}$ in platelets, and on platelet responses to BDNF.

Methods : Fourteen participants homozygous for each allele were matched for age (±5 years) and sex. Platelets were isolated from blood by centrifugation. $p75^{NTR}$ expressions were analyzed by immunoblotting, flow cytometry and ELISA. Platelet aggregation in response to BDNF was measured by optical aggregometry.

Results: Participants were on average 69 ± 5 years old. Carriers of the variant allele had a lower BMI (27 ± 3 vs 29 ± 4 kg/m^2). They were less likely to present with cardiovascular risk factors (hypertension [29% vs 57%], dyslipidemia [36% vs 64%], diabetes [0% vs 29%]) compared with carriers of the reference genome. Membrane (14.6 [10;19]% vs 9.3 [7.5;25.8]%, p=0.77) and total (19.6 [5.3;40.4]% vs 18.6 [14.2;43]%, p=0.82) $p75^{NTR}$ expression levels were similar between the 2 genotypes, which was confirmed by ELISA (124.4 ± 34.6 vs 123.6 ± 39.4 pg/2.5 x10^8 platelets, p=0.96). However on immunoblotting, in addition to the 75 kDa-band

present in all participants, carriers of the variant allele displayed a second band at a higher molecular weight (135 kDa) than carriers of the reference allele (100 kDa). The response to BDNF in aggregometry was similar between the two groups.

Conclusion : The variant causes a structural modification of $p75^{NTR}$ but does not appear to affect receptor levels in platelets or platelet aggregation responses to BDNF.

The effects of cannabinoids on male and female hypertensive vasculature.

Kayla Kitselman[1], Noriko Daneshtalab[1], Reza Tabrizchi[1]

[1]Memorial University of Newfoundland, St. John's, NL, Canada

***Purpose*:** Cannabinoids have a broad range of physiological functions including those on cardiovascular system, such as decreases in cardiac contractility, heart rate, and arterial blood pressure (1). The mechanisms of these actions are complex, show regional differences, and are mediated by their direct actions on cannabinoid receptors, CB1 and CB2, as well as, other receptors. The current studies were undertaken to explore differences in pharmacological actions of cannabinoids on small resistance arteries in male and female hypertensive rats.

***Methods*:** Blood vessels from male and female spontaneously hypertensive (SHR) rats and normotensive Wistar Kyoto (WKY) rats aged 14-16 weeks (n=6-9/group) were used. Blood pressures and biometrics were determined within one week and at the time of sacrifice. The third order mesenteric arteries were isolated, mounted on a pressure myography system and pre constricted with phenylephrine. Then, vascular responses to the action of cannabinoids, tetrahydrocannibinol (THC) and cannabidiol (CBD), (10 nM - 300 nM), were determined. Immunofluorescence imaging was conducted to determine if differences exist in the expression of CB_1 and CB_2 receptors in the third order mesenteric vessels.

***Results*:** Blood pressure was found to be significantly higher in SHRs compared to WKYs ($p<0.001$). There was a trend towards SHRs showing reduced response to THC or CBD while WKYs showed concentration-dependent vasodilatory responses. The *in vitro* semi-quantitative analysis of CB_1 in our sampled mesenteric arteries has shown no significant difference in the expression of CB_1 between the groups. The *in vitro* analysis of CB_2 in our sampled mesenteric arteries has, however, shown a significant decrease in CB_2 receptor expression in male SHRs when compared to male WKYs ($p=0.05$). There was no difference in CB_2 receptor expression between female SHRs and female WKYs.

***Conclusion*:** Overall, we observed that SHRs vascular response to cannabinoids appears to be different from that of their normotensive counterparts. The use of inhibitory proteins to block CB_1 and CB_2 in our functional studies will help to determine if alterations in CB_2 expression in male SHRs is responsible for the difference in functional responses observed in these animals.

The impact of the rs2233667 variant of Prohibitin 1 on platelet and megakaryocyte PHB1 and p75NTR levels

Yanitsa Petkova[1, 2], Charlotte Aubin[1, 2], Imane Boukhatem[1, 2], Samuel Fleury[1, 2], Jessica Blais[1, 2], Mélanie Welman[1], Georges Jourdi[1, 2, 3, 4], Marie Lordkipanidzé[1, 2]

[1]Montreal Heart Institute Research Center, Montreal, QC, Canada, [2]Faculty of Pharmacy, University of Montreal, Montreal, QC, Canada, [3]Paris Cité University, INSERM, Therapeutic Optimization in Neuropharmacology, OTEN U1144, 75006, Paris, France, [4]Department of Biological Hematology, Lariboisière Hospital, AP-HP. Nord, Paris, France

Purpose: Prohibitin 1 (PHB1) is a multifunctional protein involved in transcriptional regulation, including that of the p75 neurotrophin receptor (p75NTR). Given the association of both genes with cardiovascular diseases, we investigated the impact of the *rs2233667* variant of the PHB1 gene, linked to thrombotic disorders, on PHB1 and p75NTR expression levels, as well as on platelet and megakaryocyte function.

Methods: Platelets and hematopoietic stem cells (CD34+) were isolated from whole blood collected from carriers (C/C, n=14) and non-carriers (G/G, n=14) of the *rs2233667* variant, matched for sex and age (± 5 years), recruited from the Montreal Heart Institute Biobank. CD34+ cells were differentiated *in vitro* into megakaryocytes over a period of 14 days. The expression levels of PHB1, p75NTR and platelet activation markers (PAC-1, CD62p, and CD40L) were measured by flow cytometry. **Results:** Flow cytometry analysis revealed a significantly higher expression of PHB1 (86.3 (77.0; 91.9)% vs. 2.1 (0.7; 3.3)%) and p75NTR (87.7 (58.3; 93.8)% vs. 29.6 (17.8; 37.0)%) in megakaryocytes of carriers compared to non-carriers of the *rs2233667* variant. In platelets, PHB1 was

undetectable regardless of genotype, whereas p75NTR receptor expression was significantly higher in non-carriers (18.0 (10.0; 20.6)% vs. 38.5 (21.4; 48.9)%). Platelet count and mean platelet volume were comparable between genotypes, however, in whole blood analyses, carriers of the *rs2233667* variant showed a significantly reduced expression of the platelet activation markers PAC-1 (0.0 (0.0; 0.0)% vs. 1.1 (0.4; 6.5)%), CD62p (19.9 (13.8; 23.5)% vs 34.0 (27.9; 42.8)%), and CD40L (4.5 (0.9; 5.9)% vs 7.4 (4.2; 10.8)%). **Conclusion:** The *rs2233667* variant modulates the expression of PHB1 and p75NTR in megakaryocytes and platelets. Further studies are underway to characterize the localization of PHB1 in platelets and megakaryocytes, and to better understand the effect of the variant on thrombosis and hemostasis.

Analysis of Antibiotic Consumption Trends in healthcare systems in Kazakhstan: WHO AWaRe Classification, 2016-2023

Yuliya Semenova, Mohamad Aljofan

Nazarbayev University, Astana, Kazakhstan

Purpose: This study aimed to analyze the nationwide antibiotic consumption trends and patterns over the period 2016-2023 in Kazakhstan, an upper-middle-income country located in Central Asia. The Ministry of Health imposed several aspects of antimicrobial stewardship (AMS), with hospital-based AMS programs established in a number of hospitals across the country.

Methods: Data on systemic antibacterials (J01 code) from the Anatomical Therapeutic Chemical (ATC) classification system were extracted from public and private hospitals, covering the period from January 1, 2016, to December 31, 2023. The extraction disaggregated at the ATC5 level with details of the active ingredient(s), dosage form, route of administration, number of preparations per package, and number of packages administered were included.

Results: The results showed that consumption of antibacterials, expressed in daily doses per 1000 inhabitants per day (DID), remained stable (AAPC = 0.36%), with two peaks observed during the COVID-19 pandemic period (2020 and 2021). Interestingly, antibacterial use in Kazakhstan falls within the Watch group, and there is a growing trend in the share of antibacterials from this group.

Conclusion: Alarmingly, an increase in the consumption of Reserve group antibiotics was observed, reflecting a significant and growing issue of AMR. Thus, there is an urgent need for the expansion of the AMS programs to address these emerging challenges.

Anticholinergic Drug Burden and Polypharmacy in Older Adults Diagnosed with Delirium in a Tertiary Care Hospital: A Case-Control Study

Victoria Nguyen[1], Chris Fan-Lun[2, 3, 4], Romina Marchesano[2], Linda Ou[2], Rishabh Sharma[1], Tejal Patel[1, 5]

[1]School of Pharmacy, University of Waterloo, Kitchener, Canada, [2]Department of Pharmacy, Sunnybrook Health Sciences Centre, Toronto, Canada, [3]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada, [4]Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, Toronto, Canada, [5]Schlegel - UW Research Institute of Aging, Waterloo, Ontario, Canada

Purpose: Hospitalized older adults commonly experience delirium, a neuropsychiatric condition associated with poor outcomes and increased mortality. Medication-related factors like polypharmacy and anticholinergic burden are associated with an increased risk of delirium. This study aimed to describe prescribing practices in hospitalized older adults by comparing the prevalence of polypharmacy and anticholinergic burden at admission and discharge between patients with and without delirium.

Methods: This retrospective case-control study analyzed health records of patients aged ≥65 years admitted to general internal medicine units at a tertiary hospital from June to September 2023. Patients with delirium were identified using the CHART-DEL tool[1] and matched 1:2 by age and sex to controls without delirium. Data on demographics, medication number and class were collected. The Anticholinergic Burden (ACB) scale was used to quantify anticholinergic burden. Polypharmacy was defined as 5 to 9 scheduled medications, and hyper-polypharmacy as 10 or more scheduled medications. Statistical analyses compared differences in medication use and anticholinergic burden between cases and controls

Results: 75 cases and 150 controls were included. Median age was 85 years in both groups. The prevalence of polypharmacy was comparable between cases and controls at admission (49% vs. 59%, p=0.184) and discharge (47% vs. 56%, p=0.186). The prevalence of hyper-polypharmacy was comparable between cases and controls at admission (27% vs. 21%, p=0.311) and discharge (22% vs. 16%, p=0.222). Median ACB scores were comparable at admission (p = 0.109) but significantly higher in cases at discharge (2 [IQR 3] vs. 2 [IQR 2], p = 0.038). A greater proportion of cases had ACB scores ≥3 at discharge (43% vs. 31%).

Conclusion: Patients with delirium demonstrated significantly higher anticholinergic burden at discharge but no significant differences in polypharmacy rates compared to controls. This highlights the need to assess the appropriateness of increased anticholinergic use in older adults with delirium.

Victoria Nguyen is the recipient of the CSPS National Undergraduate Student Research Program Award from the University of Waterloo.

Description of the prevalence of actionable pharmacogenetic variants in the Montreal Heart Institute Hospital Cohort

Marc-Olivier Pilon[1, 2, 3], Jessica Hindi[1, 2, 3], Essaïd Oussaïd[2, 3], Farida Amani[4], Marie-Christyne Cyr[2, 3], Ian Mongrain[2, 3], Johanna Sandoval[2, 3], Louis-Philippe Lemieux Perreault[2, 3], David Busseuil[2], Jean-Claude Tardif[2, 3, 4], Grégoire Leclair[1], Marie-Pierre Dubé[2, 3, 4], Simon de Denus[1, 2, 3]

[1]Faculty of pharmacy, Université de Montréal, Montreal, Quebec, Canada, [2]Montreal Heart Institute, Montreal, Quebec, Canada, [3]Beaulieu-Saucier Pharmacogenomics Centre, Montreal, Quebec, Canada, [4]Faculty of Medicine, Université de Montréal, Montreal, Québec, Canada

Purpose. Pharmacogenomics enables pharmacological treatments to be tailored to individual genetic profiles, optimizing therapeutic efficacy while reducing side effects.[1] The Clinical Pharmacogenetics Implementation Consortium (CPIC) classifies gene-drug pairs according to their level of scientific evidence. Level A and B pairs are deemed "actionable", meaning that the prescriber should (Level A) or could (Level B) bring action to the therapy, for example, in the selection of the agent or in the adjustment of its dosage. The aim of this study was to assess the prevalence of actionable CPIC variants in the Montreal Heart Institute (MHI) Hospital Cohort (Montreal, Qc, Canada).

Methods. Genotyping was performed at the MHI's Beaulieu-Saucier Pharmacogenomics Centre using Agena's MassARRAY technologies (Sequenom iPLEX® ADME PGx Pro, VeriDose® Core and VeriDose® CNV CYP2D6) and Illumina Infinium's Global Screening Array v3-MD. *DPYD*, *UGT1A1*, *ABCG2*, *HLA-A*, *HLA-B*, *CYP3A5*, *CYP2C9*, *CYP2C19*, *SLCO1B1*, *VKORC1*, *TPMT*, *CYP4F2*, *CPY2B6* and *CYP2D6* genes associated with CPIC level A and B gene-drug pairs were analyzed in 10,082 participants drawn from a large cross-sectional study that evaluates the serum concentrations of 47 drugs in the MHI Hospital Cohort.

Results. Participants had an average of 4.1 genes carrying actionable genetic variation. Of the 12 genes encoding enzymes and transporters, 99.7% of participants had at least one actionable variant, and more than one-third had actionable variants in five or more genes. Among the 65 Level A or B gene-drug pairs evaluated, 57 were associated with medications used by at least one participant. For 26 drugs taken by at least 20 participants, the proportion of individuals carrying an actionable genotype specific to the drug ranged from 2.2% to 89.8% (shown in **Figure**).

Conclusion. This study shows the high prevalence of actionable genetic variants in the Quebec population and supports the implementation of preemptive pharmacogenomics in Quebec hospital settings.

Leveraging Patient Support Programs, data science, and AI to generate Fit-For-Purpose Real-World Evidence that supports population health and regulatory decision-making

Sarah Michalowicz[1], Aaron Leibtag[2], Aviva Pratzer[2], Steve Aviv[2], Ruth Moulson[2], Jerry Reid[1]

[1]Specialty Health Network, Shoppers Drug Mart, [2]Pentavere Research Group, Toronto, ON, Canada

Purpose: Generating real-world evidence (RWE) is costly, resource-intensive, and time-consuming, often constrained by small, incomplete, and expensive datasets. Despite these challenges, RWE is increasingly shaping regulatory decision-making in Canada. Patient Support Programs (PSPs), which provide direct care and services, collect a wealth of underutilized data that could significantly enhance RWE generation and population health knowledge. PSP data can also be linked with other sources, such as pharmacy records, to create globally best-in-class datasets. This pilot study examined the value of PSP data and the feasibility of linking it with pharmacy data, using artificial intelligence (AI), to generate population-level RWE suitable for regulatory use and health monitoring. **Methodology:** A national PSP provider, with access to Canada's most extensive retail pharmacy network,

analyzed data from a PSP supporting migraine patients. The study assessed data quality, completeness, and relevance in addressing key RWE questions on efficacy, treatment adherence, and patient engagement. Data were benchmarked against the Canadian Drug Agency's (CDA) RWE reporting guidelines, evaluating completeness, accuracy, and generalizability. Additionally, existing PSP systems were reviewed to explore potential integration with pharmacy data. AI algorithms were applied to enhance insights, reducing manual effort and improving data extraction efficiency. **Results:** The study demonstrated that PSP data effectively addressed key RWE questions, with over 90% completeness and accuracy across critical variables. Compared to existing migraine datasets, PSP data provided superior depth, quality, completeness, and extended patient follow-up. The dataset included a larger population and longer observation periods, covering diverse patient demographics, treatment patterns, and physician characteristics across Canada. Moreover, PSP data aligned with existing literature, ensuring representativeness. The study also highlighted the feasibility of integrating PSP data with pharmacy records, enabling a more comprehensive analysis, including dosing and dispensing data. **Conclusion:** PSPs capture high-quality data that, when integrated with pharmacy records, can address key RWE challenges. Findings suggest that leveraging AI, PSPs, and pharmacy networks can generate regulatory-grade RWE while reducing costs. This approach presents a promising pathway to enhance RWE generation, ensuring data integrity and relevance for regulatory and population health decision-making.

Patterns and determinants of statin prescribing and discontinuation in individuals aged 80 and older: a population-based study of two cohorts

Myriam Khelifi[1], Véronique Boiteau[2], Marc Simard[2], Marie-Eve Gagnon[3], Olivia Dalleur[4], Caroline Sirois[1, 2, 5]

[1]Faculté de pharmacie, Université Laval, Québec, Canada, [2]Institut national de santé publique du Québec, Québec, Canada, [3]Département des sciences infirmières, Université du Québec à Rimouski, Lévis, Canada, [4]Département de pharmacie, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Bruxelles, Belgique, [5]VITAM - Centre de recherche en santé durable, Québec, Canada

Purpose. Statin use is prevalent, with over half of Canadians 65 years and older using these cholesterol-lowering medications. Considering the limited knowledge regarding their benefits and risks in people ³80 years, use patterns in the population can be informative. Therefore, we aimed to describe the prescribing and discontinuation patterns of statin use, along with their determinants, in people ³ 80 years in Québec, Canada. **Methods.** Using the Quebec Integrated Chronic Disease Surveillance System, we built two population-based cohorts of community-dwelling adults aged ³80 years on April 1st, 2013 (2013 cohort) and April 1st, 2018 (2018 cohort). We assessed statin use in the five years before and after cohort entry to calculate the proportion of statin prevalent, incident, and discontinued users. We used multivariable Cox models to identify factors associated with initiation and discontinuation, using Hazard ratios (HRs) and 95% Confidence Intervals (95%CI). **Results.** We included 278,996 and 317,027 individuals in 2013 and 2018 cohorts, with mean ages of 84.8 and 85.2 years. At cohort entry, 49% and 51% were prevalent statin users. Within five years, 24% and 22% of current users discontinued their therapy, while about 10% initiated a statin in both cohorts. Older age was the most common factor for discontinuation (HR ranging from 1.53 to 3.65 across older groups compared to 80-84 years in both cohorts). Male sex (HRs: 1.45 and1.44) and previous statin use (HRs: 2.29 and 1.99) were the strongest factors associated with initiation in both cohorts. **Conclusion.** Statin use remained prevalent among the ³80 years with levels approximatively constant over a 15-year period. A thorough evaluation of its relevance is needed.

The effect of the linkage disequilibrium between rs7124442 and rs11030119 variants in the gene coding for the brain-derived neurotrophic factor (BDNF) on platelet function

Charlotte Aubin[1, 2], Imane Boukhatem[1, 2], Jessica Blais[1, 2], Samuel Fleury[1, 2], Mélanie Welman[1], Georges Jourdi[1, 2, 3, 4]

[1]Research Center, Montreal Heart Institute, Montreal, Canada, [2]Faculty of Pharmacy, Université de Montréal, Montreal, Canada, [3]Université Paris Cité, INSERM, Therapeutic Optimization in Neuropharmacology, Paris, France, [4]Biological Hematology Department, Hôpital Lariboisière, Paris, France

Purpose. The brain-derived neurotrophic factor (BDNF) is primarily known for its role in neuroplasticity and memory preservation, but it is also abundant in platelets, where its release promotes platelet activation, aggregation, and clot stabilization (1). Its impact on cardiovascular diseases is ambiguous, with some studies suggesting it offers vascular protection, while others indicate it may enhance

platelet aggregation and destabilize atherosclerotic plaques (2). This project aims to investigate the effects of the BDNF variants rs7124442 and rs11030119, which are linked by linkage disequilibrium, on platelet function. **Methods'.** Participants homozygous for rs712442 and the rs11030119 variants were recruited from the Montreal Heart Institute Biobank, along with sex- and age (±5 years)-matched controls. Platelet aggregation was assessed by light transmission aggregometry, while ROTEM was used to evaluate the thromboelastic properties of clots. **Results.** Carriers of the rs11030119 variant (A/A, n=20) also carried the reference allele for rs7124442 (C/C, n=20), consistent with linkage disequilibrium (r^2=0.78). Matching (n=20) on sex (50%) and age (69 [66 ; 73] vs 67 [61 ; 70] years) was successful. Carriers of the rs11030119 A/A - rs7124442 C/C genotype had a higher BMI (32 [25 ; 34] vs 26 [23 ; 29]) and more often presented with cardiovascular risk factors, e.g. hypertension (53% vs 45%), and dyslipidemia (53% vs 25%). When taken separately, rs11030119 variant carriers displayed increased platelet sensitivity in response to BDNF, which was normalized when accounting for rs7124442 genotype. Moreover, coagulation parameters (PT, INR, PTT) and clot viscoelastic properties were within normal ranges, and similar between genotypes. **Conclusion/** The rs7124442 and rs11030119 present a linkage disequilibrium, with carriers of the variant for one SNP also carrying the reference allele of the other. Taken individually, each genotype is associated with modest changes in platelet reactivity. However, when accounting for linkage disequilibrium, no aberrant phenotype in platelet function is discernible.

3D-Printing as a Compounding Tool for Personalized Point-of-Care Treatment: The Case of Hydroxyurea

Jean Laverdière[1], Carlos Bendicho-Lavilla[2, 3], Louiza Mahrouche[1], Mihaela Friciu[1], Yves Pastore[4], Grégoire Leclair[1], Alvaro Goyanes[2, 3, 5, 6], Valérie Gaëlle Roullin[1], Abdul Basit[6]

[1]Plateform of biopharmacy, University of Montreal, 2940 Chem. de Polytechnique, Montreal, Canada., [2]FABRX Artificial Intelligence, Calle Enrique Vidal Abascal 7, Santiago de Compostela, Spain, [3]FABRX Ltd., Henwood House, Henwood, Ashford, Kent, TN24 8DH, UK, [4]Pediatric Department, Hematology Section, CHU Sainte-Justine, 3175 Chem. de la Côte-Sainte-Catherine, Montreal, Canada, [5]Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, I+D Farma (GI-1645), Facultad de Farmacia, Instituto de Materiales (iMATUS) and Health Research Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain, [6]Department of Pharmaceutics, UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, WC1N 1AX, UK

Purpose. 3D printing of medication is a computer-controlled process that involves the precise, layer-by-layer deposition of components necessary for the creation of customized solid tablets (1). By altering their composition, it is even possible to control their release at specific times or sites within the body (1). Such tools could also play a crucial role in improving the acceptability of swallowing tablets among various patient populations, particularly by combining multiple medications into a single tablet of personalized color, shape, and taste (1). Although the ability to produce medications with such precision marks a pivotal step towards the advent of personalized medicine, only a limited number of pharmaceutical 3D printers are used in North America. In 2024, the Government of Canada published the *National Priority List of Pediatric Drugs*, where they call for help in the development of pediatric-adapted dosage forms for 25 drugs. We therefore sought to explore if 3D printing of medication could be used to safely and efficiently prepare these drugs at point-of-care, in child-friendly, personalized dosage forms. Herein, we present the case of hydroxyurea, a critical yet inaccessible treatment for sickle cell anemia (2).

Methods. We developed a formulation for chewable, gummy-like hydroxyurea tablets. The printed tablets were subjected to tests prescribed in the USP monograph for hydroxyurea capsules. Pharmacokinetic profiles were then compared between a single dose of the 3D formulation and a hydroxyurea suspension in Beagle dogs (n=3/group). Finally, a stability study was conducted over 90 days. **Results.** We demonstrated that it is possible to 3D-print chewable hydroxyurea tablets with customizable dose, flavor, and color. All USP tests were compliant. Pharmacokinetic studies showed a relative bioavailability of 97% for the 3D-printed tablets. Stability data indicated the formulation remains stable for at least 90 days at room temperature. **Conclusion.** 3D printing of hydroxyurea tablets offers a safe and potentially superior alternative to current compounding practices. This project, one of the first in Canada to involve 3D printing of medication, has the potential to address critical needs in quality, safety, and supply of compounded drugs.

Antisense Oligonucleotide-Loaded Liposomes for Potential Treatment of Biofilm Forming Bacteria

Emmanuel Ho, Kaidy Orellana

[1]University of Waterloo, ON, Canada

Purpose: Key pathogens in chronic wound infections are often caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*), which form biofilms via quorum sensing (QS) mechanisms [1-2]. Specifically, AI-2, produced by *S. aureus*, modulates bacterial phenotypes and enhances *P. aeruginosa* biofilm formation. Disrupting QS communication between *S.aureus* and *P. aeruginosa* is thought to hamper biofilm biomass and virulence of bacteria [3-4]. As such, this study explores nanocarriers to deliver antisense oligonucleotides (ASOs) that silence genes involved in AI-2 production, aiming to mitigate biofilm formation in a co-culture model.

Methods: Biofilms of *P. aeruginosa* (PAO1) and *S. aureus* (ATCC 25923) were grown in 24-well plates, standardized at 1 x 10^4-10^7 CFU/mL, and quantified using a crystal violet (CV) assay after 0, 24, and 48 hours. Liposomes were formulated via microfluidic mixing using DC-cholesterol-HCl and DOPE at various flow rates and total flow rate ratios. The encapsulation efficiency of ASOs was measured via Ribogreen assay. Furthermore, a *Vibrio campbellii* (*V. campbellii)* luminescence reporter assay was utilized to determine the concentration of AI-2 produced in untreated *S. aureus*. Lastly, *P. aeruginosa* was supplemented with AI-2 to determine the extent to which AI-2 impacts bacterial growth and biofilm formation when compared to controls.

Results: CV assays showed significant biofilm growth ($p<0.0001$) over 24 and 48 hours, with no significant differences among bacterial concentrations at 0 hours. Optimal liposomes had a mean size of 149.3 nm, PDI of 0.1398, and zeta potential of +41.28 mV. ASO-loaded liposomes (12.5 µM) had 78.36% encapsulation efficiency. *S. aureus* exhibited increased AI-2 production, enhancing *P. aeruginosa* growth and biofilm formation, while *S. aureus* itself showed no significant response to AI-2.

Conclusion: The polymicrobial biofilm model showed increased biomass over time, and it provides a means of tracking changes in biofilm biomass after liposomal treatment. Overall, liposomes are adequate in size and have high encapsulation efficiency for the effective internalization and administration of ASOs in bacteria. Targeting AI-2 production may be a suitable choice for decreasing biofilm biomass and bacterial growth in the co-culture model.

Assessing the Impact of Atropisomer Chirality on Drug Discovery and Development

Eleonore Delaire[1], Tanos C.C. Franca[1], Kathleen Amiens[1], Shima Rezaeian[1], Fatma Shahout[1], Salim T. Islam[1], Steven LaPlante[1]

Biotechnologie Centre Armand Frappier, Institut National de la Recherche Scientifique, QC, Canada

Purpose Mirror-image chiral compounds can have the same primary structure but are nonetheless distinct compounds. Moreover, they can also have very different biological activities, pharmacokinetics, and toxicities profiles (1)(2). Atropisomers are a class of stereoisomers that result from hindered bond rotations. Unlike classical atom-centered chiral compounds that can racemize via bond breaking, atropisomers can interconvert dynamically via simple bond rotations, with half-lives ranging from minutes to years, depending on steric and electronic factors. Thus, atropisomeric compounds are more complicated to develop (3)(4).

Methods Approximately 60% of drug discovery campaigns encounter atropisomeric properties, particularly during the lead optimization phase, where structural rigidity is often sought for optimal activity (5). However, the additional feature of hindered-bond chirality complicates the development of atropisomers because each mirror image has distinct properties that impact *in vitro* and *in vivo* behaviors, including inhibition, crystallization, racemization, and ADMET profiles. Misinterpretation of results due to bond rotation can complicate development, toxicity profiles, increase costs, and pose regulatory challenges.

Results This poster will assess the progress made by the pharma industry in developing atropisomeric drugs since the past decade, showcasing their impact and the challenges faced in their approval and commercialization.

Conclusion Future strategies focusing on atropisomer stability and selective synthesis are critical for reducing development risk. As the understanding of atropisomerism deepens, it opens the door to novel molecular designs with refind efficacy and safety profiles.

Bacterial endotoxin contamination: a factor to consider during liposome synthesis

Ece Ates[1], Antoine Labrie[1], Nicolas Bertrand[1]

[1]Faculty of Pharmacy, Université Laval, Endocrinology and Nephrology Axis, Centre de Recherche du CHU de Québec, Québec, Canada

Purpose : Bacterial endotoxins, also known as lipopolysaccharides (LPS), are molecules present in the outer membrane of Gram-negative bacteria. They are a major contaminant sources in pharmaceutical preparations. Endotoxins are known for their ability to

stimulate the immune system, thereby altering experimental results in vitro and in vivo. The objective of our study is to evaluate whether the process of liposome synthesis results in the introduction of endotoxins into our formulations. Should contamination occur, our goal is to develop formulations that are free from endotoxins.

Methods: Bacterial endotoxins are detected using the industry standard Limulus Amebocyte Lysate (LAL) test. Liposome synthesis requires the use of buffers 1) during thehydration step and 2) to dilute the formulations. We have longitudinally evaluated endotoxin concentrations in different buffers. We also assessed the concentrations of endotoxins introduced into formulations at different stages of liposome preparation.

Results: The longitudinal study carried out on sterile buffers detected no contamination after 28 days. Liposome formulations synthesized without precautions show a higher concentration of endotoxins than when precautions are taken. When liposomes are solubilized with triton, endotoxin concentration does not appear to vary. The endotoxin test carried out on formulations with a known concentration of LPS confirms that the signal increases with the amount added.

Conclusion: Taking precautions, our formulations used for in vivo studies have an endotoxin concentration below 0.5 EU/mL, the limit authorized by regulatory authorities for sterile injectable preparations. Thus, the quantities of endotoxins introduced into the formulations during synthesis appear negligible.

Combinatorial design of ionizable lipid nanoparticles for muscle-selective mRNA delivery with minimized off-target effects

Jingan (Charles) Chen[1, 2], Yue Xu[1], Bowen Li[1, 2, 3]

[1]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada, [2]Institute of Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [3]Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada

Purpose: mRNA-based therapies have emerged as a transformative approach in various medical applications, including vaccines, gene editing, and cancer treatments. Ionizable lipid nanoparticles (LNPs) are critical carriers for delivering mRNA therapeutics but often exhibit unintended off-target transfection, notably in the liver and spleen, potentially causing adverse effects. Therefore, developing ionizable LNPs with high muscle selectivity and minimal off-target delivery is imperative for enhancing the safety and efficacy of mRNA therapies. This research aims to establish a robust, high-throughput combinational platform for synthesizing biodegradable, asymmetrical ionizable lipids tailored specifically for muscle-selective mRNA delivery.

Methods: We designed an advanced Ugi-based three-component reaction (3-CR) combinational chemistry platform for high-throughput synthesis of ionizable lipids, markedly improving reaction yields (>70%) using a nontoxic catalyst. A chemically diverse library comprising various biodegradable, asymmetrical lipids was generated. High-throughput screening identified iso-A11B5C1 as a top-performing lipid. Muscle-specific transfection efficiency, biodistribution, and gene editing capabilities were assessed using luciferase and Cre recombinase mRNA in mouse models. Additionally, iso-A11B5C1-LNPs were applied for both prophylactic and cancer vaccines; the immune responses and therapeutic efficacy were evaluated and compared to Moderna's SM-102-LNP.

Results: Optimized iso-A11B5C1 LNPs achieved muscle transfection efficiency comparable to commercial SM-102 LNP but demonstrated markedly reduced off-target mRNA expression in the liver and spleen. Biodistribution studies confirmed iso-A11B5C1's localization primarily to injection sites without significant systemic dissemination. Gene editing experiments with iso-A11B5C1 showed highly selective recombination in muscle tissues, with negligible editing detected in off-target organs. Immunological assessments revealed that while iso-A11B5C1 elicited weaker humoral responses due to limited direct antigen-presenting cell transfection, robust cellular immune responses were maintained, showing therapeutic potential in melanoma vaccine models.

Conclusion: Our study presents a novel combinational chemistry platform enabling rapid synthesis of ionizable lipids tailored for muscle-selective mRNA delivery. The identified lipid iso-A11B5C1 demonstrates superior muscle selectivity, potent transfection efficiency, and minimal off-target effects, establishing it as a promising vehicle for safer mRNA therapies. Additionally, this study encourages rethinking of mRNA vaccine design principles, suggesting that achieving high immune cell transfection might not be the sole criterion for developing effective mRNA vaccines.

Core-shell bottle-brush polymers as vectors for the administration of antioxidant agents for brain diseases

Fernanda Maria de Paula Miranda[1], Quoc Thang Phan[1], Jean Michel Rabanel[2], Xavier Banquy[1]

[1]Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada, [2]School of pharmaceutical Sciences Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Purpose. Oxidative stress, either as a primary cause or a consequence of disease progression, leads to damage of essential biochemical structures in nervous system cells, contributing to various brain disorders. While antioxidants present potential therapeutic benefits, their oral administration has shown limited effectiveness due to poor stability and restricted blood-brain barrier (BBB) penetration. [1] This study aims to develop an innovative delivery system using core-shell bottlebrush (BB) polymers to encapsulate antioxidant molecules, hypothesizing that this strategy will enhance BBB penetration while maintaining therapeutic activity.

Methods. A library of core-shell BB polymer has been synthesized (Figure 1) according to QT Phan *et al.* [2]. The BB polymers were selected based on their rapid diffusion in the extracellular matrix, reduced cellular uptake in surrounding tissues, and extended blood circulation time. The study comprises four sequential phases: (1) Optimization of payload encapsulation using solvent exchange methods, along with in vitro drug release profiling via HPLC and colloidal stability assessment by dynamic light scattering (DLS); (2) In vitro evaluation of antioxidant properties through measurement of intracellular ROS levels after treatments with formulations and free drug; (3) Assessment of BBB penetration capability using fluorescence-based tracking after injections in zebrafish larvae; and (4) In vivo biodistribution and efficacy studies using zebrafish larvae and a Parkinson's disease mouse model.

Results. Preliminary analyses suggest that BB polymers provide a promising platform for antioxidant delivery due to their unique architectural properties and ability to form stable complexes through hydrophobic interactions. The systematic approach to formulation development and characterization is expected to yield stable compositions with optimal loading efficiency and preserved antioxidant activity.

Conclusion. This innovative approach to antioxidant delivery using BB polymers presents a potential breakthrough in treating brain disorders associated with oxidative stress. The comprehensive evaluation pipeline, from formulation to in vivo testing, will provide crucial insights into the effectiveness of this delivery system for CNS therapeutic applications.

Core-Shell Bottlebrush Polymers: Superior Nanocarriers for Enhanced Drug Loading and Tissue Penetration

Thang Phan Quoc[1], Jean-michel Rabanel[2], Dikran Mekhjian[1], Justine Saber[1], Araceli Garcia Ac[1], Hu Zhang[1], Victor Passos Gibson[3], Charlotte Zaouter[4], Pierre Hardy[3], Shunmoogum Aroonassala Patten[4], Daria Boffito[5], Xavier Banquy[1]

[1]Faculty of Pharmacy Université de Montréal 2940 Chemin de Polytechnique, Montréal, Québec H3T 1J4, Canada, [2]School of Pharmaceutical Sciences Faculty of Medicine University of Ottawa Roger-Guindon Hall, 451Smyth Rd, Ottawa, Ontario K1H 8M5, Canada, [3]Department of Pharmacology and Physiology Université de Montréal Montréal, Québec H3T 1J4, Canada, [4]INRS Centre Armand-Frappier Santé Biotechnologie 531, boul. des Prairies, Québec, Canada, H7V 1B7, [5]Department of Chemical Engineering Polytechnique Montréal 2500 Chemin de Polytechnique Montréal, Québec H3C 3A7, Canada

Purpose

The physiochemical organization of a drug delivery nanovector is a critical determinant of the efficiency of drug delivery in the body. Recently, bottlebrush (BB) polymers have garnered significant attention due to their distinctive characteristics pertaining to tissue diffusion and cell uptake modulation. The present study aims to elucidate the influence of structural properties on biological behavior by introducing variations in backbone length, grafting density, and self-assembly morphology in BB polymers.

Methods

In this study, a drug delivery system was developed based on core-shell BB polymers synthesized using a "grafting-from" strategy. A suite of characterization techniques, including nuclear magnetic resonance (NMR), gel permeation chromatography (GPC), and atomic force microscopy (AFM), were employed to verify the structural integrity of the polymers. The BB polymers were evaluated as carriers for molecules with differing hydrophobicity profiles, namely Rhodamine B and Paclitaxel. We systematically assessed these nanocarriers for drug loading efficiency and penetration capabilities, and compared them to polymeric micelles (PM) formed from linear amphiphilic polymers in their ability to interact with cells, tumoral spheroids, and to distribute in zebrafish larvae.

Results

BB-based nanocarriers demonstrated superior cellular uptake in two-dimensional (2D) and three-dimensional (3D) cell culture models

when compared to PM. Furthermore, analysis of drug distribution and particle penetration highlighted the profound influence of polymer morphology on biological interactions.

Conclusion

A library of cylindrical brush core-shell BB polymers and spherical micelles were used as nanocarriers for RhB and PTX. These unimolecular brushes showed higher molecule loading capacity than conventional micelles. The worm-like BB polymer improved cellular uptake and penetration. These findings underscore the potential of unimolecular carriers with precisely defined structures as promising drug delivery platforms for a wide range of biomedical applications.

Development of an in silico model using in vitro data to evaluate the feasibility of aspirin-loaded polymeric microneedles for HIV prevention

Alistair K.C. Chan, Emmanuel A. Ho

School of Pharmacy, University of Waterloo, ON, Canada

Purpose: HIV remains a major healthcare challenge particularly in developing countries because current preventative methods face accessibility and stigma issues. We developed a dissolving poly(lactic-co-glycolic) acid (PLGA) microneedle (MN) patch for sustained delivery of acetylsalicylic acid (ASA) as a novel, painless, and gender-neutral means of HIV prevention[1]. Since activated T-cells (primary HIV target cells) are 1000x more susceptible to HIV infection compared to their quiescent, resting counterparts (referred to as immune quiescence; IQ), this strategy relies on ASA to induce IQ in these target cells[2]. We postulate that our MN patch can penetrate the epidermis and release therapeutically relevant amounts of ASA to the dermis for at least 7 days.

Methodology: ASA and PLGA were casted into MN moulds and dried at room temperature. Patches were applied to dermatomed pig skin to assess epidermal penetration. To determine release kinetics, aliquots of ASA-loaded MNs incubated in PBS under physiological conditions were collected at various timepoints and analysed using UPLC. To ascertain encapsulation efficiency, ASA from MN patches that had been dissolved in acetonitrile were enriched and extracted for UPLC analysis. A Python-based differential equation model was developed to evaluate *in silico* whether patches could deliver therapeutically relevant ASA amounts.

Results: PLGA MNs fully penetrated 350 µm thick porcine skin that was confirmed with X-CT imaging. ASA encapsulation efficiency was 79% ±1.76% with an average release rate of 16.5529 ±4.11 µg/hour. Release data combined with *in silico* simulation indicated potential ASA delivery to the bloodstream at peak concentrations of up to 24 times higher than conventional delivery methods. However, *in vivo* studies with mice are required to confirm these findings.

Conclusion: This proof-of-concept demonstrates the feasibility and effectiveness of PLGA MN patches for transdermal ASA delivery to the dermal layer as a potential strategy for HIV prevention.

Development, Optimization and Drug Release Evaluation of a Liposome-based Long-acting Injectable Hydrogel Formulation

Tao Wang, Yongqiang Li

Candoo Pharmatech Company Inc., Mississauga, Ontario, Canada

PURPOSE To develop, optimize and evaluate a liposome-based, long-acting injectable hydrogel formulation containing 0.4% Valbenazine Tosylate as a model drug.

METHODS The hydrogel formulations were developed using natural and/or synthetic phospholipids by thin-film hydration method. Valbenazine Tosylate was selected as a model drug. The effect of different phospholipids and Cholesterol on liposomal particle size distribution and drug release kinetics was studied. The lipid thin film was hydrated with 0.1M citrate buffer pH4.0 or fresh MilliQ water pH7.0 to evaluate the impact of hydration solution pH on drug release. PLM was used to evaluate the morphology and particle size distribution of liposome. 0.5g hydrogel was placed into a dialysis tube with a MWCO 20K Da which was then immersed in 50mL phosphate buffer pH2.3 at 37 ºC and under stirring at 600 rpm. Drug release was monitored over 7 days.

RESULTS Three different liposome-based hydrogel formulations (F1, F2, F3, Table 1) were prepared. Drug release from F1 with both synthetic and natural phospholipids showed more sustained drug release than F2 with only natural phospholipids. The burst release from F1 was reduced to 7.6% at 4 hours, in comparison with 22.8% for F2 (Figure 1). PLM revealed spherical vesicle

morphology for liposome-based hydrogel. The liposomes containing both synthetic and natural phospholipids have significantly smaller particles (≈150 nm) than those composed natural phospholipids alone (< 10µm). Encapsulation efficiency for the three formulations is: F1: 92.36%; F2: 77.25%; and F3: 99.72%. Formulation F3, hydrated with fresh MilliQ water pH7.0, showed much slower drug release profile compared to Formulation F2 hydrated with 0.1M citrate buffer pH4.0. No significant difference in particle size distribution was observed between liposome hydrated with MilliQ water pH7.0 (F3) and those with 0.1M citrate buffer pH4.0 (F2). Formulation F1 was selected as the lead formulation.

CONCLUSIONS A liposome-based, long-acting injectable hydrogel containing 0.4% Valbenazine Tosylate was successfully developed. The combination of phospholipids, addition of cholesterol and hydration method affect the quality attributes and release kinetics of the hydrogel formulation.

Emulsion-based Hydrogels for the Dermal Delivery of Hydrophobic Drugs

Gurpreet K Randhawa, Mandana Tavakolian, Manar Ali, Todd Hoare

McMaster University, Hamilton, Ontario, Canada

Purpose: Hydrophobic drugs account for nearly 50% of currently approved therapeutics as they have the advantage of easily interacting with biological membranes enabling tissue penetration.[1] However, challenges arise in carrying the drugs to the target tissue to ensure effective bioavailability and consistent pharmacokinetic profiles for therapeutic efficacy.[1] In the context of dermal drug delivery, all-trans retinoic acid (commonly known as tretinoin, TRE) is a hydrophobic drug administered topically for acne vulgaris and photoaging based on its ability to increase collagen production, increase cellular turnover, and reduce inflammation.[2] However, current TRE delivery systems are associated with significant side effects such as skin irritation and severe skin dryness.
Methods: Herein, we have developed adhesive and hydrating emulsion-based hydrogel (emulgel) patches to deliver TRE through the skin overnight. TRE was dissolved in a non-comedogenic and highly penetrative castor oil, after which oil-in-water Pickering emulsions were created, stabilized by bifunctional hairy nanocellulose crystals (BNCC) and cross-linked with carboxymethyl chitosan (CMCh) to form emulgels.
Results: Emulgels prepared at 2 wt% and 3 wt%, from CMCh and BNCC precursor solutions, were fabricated using a double barrel syringe and exhibited gelation times between 14 - 56 s. 2 wt% emulgels were delicate under physical manipulation with tensile strength <1 kPa while 3 wt% emulgels had effective tensile strengths of ~3 kPa and were significantly easier to handle. The 3 wt% emulgels released ~50% of encapsulated TRE (0.05 wt% dose) via first order release over 8 hours. After less than 2 hours of application to porcine skin, the skin was visibly hydrated while the untreated skin was dry. Over 8 hours, the emulgels lose only 20% of their water upon drying and remain stable in storage conditions for over 1 month.
Conclusion: By delivering TRE through a hydrated emulgel, risks of irritation and burning associated with conventional methods are minimized to improve patient compliance and overall treatment experience. This system can extend beyond TRE, offering a versatile platform for delivering hydrophobic drugs to meet patient needs.

Encapsulation of Breu Branco Essential Oil via Nanoprecipitation

Debora Freitas Silva[1], Kaidy Orellana[2], Francisco Xavier Junior[3], Emmanuel Ho[2], Marcele Fonseca Passos[1]

[1]Federal University of Para, Brazil, [2]University of Waterloo, Kitchener, ON, Canada [3]Federal University of Paraiba, Brazil

Purpose: The Amazon rainforest is one of the most biodiverse regions on the planet, yet many of its bioactive compounds remain underexplored for high-value biomedical applications. Among these, the essential oil of *Protium heptaphyllum* (Breu Branco) is a powerful natural resource, traditionally used for its potent antimicrobial, anti-inflammatory, and antioxidant properties. Despite its immense therapeutic potential, its direct application remains limited due to high volatility, poor solubility, and rapid degradation. To unlock the full potential of this Amazonian treasure and bridge the gap between traditional knowledge and advanced biomedical technologies, this study proposes a nanotechnological approach to enhance stability and encapsulation. This research aimed to obtain polymeric nanocapsules containing *Protium heptaphyllum* essential oil, employing nanoprecipitation (NP).

Methods: Polymeric nanocapsules were prepared using poly-ε-caprolactone (PCL) as the encapsulating matrix. A Box-Behnken experimental design was applied to optimize the NP formulation, evaluating the influence of polymer, oil, and surfactant

concentrations on particle size, polydispersity index (PDI), and zeta potential (ζ). Nanocapsules were characterized by dynamic light scattering (DLS), thermal stability analysis, encapsulation efficiency (EE%), and cell viability assays (MTS).

Results: The NP method yielded nanocapsules with a mean hydrodynamic diameter of 172.07 nm ± 2.9, PDI of 0.14 ± 0.04, and ζ of -25.49 ± 1.08 mV, with EE of 98.9%. Stability assessments indicated that NP nanocapsules maintained their size and PDI with minimal variation over 30 days at 4°C and 37°C, confirming their physical stability under different storage conditions. The MTS assay demonstrated that the nanocapsules achieved >80% cell viability at a 1:8 dilution.

Conclusion: The nanoprecipitation (NP) technique successfully produced stable and biocompatible polymeric nanocapsules for *Protium heptaphyllum* essential oil, demonstrating its potential as an effective delivery system for bioactive compounds. Further studies should focus on antimicrobial activity, and cellular uptake to fully explore the therapeutic potential of these nanocapsules. Additionally, investigating and optimizing formulation parameters could enhance their applicability in biomedical and pharmaceutical fields, promoting the sustainable valorization of Amazonian biodiversity.

Acknowledgment to Project: Bionanovectors: Sustainable Technological and Bioeconomic Strategy. FAPESPA, AWARD TERM No. 106/2024

Experimental design and development of lipid nanocarriers as a bioactive delivery system

Atos Matos[1], Caio Canelas[1], Luís Silva[2], Francisco Xavier-Junior[2], Marcele Passos[1], Debora Silva[1]

[1]Federal University of Pará, Belém, PA, Bazil. [2]Federal University of Paraíba, João Pessoa, PB, Brazil

Purpose: Nanocarriers, particles smaller than 1 μm, are effective in controlling bioactive release systems across various fields, including medicine and cosmetics [1]. However, producing these particles is complex, as their physicochemical properties are influenced by factors like reagent concentration, preparation method, and material type. Hence, statistical experimental designs are essential to achieve consistent and reproducible results [2]. This study aimed to develop lipid nanocarriers using a Box-Behnken experimental design (BBED) strategy to optimize nanocarrier production.

Methods: A BBED was employed with three factors with 3 levels: beeswax (1, 5.5 and 10% w/w), Tween 80 (1, 2 and 3% w/w), and copaiba (CO) or lemongrass (LGO) essential oils (1, 4 and 7% w/w). Each oil underwent 15 experimental runs. Nanocarriers were produced via hot emulsion followed by sonication (80% power/30 s) and characterized by particle size (PS), polydispersity index (PDI), and zeta potential (ZP). Data was analyzed with response surface graphs using Statistica v12 software.

Results: The formulations demonstrated PS ranging from 114-335 nm for CO and 101-394 nm for LGO; ZP ranging from -37.83 to -28.48 mV for CO and -30.94 to -19.78 mV for LGO. Smaller particle sizes are generally preferable, but other parameters are equally crucial. Ideally, PDI values near zero indicate uniform particle distribution, while ZP values of at least 30 mV (in modulus) ensure particle stability by reducing aggregation through electrostatic repulsion. Response surface analyses (Figure 1) revealed that particle size significantly depends on wax and oil concentrations. For CO, increased oil content reduced particle size, whereas increased wax led to particle growth due to coalescence. Conversely, for LGO, smaller oil amounts resulted in smaller particles.

Conclusion: BBED was effective in developing lipid nanocarriers, clarifying how each variable affects key characteristics like PS. Despite the method's efficacy, various challenges remain in nanoparticle production. Therefore, preliminary screening studies identifying critical factors are recommended. Future research should include stability assessments, bioactive release studies, and cytotoxicity evaluations.

Acknowledgments: The authors thank the Amazon Foundation for the Support of Studies and Research (FAPESPA, award term 106/2024) for funding this research.

Gemini Surfactant-Based Lipid Nanoparticle Delivery of Transcription Factors for Astrocyte-to-Neuron Conversion

Archer Nelson[1, 2, 3], Dr. Ildiko Badea[1, 2, 3], Jonathan Rekve[1, 2, 3]

[1]University of Saskatchewan, Saskatoon, [2]College of Pharmacy and Nutrition, [3]Pharmaceutical and Nutritional Sciences Research Group, Saskatoon, Canada

Purpose: Neuronal loss due to neurodegenerative diseases (e.g., Alzheimer's disease) or ischemic injury (e.g., stroke) remains irreversible, as mature neurons lack regenerative capacity. Astrocytes outnumber neurons by approximately 3:1 in the brain, presenting a promising target for neuron regeneration therapies. The controlled conversion of astrocytes into induced neurons offer a favorable trade-off, where the functional gain of induced neurons outweigh the loss of astrocytes, which mainly act as glial support cells. This study explores the use of gemini surfactant-based lipid nanoparticles (LNPs) for the targeted delivery of transcription factors to induce astrocyte-to-neuron conversion.

Methods: As part of this multi-center, interdisciplinary collaboration, we aim to explore the delivery of transcription factors produced off-site. Gemini surfactants are a class of amphiphilic molecules with configurable properties allowing for targeted delivery to astrocytes. Compared to conventional LNP formulations, gemini surfactants offer reduced cytotoxicity, improved transfection efficiency, enhanced nucleic acid payload protection, and enhanced nanoparticle stability. To evaluate their effectiveness, immortalized astrocytes will be cultured and transfected with gemini surfactant-LNP formulations containing fluorescently tagged nucleic acids. Transfection efficiency, cell viability, and neuronal marker expression will be assessed.

Results / Conclusion: Preliminary results indicate successful nucleic acid uptake, supporting the feasibility of this approach. This study contributes to the advancement of neurodegenerative medicine, with potential applications in treating neurodegenerative diseases, stroke recovery, and brain injury repair.

Archer Nelson is the recipient of the 2025 CSPS National Undergraduate Student Research Program Award for the University of Saskatchewan.

Hydrogel containing liposomal enzyme microreactors for molecular diagnostics in diabetic foot ulcers

Gabriela Manrique[1], Davide Brambilla[1], Simon Matoori[1]

[1]Université de Montréal, Montréal, QC, Canada

Purpose: Diabetic foot ulcers (DFU) are a serious diabetes complication linked to high rates of amputation and mortality. Current DFU diagnostics rely on visual assessments such as wound depth and signs of infection. However, this visual assessment does not consider the complexity of the ulcer and is rather subjective [1]. Overexpression of hydrogen peroxide (H_2O_2) contributes to chronic inflammation and the non-healing state of DFUs[2].

Therefore, we are developing a hydrogel with liposomal microreactors to monitor H_2O_2 levels in wounds. Upon application, wound fluids containing H_2O_2 diffuse into the hydrogel, where they interact with the liposomal microreactors. This interaction triggers an enzyme-mediated fluorescence decrease, which is proportional to H_2O_2 levels in diabetic ulcers (Fig 1A).

Methods: The methodology involves the development of liposomal microreactors via the thin-film rehydration method. Liposomes are loaded with horseradish peroxidase (HRP) and fluorescent dye sulfo-cyanine 7 (S7). The reaction kinetics of H_2O_2, HRP, and S7 were studied. Additionally, HRP loading in liposomes for H_2O_2 sensing was optimized. Finally, liposomes were encapsulated in an alginate-based hydrogel and used for H_2O_2 quantification.

Results: In liposomal assays, the HRP-mediated fluorescence decrease of S7 occurs within 40 seconds in an H_2O_2-dependent manner, showing a 60% reduction at the highest concentration after 20 minutes. The quantity of HRP loaded in the liposomes was optimized for detecting H_2O_2 within a clinically relevant range of 1 to 10 µM (Fig 1B). Additionally, alginate hydrogels have been developed and are being evaluated for their H_2O_2 sensing capability.

Conclusion: DFUs remain challenging due to the lack of objective diagnostics. The proposed hydrogel system enables sensitive *in situ* H_2O_2 detection within clinically relevant ranges. The next steps include optimization and characterizing hydrogels, testing in biologically relevant media, and *in vivo* tests in diabetic wound mouse models. Initial results are promising, with further optimization and validation in animal models required for clinical translation.

Immunomodulatory yeast beta-glucan microparticles prepared by Pressurized Gas eXpanded liquid technology (PGX-TEC) as an inhalable therapeutic for idiopathic pulmonary fibrosis

Nate Dowdall[1], Safaa Naiel[2], Emily Y. Wong[3], Ricardo Couto[3], Byron Yépez[3], Bernhard Seifried[3], Paul Moquin[3], Myrna Dolovich[4], Martin R. Kolb[5], Kjetil Ask[2], Todd Hoare[1]

[1]Department of Chemical Engineering, McMaster University, Hamilton, ON, [2]Department of Medicine, McMaster University, Hamilton, ON, [3]Ceapro, Inc., Edmonton, AB, [4]Firestone Aerosol Laboratory, St Joseph's Healthcare, Hamilton, ON, [5]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON

Purpose. Idiopathic pulmonary fibrosis (IPF) is a deadly lung disease characterized by excessive extracellular matrix (ECM) deposition in the alveolar regions which alters lung architecture and impedes gas exchange. Pro-fibrotic macrophages are implicated in IPF progression via cytokine production that promotes fibroblast differentiation, proliferation, and ultimately ECM deposition. While current IPF drugs can slow the rate of disease progression, prognosis remains poor, with a median survival rate of 3-5 years. Yeast beta-glucan (YBG) microparticles are known to target and modulate macrophages, and their size and density offer potential for direct inhalation to the diseased tissues. However, conventional YBG spray-drying processes are limited by their achievable sizes, purities, and densities, thus limiting YBG's translation into an immunomodulatory therapeutic.

Methods. YBG microparticles were processed via PGX^{TEC} or spray-drying (SD) and characterized for their size, morphology, density, and porosity. Modulation of macrophage markers was assessed *in vitro* and *ex vivo* using THP-1 macrophages and murine lung slices, respectively. PGX^{TEC}-YBG and SD-YBG were blended with inhalation grade lactose, loaded into a dry powder inhaler, and characterized for aerodynamic size via cascade impaction.

Results. PGX^{TEC}-YBG exhibited optimal size (5.7 µm vs. 10.7 µm for SD-YBG), specific surface area (135 m^2/g vs. <5 m^2/g for SD-YBG), and density (0.056 g/mL vs. 0.195 g/mL for SD-YBG) for macrophage uptake and aerosolization. PGX^{TEC}-YBG was preferentially phagocytosed by pro-fibrotic macrophages and prevented pro-fibrotic macrophage polarization while promoting anti-fibrotic phenotypes *in vitro* and in *ex vivo* lung slices. While both PGX^{TEC}-YBG and SD-YBG formed aerosolizable blends with lactose, PGX^{TEC}-YBG demonstrated superior aerosol properties, exhibiting a mass median aerodynamic diameter of 3.4±0.3 µm and a fine particle fraction of 57±3% (approximately double that achievable with SD-YBG).

Conclusion. PGX^{TEC}-YBG offers the potential to be inhaled directly to the disease site and specifically target and modulate pro-fibrotic macrophages toward an anti-fibrotic state.

Impact of Mucosal Thickness Variability on Ex Vivo Nasal Drug Permeability: A Simulation-Based Correction Approach

Shengnan Zhao[1, 2], Yuanheng Zhao[2, 3], Jieyu Zuo[1, 2], Neal M. Davies[1, 2], Raimar Loebenberg[1, 2]

[1]Pharmacy and Pharmaceutical Science, [2]University of Alberta, Edmonton, Alberta, Canada, [3]Department of Laboratory Medicine and Pathology

Purpose: *Ex vivo* nasal mucosa is commonly used to study drug permeability for nasal drug delivery. However, inherent variations in porcine nasal mucosal thickness (0.26 to 1.47 mm) introduce variability in Franz diffusion cell experiments, complicating data interpretation. This study aimed to develop a numerical simulation method based on the classical diffusion equation to normalize permeation curves to a standardized mucosal thickness, thereby improving data reliability.

Methods: Franz diffusion cell experiments were conducted using porcine nasal mucosa to assess drug permeation under varying mucosal thickness conditions. A computational model was developed to account for mucosal thickness variations and to correct permeability data accordingly. Using this approach, the permeability of three compounds with distinct solubility and lipophilicity profiles—melatonin, triamcinolone acetonide, and mitragynine—was evaluated. Apparent permeability coefficients (Papp) were determined before and after thickness normalization to assess the impact of the correction method.

Results: Mucosal thickness variations significantly impacted drug permeability, masking statistical differences in Papp values for mitragynine, melatonin, and triamcinolone acetonide. Before normalization, permeability differences were not statistically significant due to thickness variability. After normalization, statistically significant differences emerged. The simulation-based method effectively minimized thickness-related variability, improving the sensitivity in distinguishing permeability differences and enhancing the accuracy of comparative permeability assessments.

Conclusion: These findings emphasize the importance of mucosal thickness correction in ensuring accurate and comparable permeability data in ex vivo nasal drug delivery studies. This method enhances data consistency across different experimental setups and drug candidates, contributing to the development of reliable nasal drug delivery systems. Furthermore, this approach can be extended to permeability studies involving other species or tissue types, broadening its application in drug delivery research.

jpps Journal of Pharmacy and Pharmaceutical Sciences

Lipid Nanoparticle-Driven Precision Targeting of Hematopoietic Stem Cells for Gene Therapy

Rita Maria Kenaan El Rahbani[1], Blandine Monjarret[1, 2], Sabrina Schlienger[2], Romain Gioia[2], Fabien Touzot[1, 2], Simon-pierre Gravel[1], Davide Brambilla[1]

[1]Université de Montréal, [2]CHU Sainte-Justine

Purpose: The hematopoietic niche is a microenvironment that regulates hematopoietic stem cells (HSCs), which are crucial for blood homeostasis and tissue regeneration. Defects in HSCs, often genetic, can cause various hematological diseases[1]. Familial hemophagocytic lymphohistiocytosis (fHLH) is a rare genetic disorder causing uncontrolled immune activation, leading to severe symptoms (persistent fever, organ enlargement, neurological issues) in children under 2 years old, often fatal within months. 30-40% of patients with fHLH carry a missense mutation in the *PRF1* gene, resulting in deficient perforin expression—a cytolytic protein critical for immune function. Current treatment—allogeneic HSC transplantation—faces limitations like donor scarcity, toxicity, and high costs, resulting in a 33% mortality rate despite therapy[2]. Designing lipid nanoparticles (NPLs) for precise targeting and gene editing of defective HSCs could enhance treatment efficacy and reduce side effects, leading to safer, more effective therapies.

This study aims to design NPLs encapsulating a prime editor mRNA (PE) engineered to correct the mutation in *PRF1* and restore normal perforin function. These NPLs are functionalized with antibodies to target HSCs in vivo after intravenous injection, bypassing the need for HSC harvesting or mobilization.

Methodology: Antibody-functionalized PEGylated NPLs are synthesized using ionizable lipids and anti-CD117 conjugation. PE mRNA is encapsulated via microfluidic mixing, optimizing size, charge, and payload. In vitro assays will assess mRNA delivery, PE translation, and *PRF1* correction in HSC models. Future in vivo studies in a murine fHLH model will evaluate systemic delivery efficiency, perforin restoration, and disease phenotype rescue.

Results: Preliminary data demonstrate successful NPL formulation (size: ~100 nm, charge: -1 mV) with >90% mRNA encapsulation. PE mRNA delivery to HEK293 cells confirmed protein expression. Ongoing work focuses on NPL functionalization, optimizing transfection efficiency, and correction of the *PRF1* mutation to restore perforin expression and activity in an HSC model.

Conclusion: This study establishes antibody-targeted NPLs for in vivo HSC gene editing, offering a precise, non-invasive therapeutic strategy for fHLH. By restoring perforin via *PRF1* correction, this approach addresses current treatment limitations and holds promise for broader hematological applications.

Metformin Conjugation for Improved Dual Drug Therapeutics

Esha Gahunia[1], Saras Tiwari[1], Emma Durocher[1], Michelle Gandelman[1], Morgan Fullerton[1], Suresh Gadde[1]

[1]University of Ottawa, Ottawa, ON

Purpose: Dysregulation of various biological pathways has been linked to many diseases. Thus, combination therapeutics have become an increasingly popular form of treatment for these complex diseases, including cancer and cardiovascular diseases (CVDs). Notably, CVDs, caused primarily by atherosclerosis, are the leading cause of mortality and morbidity worldwide[1]. Thus, for synergistic therapies, nanotherapeutics are an ideal drug delivery platform, as they have high bioavailability, minimize off-target effects and are generally non-toxic. Furthermore, gold-based nanomaterials are highly versatile, in that they are highly stable, can be easily functionalized and have tunable electrochemical properties[2].

Methods: To this end, we developed three nanoparticle (NP) systems, termed nanoassemblies (NAs), with intrinsic therapeutic capacities. Elaborating, a gold NP core was capped with citric acid, which allowed for its interaction with a carbon conjugated metformin derivative (C_6-metformin, C_{16}-metformin or C_{18}-metformin) through hydrogen bonding. To establish the bioefficacy of our NAs for atherosclerosis, model drug GW 3965 (GW) was loaded in the platforms.

Results: Metformin derivatives were synthesized and characterized via ^{1}H NMR. NAs were developed using self-assembly methods and coated with helper lipids. Particle size/surface charge and morphology was established using the Malvern Zetasizer and Transmission Electron Microscopy (TEM) respectively. By increasing the length of the carbon chain, we were able to increase NA size. Additionally, C_6-NAs had a negative zeta potential while both C_{16} and C_{18}-NAs had a positive zeta potential. Stability and validation of the NAs structure was supported through stability studies at physiological conditions and absorbance binding plots.

In vitro studies involved establishing the bioactivity of metformin conjugates via western blotting. Furthermore, RT-qPCR studies confirmed synergistic upregulation of the anti-atherogenic receptor, ABCA1, following its treatment with the dual drug NAs. Lastly,

through the lactate dehydrogenase (LDH) assay we demonstrated that the NAs were non-toxic in relevant cardiovascular and cancer cell lines.

Conclusion: The synthesized NAs can be readily modulated by varying the length of the metformin derivative. The NAs may also be applicable in various diseased conditions, including cancer and cardiovascular diseases, where metformin is used therapeutically.

Methods to study the intracellular fate of polymer nanoparticles

Vanessa Maio[1], Nicolas Bertrand[1]

[1]Laval University, Quebec, QC

Purpose. Developing novel drug delivery technologies requires understanding the toxicology of their excipients. The present project aims to study what happens to the components used to prepare nanoparticles once they are internalized by cells. **Methods.** In this preliminary study, we established a flow cytometry method to study the fate of fluorescent excipients after internalization by replicating cells. In parallel, we also used size exclusion of radioactive polymers (SERP) to monitor the degradation of polymer excipients inside the cells.[1] Polymer nanoparticles were prepared by nanoprecipitation, using fluorescent or radioactive labels. Cell internalization was conducted *in vitro* for 2 h, using RAW 264.7 leukemic macrophages. For flow cytometry experiments, cells were 1) incubated with nanoparticles and a fluorescent control while in suspensions[2], 2) seeded in 24-well plates, and 3) cultured for 24, 48, or 72 h. At each time point, the intensity of the signal was evaluated by flow cytometry, using standardized parameters allowing comparisons between experiments. For SERP analyses, cells were treated with radioactive nanoparticles, the media was replaced, and cells were left to proliferate for 24, 48, or 72 h. The amount of radioactivity in the cells and the external media was measured by scintillation. **Results.** Adjusting the concentration of nanoparticles, we can obtain conditions where more than 98% of cells internalize nanoparticles within 2 h. In comparison with a control dye, the nanoparticle fluorescence decreases more rapidly than solely due to cell proliferation. This suggests that the dye is degraded in the endosome/lysosome. Using a radioactive polymer, it was possible to quantify the extent of polymer degradation over the first 72 h that followed internalization. **Conclusion.** The combination of flow cytometry and SERP informs on the intracellular degradation of polymer nanoparticles. In the future, these methods will be combined to explore the intracellular fate of nanoparticles of different compositions.

Microbubble-Assisted Drug Delivery for Glioblastoma

Isabella Zittlau[1], Steven Machaler[1], Ildiko Badea[1]

[1]University of Saskatchewan, Saskatoon, SK

Purpose. Glioblastoma is the most common and aggressive brain tumor in adults. One significant challenge for therapy is the blood-brain barrier (BBB), a significant obstacle that maintains the integrity of the brain. Microbubbles (MBs) are colloidal particles that consist of a gas core surrounded by a protective shell, ranging from 1 to 10µm in diameter. They have been used as a contrast agent in medical imaging and, when combined with focused ultrasound, MBs cross physiological barriers, allowing the delivery of drugs to a specific area. Gemini lipids (GL) are a new class of surfactants, presenting unique structures and properties compared to conventional surfactants. GLs are versatile drug and gene delivery systems that, if incorporated into MBs, could offer advantages in the formulation. This project aims to synthesize MBs combined with GL, to increase the delivery of model chemotherapeutic agent temozolomide aiding crossing the BBB into glioblastoma and maintaining low toxicity.

Method. MBs were produced using the mechanical agitation method and formulated with various GLs and molar ratios. Size, bubble concentration, and surface properties were assessed.

Results. The results revealed that the presence of GL in the formulation favored the formation of MBs, increasing not only the number of bubbles per mL but also the zeta potential. In addition, a reduction in size from 1.2 to 0.9 µm was observed. Among the GLs GL 16, yielded formulations with a highest concentration of bubbles, as well as an increase in the positive zeta potential of the formulations tested.

Conclusion. MBs with optimal physicochemical properties were formulated. Since MBs enable the release of the drug through the BBB more easily, a lower drug concentration is necessary to be injected, thus minimizing drug adverse effects and circumventing drug resistance caused by high doses of drugs.

Modified Starch Nanoparticles for improved Anti-Bacterial Pickering Emulsions

Cameron Macdonald[1], Asha Bouwmeester[1], Ben Kertesz[1], Andrew Lofts[1], Todd Hoare[1]

[1]McMaster University, Hamilton, ON, Canada

Purpose. Emulsions have a variety of uses in biomedical, food, cosmetics, and agriculture. However, traditional surfactants used to create stable emulsions can be a detrimental cause of inflammation in biomedical applications and environmental toxicity in agrochemical applications. Pickering emulsions are nanoparticle-stabilized emulsions that avoid the use of traditional surfactants and can result in higher stability emulsions, providing both higher efficacy and lower downstream consequences. Herein, we explore the use of starch nanoparticles (SNPs) to create these emulsions, leveraging the low toxicity, tuneable surface chemistry, sustainable manufacturing, and opportunity for multi-kinetic controlled release enabled by SNPs as the stabilizer. Specifically, multi-functionalized SNPs are applied to create antibacterial Pickering emulsions that offer the benefits of improved release kinetics and decreased side effects over traditional drug delivery vehicles for biomedical applications and decreased environmental impact of pesticides and fertilizers in agrochemical applications.

Methods. Starch nanoparticles were multi-functionalized with a combination of hydrophobic chains and hydrophilic antibacterial groups. These antibacterial and amphiphilic nanoparticles were then used to generate an antibacterial Pickering emulsion with an oil phase loaded with an antibacterial bioactive. The two-stage emulsion was then tested against a suite of biomedically and agriculturally relevant bacteria, including *Escherichia coli, Pseudomonas aeruginosa,* and *Pseudomonas syringae*.

Results. The multi-functionalized starch nanoparticles could generate stable emulsions that remained re-dispersible over 50 days, improving emulsion stability over a model commercially available surfactant. These emulsions were able to inhibit bacterial growth, and the multi-functional starch nanoparticles were able to work synergistically with the bioactive to greatly improve the antibacterial potential of the drug delivery system.

Conclusion. This work demonstrates the capability of starch nanoparticles to generate stable anti-bacterial emulsions. This provides the foundation of creating highly effective and low toxicity drug delivery vehicles for multiple potential applications.

Nano-delivery of B9, a novel ERCC1/XPF inhibitor for sensitization of cancer cells to cisplatin

Ahmed Abdelfattah[1, 2], Parnian Mehinrad[1], Sameen Sadaf[1], Tulell Kerr-Benoit[3], Ahmed S. Al-Behery[4, 5], Ahmed H. Elmenoufy[4, 6], Michael Weinfeld[7, 8], Frederick G West[4, 8], Afsaneh Lavasanifar[1, 9]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada., [2]Department of Industrial Pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt., [3]Department of Nanotechnology Engineering, University of Waterloo, Waterloo, ON, Canada, [4]Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, Canada., [5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Tanta University, Tanta 31527, Egypt, [6]Department of Pharmaceutical Chemistry, College of Pharmacy, Misr University for Science and Technology, P.O. Box 77, 6th of October City 12568, Egypt, [7]Department of Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada., [8]Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada., [9]Department of Chemical Engineering, University of Alberta, Edmonton, AB, Canada.

Purpose. DNA repair pathways contribute significantly to cancer treatment resistance. ERCC1-XPF is a DNA repair enzyme, the overexpression of which correlates with poor treatment outcomes in many tumors (1). Small molecule inhibitors of ERCC1/XPF dimerization developed by our research team, are shown to increase the susceptibility of cancer cells to DNA damage. B9 is a novel inhibitor of ERCC1/XPF that sensitizes colorectal cancer (CRC) and lung cancer cells to cisplatin and mitomycin C (2). This study aimed to investigate the synergistic activity of free versus lipid and polymer-based nanocarriers of B9 in combination with cisplatin across multiple cancer cell lines.

Methods. Polymeric micelles (PM) made from different poly (ethylene oxide)-poly(ester)s, or liposomes composed of DSPC, DSPE-PEG2000 and cholesterol were developed to encapsulate B9. The formulations were subsequently characterized for average diameter, polydispersity index (PDI), encapsulation efficiency (EE%), and *in vitro* drug release. The effect of combining B9 as free or its nanoformulations on the cytotoxicity of cisplatin was evaluated in head & neck cancer (HNC) FaDu and non-small cell lung cancer (NSCLC) A549, and H1299 cells.

Results. Liposomal B9 exhibited a uniform average diameter of 73.6± 0.4 nm and an EE% of 89%, whereas B9 PM exhibited an average diameter range of 47-76 nm and an EE% between 80-90%. Within 6 hours, about 60 and 80 % of the encapsulated B9 was released from the liposomal and PM formulations, respectively. The IC_{50} of cisplatin was significantly reduced from 7.9 to 2.7 µM in FaDu, 3.5 to 0.6 µM in A549 and 28.4 to 20 µM in H1299 cells when combined with 0.5 and 1 µM of B9.

Conclusions. The preliminary results indicate a potential for B9 and its nanocarrier formulations, particularly B9 liposomal formulations, as viable sensitizers of HNC and NSCLC cells to cisplatin.

Novel ROS-activated nanoparticle system for ROS reduction and anti-inflammatory purposes

Amandine Courtemanche[1, 2, 3], Suresh Gadde[1, 2, 3]

[1]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Canada, [2]Center for Infection, Immunity and Inflammation, Faculty of Medicine, University of Ottawa, Ottawa, Canada, [3]Ottawa Institute of Systems Biology, Faculty of Medicine, University of Ottawa, Ottawa, Canada

Purpose. Inflammation is a normal process mediated by signaling molecules, including reactive oxygen species (ROS). In addition to their signaling roles, ROS' high reactivity may lead to oxidative stress and inflammation via the damage of macromolecules when ROS levels are abnormally elevated. Overtime, this redox imbalance may lead to tissue damage and chronic inflammatory diseases (CIDs). Thus, developing novel ROS-activated and ROS-reducing therapies could result in beneficial anti-inflammatory effects in CID cases.

Methods. This study focuses on the development of a novel self-assembling, ROS sensitive and ROS scavenging nanoparticle (NP) system. Chemical synthesis of boronic acid polymers enabled the formation of boronic ester-based NP systems with 3 ROS scavenging polyphenols of interest. Additionally, chemistry methods enabled the synthesis of a mitochondria targeting surface molecule in an attempt for increased ROS reduction. In high ROS conditions, the boronic ester bonds disassemble, releasing the ROS scavenger to stabilize ROS and reduce its damaging and signaling potential. These optimized systems will be assessed for ROS and inflammation reduction in inflammatory conditions.

Results. Biomaterials were synthesized via a ring opening reaction to conjugate boronic acid groups or an esterification reaction to conjugate a mitochondria targeting molecule to a PLGA-based surface molecule. Fluorescence and ^{1}H NMR confirmed formation of boronic ester bonds between the boronic acid polymers and ROS scavengers. Polymeric NP formulations were optimized, presenting a size of 60-200nm and a suitable polydispersity index. NPs showed ROS sensitivity properties via size variation following H_2O_2 incubation and ROS scavenging properties via the reduction of H_2O_2 levels measured via a luminescence assay. In vitro assays using RAW264.7 cells showed high NP cellular uptake and no cytotoxicity. A DCFH-DA assay confirmed ROS scavenging in vitro and RT-qPCR showed promising anti-inflammatory effects.

Conclusion. We have developed a ROS-dependent system capable of in vitro ROS scavenging and showing promising anti-inflammatory effects. As the system retains its drug encapsulation properties, it may be adapted for use in various ROS-dependent processes and disease conditions. Thus, this versatile NP system created has potential for the development of new treatments for CIDs.

One Stone, Two Birds: Harnessing Microfluidics to Develop Placental-Targeted Nanoparticles and to Assess their Efficacy in a Placenta-on-a-Chip Model

Luis A Pérez Dávalos[1, 2], Pauline Chavrier[3], Ricardo González Hidalgo[4], Hagar Labouta[1, 2, 5]

[1]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto ON, [2]Keenan Research Centre for Biomedical Science, Unity Health Toronto, [3]Unité des Technologies Chimiques et Biologiques pour la Santé, Université Paris Cité, Paris, FR, [4]Universidad de las Américas, Puebla MX, [5]Institute of Biomedical Engineering, University of Toronto, Toronto ON

PURPOSE Medication management during pregnancy is challenging as many drugs can have adverse effects on the mother and the fetus. This limitation results in poor outcomes for people affected by pregnancy-associated diseases like pre-eclampsia (PE). PE is a leading cause of maternal and perinatal morbimortality worldwide, and tragically, there is no treatment for it besides pre-term delivery

of the baby[1]. Lipid nanoparticles (LNPs) are drug delivery vehicles that can transport therapeutic cargoes such as siRNA to specific sites in the body where they are needed, minimizing the risk of fetal exposure and off-target adverse effects[2].

METHODS Using a microfluidic method (hydrodynamic focusing), we prepared a library of LNPs to deliver fluorescently tagged siRNA to placental cells. In addition, LNPs were functionalized with different peptides to increase their affinity to placental cells. Transfection efficiency was assessed by loading siRNA to downregulate TBP, a housekeeping gene. To obtain a relevant placenta-on-a-chip (PLOC) model of PE, placental cells (BeWo b30) were cultured in single-channel microfluidic chips under dynamic flow to expose the cells to shear stress (~0.01 dyn/cm^2) and to either chemical or physical hypoxia. After the cells were conditioned, siRNA-LNPs were administered to assess their transfection efficiency.

RESULTS The LNPs prepared via hydrodynamic focusing showed good monodispersity, stability, biocompatibility, and encapsulation efficiency. Coating LNPs with P4L2 peptide improved significantly their uptake by placental cells. The PLOC model reproduced biomarkers of PE such as decreased human chorionic gonadotropin (β-HCG), decreased fusion of placental cells, and increased concentrations of anti-angiogenic factors like sENG. Compared to untreated chips, the concentration of TBP decreased with the administration of siRNA-LNPs, without impacting cell viability.

CONCLUSION Microfluidics enabled the development of LNPs that can deliver nucleic acid cargoes to placental cells, and to generate a placenta-on-a-chip model of PE. Further experiments will explore the delivery of different siRNAs to downregulate proteins involved in the pathogenesis of PE. The effect of such interventions will be evaluated in the placenta-on-a-chip model of PE to identify potential therapeutic targets that can yield better treatments and clinical outcomes for patients with PE.

Optimization of size exclusion chromatography to study the fate of polymers

Anais Deleglise[1, 2], Nicolas Gaudreault[1, 2], Nicolas Bertrand[1, 2]

[1]Endocrinologie et Néphrologie, Centre de Recherche du CHU de Québec - Université Laval, Québec, QC, [2]Faculté de Pharmacie, Université Laval, Québec, QC

PURPOSE: In the past, Pr. Bertrand's laboratory proposed an analytical method to study the biological fate of polymers: the SERP method (for *Size Exclusion Chromatography of Radiolabeled Polymers*). This method allows evaluating the degradation of polymers by combining two well-known techniques: the size exclusion chromatography (SEC) and the radiolabeling. Our hypothesis is that the SERP method can be adapted to: 1) confirm the integration of amphiphilic lipid-polymers to liposomes, 2) monitor the polymerization process, or 3) monitor interactions between lipid-polymers and plasma proteins. In this preliminary work, we optimized the SEC column to expand the capabilities of our SEC separation.

METHOD: In this study, we used commercial polyethylene glycol (PEG) polymers, PEGylated phospholipids, as well as poly(PEG methacrylate) p(PEGMA) polymers synthesized by Reversible Addition-Fragmentation Transfer (RAFT) polymerization. The void volume of the columns were determined using a radioactive ^{14}C-labelled nanoparticle. Then, polymers of different sizes (4 and 14 kDa) were loaded individually on the column and fractions were collected. Fractions were analyzed with a colorimetric assay to reveal the presence of PEG.

RESULTS: Polymers of different sizes were synthesized and characterized. By comparing SEC gels of distinct composition, we found conditions to retain or exclude the different macromolecules. These SEC separation methods allowed measuring the association of PEG polymers to liposomes in different conditions.

CONCLUSION: Carefully choosing the separation column expands the capabilities of SERP. Notably, SEC columns that retain polymers allow monitoring their association with nanoparticles.

Optimizing Bottlebrush Polymers for mRNA Delivery: Structure-Function Insights

Alessia Filippini[1], Hu Zhang[1], Jean-Michel Rabanel[2], Davide Brambilla[1], Xavier Banquy[1]

[1]Faculty of Pharmacy, Université de Montréal, Montréal, QC, Canada, [2]School of Pharmaceutical Sciences, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

Purpose. Messenger RNA (mRNA) therapeutics have emerged as transformative tools for applications in vaccines, protein replacement therapies, and cancer immunotherapy. Successful delivery relies on specialized formulations that shield mRNA from

degradation and improve its cellular uptake. Over the last decade, cationic polymers have gained attention for their tunable architecture and ability to form nanoscale complexes with nucleic acids, called polyplexes. Among these, hyperbranched polymers have been shown to positively influence the physicochemical properties of such complexes, thereby improving their efficiency of genetic material transport. Bottlebrush polymers (BBs), in particular, have emerged as promising candidates for mRNA delivery because of their unique and versatile architecture, which allows for precise control over backbone length, branching density, and charge distribution. This study investigates how structural variations in BBs impact their performance as vectors for mRNA delivery.

Methods. A library of BBs with poly(2-(dimethylaminoethyl)methacrylate) (PDMAEMA) side chains, varying in backbone length and amine's charge state, was synthesized and, subsequently, complexed with Lysosomal Acid Lipase (LAL) mRNA. Polyplex size distribution was characterized using Dynamic Light Scattering, while encapsulation efficiency was assessed quantitatively and qualitatively through RiboGreen assay and gel electrophoresis. The most promising formulations were further evaluated for stability, cytotoxicity, enzymatic activity restoration, and cellular uptake *in vitro*, followed by biodistribution and pharmacokinetics *in vivo*.

Results. Our findings reveal a distinction between quaternary and non-quaternary BBs of identical backbone length. Permanent positive charges resulted in polyplexes with more favorable size distribution for *in vivo* delivery, higher mRNA encapsulation efficiency at lower mass ratios, and reduced cytotoxicity. *In vitr*o studies highlighted the impact of polymer architecture on cellular uptake and LAL activity restoration, while *in vivo* evaluations offered key insights for the synthesis of next-generation BBs for mRNA delivery.

Conclusion. This study provides valuable design principles for next-generation BB carriers, highlighting quaternized, highly branched structures as a strong foundation for advancing their clinical potential in mRNA therapeutics.

Optimizing immunoprecipitation to extract lipid nanoparticles (LNPs) from complex biological media.

Nicolas Gaudreault[1, 2], Nicolas Bertrand[1, 2]

[1]Faculté de Pharmacie, Université Laval, Québec, Qc, [2]Centre de Recherche du CHU de Québec, Axe Endocrinologie et Néphrologie, Québec, Qc

PURPOSE Proteins that adsorb on nanoparticles influence their *in vivo* distribution and pharmacology. [1] Probing the protein corona (PC) on nanoparticles requires extraction procedures that maintain a dynamic and fragile equilibrium. This remains a challenge for Lipid Nanoparticles (LNPs), because of their low density and their similarity to endogenous blood components. Our lab has previously developed a method to extract pegylated nanoparticles using immunoprecipitation. [2] With this study, we sought to understand 1) if this method was suitable for LNPs and 2) what were the relevant parameters to consider.

METHODS LNPs were extracted by immunoprecipitation, using antiPEG or Isotype-control antibodies immobilized on magnetic beads (MB). LNP formulations were labelled with either DiIC18(5) or radiolabeled cholesteryl oleate to yield quantitative results. We studied the influence of LNP characteristics on extraction efficiencies, such as the type of ionizable cationic lipid (DODAP, SM-102 and MC3) and the nature of PEG-lipid (distearoyl vs dimyristoyl). The effects of incubation time in plasma and extraction time were also evaluated.

RESULTS In PBS and plasma, the type of ionizable lipid had minimal impact on extraction efficiency. For LNPs incubated in plasma, maximum extraction was achieved within 15 - 60 minutes, regardless of the PEG-lipid used in the formulation. Incubation of LNPs containing dimyristoyl-derivatives in plasma reduced extraction.

CONCLUSION

Irrespective of their composition, all the tested formulations could be extracted from plasma, demonstrating that immunoprecipitation is a specific, soft and rapid method to extract LNPs. In biological media, the desorption rate of PEG-lipid from the LNP is a critical parameter, as the success of this method depends on the presence of PEG on the LNP's surface.

Organic synthesis and characterization of bone targeting beta-lactamase inhibitors for the treatment of bone infections

Ruikun Tang, Ranga Nakandalage, Prasanna Neelakantan, Neal Davies, Nathan Beahm, Carlos Velazquez, Michael Doschak

University of Alberta, Edmonton, Canada

Purpose: The effective antimicrobial treatment of bone infections are often compromised by bacterial beta-lactamase enzyme production, which degrade beta-lactam antibiotics such as penicillins, cephalosporins, and carbapenems. Accordingly, beta-lactamase inhibitor drugs, such as Tazobactam, are commonly co-administered to neutralize enzymatic degradation of the otherwise effective antibiotic. However, the systemic delivery of beta-lactamase inhibitors often results in suboptimal bioavailability in bone tissue - the primary site of infection in conditions such as osteomyelitis. This study aimed to enhance drug delivery of Tazobactam to bone tissues by conjugation to a bisphosphonate (BP) carrier, thereby improving bone affinity and local drug concentration in bone.

Methods: Tazobactam-bisphosphonate (Tazo-BP) conjugates were synthesized using a Steglich esterification linkage strategy to a protected hydroxybutane-1,1-diyl-bisphosphonate carrier. The structural identity of these prodrug conjugates was confirmed via Liquid Chromatography-Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. The enzymatic inhibition activity of the Tazo-BP conjugates was evaluated *in vitro* using a nitrocefin-based colorimetric assay. Bone mineral affinity of Tazo-BP conjugates was assessed using *in vitro* hydroxyapatite binding assays. Antimicrobial activity was tested against beta-lactamase producing strains of *Escherichia coli* with co-administered Piperacillin antibiotic, using Minimal Inhibitory Concentration (MIC) microbroth dilution assays.

Results: Tazo-BP conjugates were successfully synthesized and structurally validated. The conjugate retained beta-lactamase inhibitory activity and exhibited affinity for hydroxyapatite, supporting its bone targeting potential. When Tazo-BP was co-administered with Piperacillin, 80% bacterial killing was evidenced at 62.5 µg/ml and 100% bacterial killing at 125 µg/ml, indicating bisphosphonate conjugation did not interfere with BP-Tazo support of Piperacillin antimicrobial activity.

Conclusion: This study confirms the synthesis of a first-in-class bone-targeting beta-lactamase inhibitor drug, which offers synergistic beta-lactamase enzyme inhibition and bone-binding properties. The increased bone tissue bioavailability of Tazobactam, after BP-conjugated formulation, may serve to overcome current limitations of high systemic drug exposure, by achieving increased local drug concentrations in bone.

Plasma Protein and Lipoprotein Distribution of RM-581, an aminosteroid derivative, within Normo- and Hypercholesterolemic Human Plasma.

Mitali Pandey[1], Hans Adomat[1], René Maltais[2], Donald Poirier[2], Michael Cox[1], Kishor Wasan[1]

[1]University of British Columbia, Vancouver, BC, [2]Université Laval, QC, Canada

Purpose. The purpose of this study was to determine the plasma protein and lipoprotein distribution of RM-581 within representative normocholesterolemic and hypercholesterolemic human plasma.

Methods. RM-581 at a concentration of 2000 ng/ml was incubated in representative normocholesterolemic and hypercholesterolemic human plasma (3 ml; 6000 ng RM-581 total incubated) from healthy male subjects for 60 minutes at 37°C. RM-581 was incubated at this concentration and incubation time to reflect the Cmax and Tmax seen following administration to mice where we observed an effect of RM-581 in reducing prostate cancer tumour growth (1). Following incubation, the human plasma was separated into their different plasma protein (lipoprotein deficient plasma fraction (LPDP) which contains albumin and alpha-1 glycoproteins) and lipoprotein (VLDL, LDL, IDL and HDL) sub-fractions by density gradient ultracentrifugation and the amount of RM-581 recovered in each fraction was determined by tandem LC-MS-MS. Total plasma and lipoprotein cholesterol concentrations in each sample were determined by enzymatic assays.

Results. When RM-581 was incubated in either normocholesterolemic (Total cholesterol concentration of 3.4 mmole/L) or hypercholesterolemic (Total cholesterol concentration 5.6 mmole/L) human plasma much of the drug was recovered within either the LPDP or HDL sub-fractions (**Table 1**).

Conclusions. These findings suggest that changes in plasma cholesterol concentrations did not significantly modify the lipoprotein distribution of RM-581 with most of the drug exclusively retained within the HDL subfractions. We have recently shown that SR-BI, a scavenger receptor involved in the cellular uptake of HDL cholesterol, is up-regulated in prostate cancer cells (2). Thus, these findings may provide further insight to explain the pharmacokinetic and pharmacodynamic profile of RM-581 within prostate cancer subjects that do experience a rise in their baseline plasma cholesterol concentrations potentially through the SR-BI pathway.

Acknowledgements: Funding was provided by CIHR Project Grants to MEC and KMW, and to DP and KMW.

PLGA nanoparticles for oral delivery: optimization of a method to track excretion through radiolabeled polymers

Antoine Labrie[1, 2], Alyssa Lavoie-Girard[2, 3], Pierre-Olivier Sirois[2, 3], Nicolas Bertrand[1, 2]

[1]Faculty of Pharmacy, Université Laval, Quebec, QC, [2]CHU de Québec Research Center, Quebec, QC, [3]Faculty of Medicine, Université Laval, Québec, QC

Purpose: The oral delivery of medicine is a non-invasive and generally preferred path of administration by patients, yet many disadvantages are associated with the oral route: the harsh conditions of the gastrointestinal tract (GIT), as well as the first pass effect, make it difficult to develop new medicines that employ this administration method.

Nanomedicines consist of particles that are nanometric in diameter and size with modulable characteristics, and are used in the clinic to ameliorate the biopharmaceutical properties of active ingredients, and allow better targeting of specific anatomical structures. Whilst most nanoparticles (NPs) currently used in the clinic are administered intravenously, their usage through the oral delivery route is currently studied. In this study, we explore the possibility of using poly(lactic-*co*-glycolic) acid (PLGA) based drug delivery carriers to treat disorders that affect distal parts of the GIT, such as the cecum or the colon. We developed and optimized a method of tracking excretion, using feces at different time points in pharmacokinetics studies.

Method: Radiolabeled PLGA nanoparticles were synthesized with poly(ethylene) glycol (PEG) as a stabilizer, and administered orally to healthy mice to analyze the pharmacokinetics of the NPs as a drug delivery system. Blood and tissue samples were taken throughout the experiment to trace a portrait of absorption and excretion of PLGA NPs. We developed and optimized a simple method of analyzing radioactivity in feces, using glass beads and sulfuric acid, and compared it to previous methods we used.

Results: PLGA nanoparticles were not efficiently absorbed in systemic circulation and displayed low biodistribution. The optimized feces analysis method is able to retrieve a high amount of radioactivity from high doses, and shows complete recovery for low doses of radioactivity.

Conclusion: PLGA NPs have the potential to act as drug delivery carriers for active ingredients that need to reach distal parts of the GIT, through low absorption after administration. Our method of tracking excretion through feces shows promising results and gives a better portrait of the pharmacokinetics of radiolabeled NPs.

Poly (ethylene oxide)-poly(D, L-lactide) (PEO-PDLLA) polymeric micelles for nano delivery of S4, a novel competitive inhibitor of polynucleotide kinase/phosphatase (PNKP), for colorectal cancer therapy.

Sara Abd El-Hafeez[1, 2], Cameron Murray[3], James Donnelly[4], Mark Glover[5], Frederick West[4], Kristi Baker[6, 7], Michael Weinfeld[8], Afsaneh Lavasanifar[1]

[1]Department of Pharmacy and pharmaceutical science, University of Alberta, Edmonton, AB, [2]Department of Pharmaceutics, Assiut university, Assiut., [3]Department of Biochemistry, University of Alberta Edmonton, [4]Department of Chemistry, University of Alberta, Edmonton,AB., [5]Department of Biochemistry, University of Alberta Edmonton, AB., [6]Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, AB., [7]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB., [8]Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, AB..

Purpose: Inhibitors of polynucleotide kinase/phosphatase (PNKP) can make cancer cells more sensitive to DNA damage by ionizing radiation or topoisomerase I inhibitors (1,2). Inhibitors of PNKP are also known to be synthetic lethal partners of Phosphatase and tensin homolog (PTEN) deficiency leading to targeted cytotoxic effects in PTEN negative cancer cells . The aim of this study was to develop nanocarriers of S4, a potent competitive inhibitor of PNKP (IC50= 170 nM), that can potentially redirect the encapsulated S4 towards colorectal cancer (CRC) and reduce its exposure to normal tissues. **Methods:** Encapsulation of S4 was accomplished by dissolving S4 and poly(ethylene oxide)-poly(D, L-lactide) (PEO-PDLLA) in DMSO followed by dropwise addition to water and dialysis against water at 1:2.5,10 or 20 w/w ratio . The prepared formulations were characterized for the level of encapsulated S4 using UV/Vis spectroscopy at 440 nm and average diameter using dynamic light scattering. Cytotoxicity of S4 as free or part of nanocarriers was measured in wild type HCT116 (WT HCT116) and its (PTEN) knock-out (HCT116 PTEN$^{-/-}$) phenotype using MTS and colony forming assay. **Results:** The average diameter of all formulations under study was< 150 nm. Highest encapsulation efficiency of 25.1 % for S4 was achieved in PEO-PDLLA nanocarriers using S4/polymer 1:2.5 w/w ratio. After 8 hours, 51.6% of encapsulated S4 was released from PEO-PDLLA nanocarriers in water. On the one hand, higher IC50 for S4 free dug in WT HCT 116 (IC50=2.9 µM) compared to HCT116 PTEN$^{-/-}$ cells (0.07 µM) was observed indicating synthetic lethality. PEO-PDLLA nanocarrier formulations of S4 showed significantly higher cytotoxicity to HCT116 PTEN-/- cells in S4 concentration range of 0.78 :12.5 µM [1]. Clonogenic survival assay showed HCT116 /PTEN$^{-/-}$ to be more sensitive to S4 , than WT HCT116 at the same concentration range. **Conclusions:** Data confirms the anti-cancer activity of S4 in PTEN negative CRC and a good potential for PEO-PDLLA nanocarriers for S4 solubilization and delivery leading to synthetic lethality in PTEN negative CRC cells.

Polymeric microreactor for blood urea sensing at the point-of-care

Emma Lenglet[1], Cléa Belin[1], Marie-Lynn Al-Hawat[1], Justine Caron[1], Sarah Djebbar[1], Simon Matoori[1]

[1]Montreal University, Montreal, QC, Canada

Purpose. Urea is a critical biomarker for monitoring dialysis efficiency in renal disease patients, requiring rapid point-of-care testing to reduce reliance on centralized laboratories. Vesicular microreactors with selective membrane permeability are increasingly used in diagnostics [1]. Among these, IRDye-functionalized polymeric microreactors show promise for indirect urea detection by selectively sensing ammonia in whole blood [2]. We aimed to develop a portable fluorescent assay for rapid and accurate blood urea detection, hypothesizing that ammonia-sensitive polymersomes loaded with a near-infrared fluorescent dye could detect urea via urease-mediated degradation.

Methods. Polymersomes composed of polystyrene-polyethylene glycol were prepared in an acidic buffer containing a pH-sensitive fluorescent dye (IRDye680) or pyranine dye, using emulsification. These polymersomes, featuring an ammonia-sensitive transmembrane pH gradient, were tested in urea-spiked buffer with urease, which degrades urea into ammonia. The released ammonia diffuses into the acidic core of the polymersomes, is protonated and raises the pH enhancing fluorescence intensity from the pH-sensitive dye (Figure 1). Fluorescence was measured with a plate reader.

Results. Assays with polymersomes, pyranine dye and urease in urea-spiked buffer showed high linearity ($R^2 = 0.9925$) and fluorescence ratios (0.8826) across 0 to 30 mM of urea. When using IRDye, more promising results were obtained with 1 mg/mL urease incubated with 3 µL of urea at concentrations between 0 and 10 mM, yielding high linearity ($R^2 = 0.9742$) and fluorescence ratios up to 1.34 within 2 minutes.

Conclusion. The polymersome-based assay detects urea through ammonia formation, offering rapid, sensitive, and linear responses in clinical ranges. The IRDye-based system shows strong potential for point-of-care applications but further optimization in human blood is necessary for clinical reliability. This portable, easy-to-use assay holds promise for real-time urea monitoring in dialysis patients, encouraging preclinical validation and testing in resource-limited or home-based care scenarios.

Poly(monoglycerol acrylate) promotes fast endophilin-mediated endocytosis of liposomes

Lucia Carrera Fragoso[1, 2], Sabrina Roussel[1, 2], Nicolas Bertrand[1, 2]

[1]Faculté de Pharmacie, Université Laval, Québec, QC, [2]Endocrinologie et Néphrologie, Centre de Recherche du CHU de Québec - Université Laval, Québec, QC

Purpose: Nanocarriers are designed to modify the pharmacology of active ingredients. However, they require efficient cell internalization to deliver their payload. To improve cellular uptake, we devised a polymer, poly(monoglycerol acrylate) (PMGA), to modify surface of liposomes. The objective of this study was to evaluate how the attachment of PMGA on the surface of liposomes affected their cellular internalization pathway.

Methods: Fluorescent liposomes, with and without PMGA, were prepared by hydration of lipid films followed by extrusion through polycarbonate membranes. We evaluated cellular uptake in RAW 264.7 cells (macrophage-like). To inhibit specific cellular internalization pathways, cells were treated with specific inhibitors: chloroquine, Dyngo-4a, Methyl-β-cyclodextrin (MβC), 7-ketocholesterol (7KC), Ethyl-isopropyl amiloride (EIPA), and Cytochalasin D. After 30 minutes, a range of concentrations (from 0.04 to 6 µg/mL) of PMGA or control liposomes were incubated with cells for 2 hours. To measure cellular internalization, cells were analysed by high throughput flow cytometry. Median effective concentrations (EC_{50}) were calculated for each condition.

Results: Cellular inhibitors had different effects depending on liposomal composition. For control liposomes, internalization was reduced by chloroquine, an inhibitor blocking clathrin-mediated endocytosis (CME). This inhibitor only moderately impacted the cellular internalization of PMGA liposomes. In contrast, PMGA liposomes uptake was significantly decreased by treatment with Dyngo-4a, an inhibitor of the fast endophilin-mediated endocytosis (FEME) and CME. These different effects of the inhibitors on cellular internalization suggest that PMGA liposomes are internalized strongly via the FEME pathway. Both formulations were unaffected by MβC, EIPA and 7KC. Finally, uptake of both types of liposomes was reduced by Cytochalasin D, which inhibits micropinocytosis and phagocytosis.

Conclusion: Results indicate that the mechanisms of cellular uptake for PMGA liposomes are different from those regulating the internalization of control liposomes. PMGA seems to promote internalization by the FEME pathway compared to control liposomes that seem to be internalized through the CME pathway.

Porous silica cork-shell microcapsules for ultrasound-triggered insulin delivery

Kayla Baker[1], Andrew Singh[1], Tahirah D'Costa Correia[1], Todd Hoare[1]

[1]McMaster University, Hamilton, ON, Canada

Purpose: Effective treatment of Type I and II diabetes requires certain basal levels of insulin that must be supplemented in a pulsatile fashion ~2 hours before eating [1]. In this context, an externally addressable, reliable, and repeatable insulin delivery system is critical for increasing the quality of life of diabetic patients worldwide.

Methods: An immersion electrospraying technique was used to fabricate microcapsules with an aqueous, insulin-loaded core and a poly(lactic-co-glycolic acid) shell into which porous silica microparticles were incorporated. Under ultrasound stimulation, the soft polymer shell expands while the hard silica particles vibrate in place, creating transient gaps in the shell for a pulse of insulin to release. Between pulses, the interconnected porous network in the silica particles allows for low-level constant release via a diffusive mechanism. Ultrasound-triggered drug release was studied using an egg-white based tissue-mimic and an Olympus 5077PR pulse generator set at 400 V and 3.5 MHz.

Results: The microcapsules had an average diameter of 134 µm, with silica particles covering ~4% of the total surface area. The insulin encapsulation efficiency was 98%. The porous microparticles allowed for low basal release in the absence of stimuli; when a 20-minute focused ultrasound pulse was applied, the release rate increased 7-fold and was repeatable over at least five cycles, returning to the low basal release between pulses.

Conclusion: The dual basal/pulsatile drug release kinetics achievable with this cork-shell system represents a potential solution for insulin delivery in treating diabetes, replacing frequent injections with non-invasive ultrasound applications.

Spatially defined post-manufacturing loading of nanoparticles into hydrogel-forming microneedles

Davide Brambilla[1], Alfonso Nieto Arguello[1], Gaurav Sadhnani[2], Jerry Leung[3], Fatma Moawad[1], Pieter R. Cullis[3], Sarah Hedtrich[2, 4, 5, 6]

[1]University of Montreal Faculty of Pharmacy, [2]Berlin Institute of Health at Charité Universitätsmedizin, [3]Department of Biochemistry and Molecular Biology, University of British Columbia, [4]Faculty of Pharmaceutical Sciences, The University of British Columbia, [5]School of Biomedical Engineering, The University of British Columbia, [6]Centre for Blood Research & Life Science Institute, University of British Columbia

Purpose. As an approach for transdermal drug delivery, hydrogel-forming microneedle patches have garnered significant attention. Lipid nanoparticles are highly effective carriers for delivering genetic material such as mRNA, due to their ability to protect fragile payloads from enzymatic degradation and enhance cellular uptake. This study presents a method for spatially controlled loading of mRNA-encapsulated lipid nanoparticles into manufactured hydrogel-forming microneedle patches.

Methods. Key parameters of the method, such as microneedle insertion depth, insertion duration, insertion repetition, and dye concentration in the loading gels, are systematically evaluated and optimized to maximize loading while preserving needle structural integrity.

Results. Functional studies confirmed effective lipid nanoparticle uptake, and ex vivo experiments demonstrated the successful delivery of active lipid nanoparticles into human skin. This method allows minimal loss of lipid nanoparticles during manufacturing.

Conclusion. Thus, expanding the potential for delivering genetic material to skin cells.

Stabilizing mRNA-Lipid Nanoparticles with Carbohydrates for Inhalation

Naga Suresh Kola[1], Dr. Aneesh Thakur[2]

[1]1. Vaccine and Infectious Disease Organization, University of Saskatchewan 2. School of Public Health, University of Saskatchewan, [2]1. Vaccine and Infectious Disease Organization, University of Saskatchewan, 2. Department of Biochemistry, Microbiology and Immunology, University of Saskatchewan

Purpose: Pulmonary delivery of messenger ribonucleic acid (mRNA) vaccines via inhalation holds promise for eliciting comprehensive mucosal and systemic immunity, which is pivotal for providing frontline protection against respiratory pathogens. However, delivering mRNA to the lungs presents challenges, particularly with conventional lipid nanoparticles (LNPs), which while effective in systemic delivery of mRNA, are prone to instability during nebulization owing to high shear stress. To address this challenge, we aimed to test carbohydrates as excipients in LNPs to improve their stability during nebulization. We hypothesized that incorporating saccharides into LNP formulation would prevent nanoparticle aggregation and protect the encapsulated mRNA from degradation during nebulization.

Methods: We formulated firefly luciferase (FLuc) encoding mRNA-loaded LNPs by microfluidic mixing and incorporated carbohydrates (mono-, di-, and polysaccharides) as excipients during dialysis (Figure 1). The FLuc mRNA-LNPs were aerosolized into fine mist by vibrating mesh nebulizer. The mRNA-LNPs were characterized for physicochemical properties, *i.e.*, size, polydispersity, morphology, and mRNA encapsulation. To assess the efficacy of nebulized LNPs for mRNA transfection, we performed *in vivo* bioluminescent imaging in mice following intranasal administration.

Results: FLuc mRNA-LNPs exhibited monodispersed, spherical oligolamellar structures with sizes <150 nm. Nebulization differentially impacted LNPs stability: conventional LNPs without excipients, *i.e.*, salt-based formulations underwent significant physicochemical alterations with almost 50% of encapsulated mRNA degradation. While formulations incorporating mono- and disaccharide excipients effectively preserved LNPs integrity. Conversely, polysaccharides caused nanoparticle aggregation with polydispersity >0.3. *In vivo* luciferase expression studies in mice demonstrated sustained protein expression for 7 days and modified LNPs exhibited a three-fold increase in FLuc expression compared to the unmodified counterparts. Among all sugars, lactose-based mRNA-LNPs showed higher protein expression in the nasal cavity while sucrose-based formulations induced expression predominantly in the lungs.

Conclusion: Our findings demonstrate that incorporating non-reducing disaccharides as excipient is a promising strategy to mitigate nebulization-induced degradation of mRNA-LNPs and promote higher protein expression in the mice lungs. This suggests that the strategic selection of excipients can significantly improve the efficacy of inhaled LNP-based mRNA therapies by promoting both LNP stability and efficient mRNA transfection across the respiratory epithelium.

Structural Optimization of Lipid Nanoparticles Using Synchrotron SAXS for Improved Nucleic Acid Delivery

Jonathan Rekve[1], Lee Wilson[2], Ildiko Badea[1]

[1]College of Pharmacy and Nutrition, University of Saskatchewan, [2]Department of Chemistry, University of Saskatchewan, Saskatoon, Canada

Purpose. Lipid nanoparticles (LNPs) are an advanced platform for nucleic acid delivery, offering protection and efficient cellular uptake. The incorporation of excipients such as glucose and sucrose can influence LNP structural integrity, stability, and nucleic acid release. This study explores the impact of lipid composition and excipients on LNP formulation and performance.

Methods. LNPs were formulated with DNA, saRNA, or mRNA using Gemini lipids, incorporating either 5% glucose or 9.25% sucrose. Small-angle X-ray scattering (SAXS) was employed to analyze structural organization, lipid packing, and nanoparticle stability. The effects of varying lipid molar ratios were assessed to determine their influence on encapsulation efficiency and nucleic acid release kinetics.

Results. SAXS analysis revealed that glucose-based formulations exhibited higher structural ordering and enhanced stability compared to sucrose-based LNPs. Increased peak intensities and hexagonal nanostructure formation in glucose formulations suggest improved nucleic acid loading and sustained-release potential.

Conclusion. By optimizing LNP structural organization and stability, this study contributes to the development of more effective nucleic acid delivery systems. The findings have implications for improving therapeutic efficacy, patient compliance, and advancing targeted treatments for neurodegenerative diseases.

Subcutaneous implant containing pH-dependant near infrared fluorescent liposomes for continuous lactate detection

Justine Caron[1], Emma Grande[1, 2], Simon Matoori[1]

[1]Faculty of pharmacy, Université de Montréal, [2]Faculté sciences et ingénieurie, Université de Toulouse, Toulouse

Purpose: Lactate is a key biomarker of tissues undergoing hypoxia, and by extension sepsis. It is used to assess the disease severity and the efficacy of administered treatments. As such, faster testing (median time for standard lab testing: 3 h) and continuous monitoring would greatly benefit patients. Building on reports of a Near InfraRed (NIR) portable blood lactate assay[1], we aim to develop a subcutaneous implant based on liposomes containing no enzymes, immobilized in a hydrogel. Liposomes are already established as vesicles used for a variety of purposes, including FDA-approved drug delivery. Achieving a reliable assay containing no enzymes would circumvent the issues created by their use (immunogenicity, stability, cost) and allow for a better assessment of the patient's health at the bedside.

Methods: A liposomal formulation containing DPPC, DSPE-PEG(2000) and cholesterol was prepared by rehydrating a dried thin film. The NIR fluorophore (PEGylated pip-cyanine 7)-containing HEPES buffered rehydration solution was then removed from the liposome suspension outer phase with a pre-packed Sephadex size-exclusion column. Liposome fluorescence was measured by a plate reader in citrate and HEPES buffers at different pHs and finally in presence of clinically relevant concentrations of lactate in sodium chloride containing phosphate buffer, optimizing the system by modulating the outer and inner phases pHs.

Results: PEGylated pip-cyanine 7 fluorescence showed a strong pH dependence between 4 and 9, with a sigmoid shape centred around an inflection point of 7.4, the physiological pH of the body, which makes it highly suited to an *in vivo* application. Then, the best result of lactate detection showed a high linearity (R^2=0.974) and a fluorescence decrease of 25%, with parameters including an inner phase with HEPES buffer (concentration of 1,25 mM, pH 8.5) and an outer phase with citrate buffer (concentration 50 mM, pH 5).

Conclusion: These results are promising for a future implant. Further optimization is needed to achieve a high linearity and a strong response for better accuracy. The use of a portable fluorometer is also a requirement for a successful translation to the clinical setting.

Synthesis and Evaluation of a Lipid-Dendrimer-Hybrid Nano Delivery System for Targeted Delivery of Urolithin A to Adipocytes

Qi Sun[1], Emmanuel A. Ho[1]

[1]University of Waterloo, Waterloo, ON, Canada

Purpose: Obesity affects nearly 25% of Canadians, leading to substantial healthcare costs and posing a significant threat, such as causing type-II diabetes and metabolic disorders, to Canadian society. Although drugs such as Semaglutide have demonstrated efficacy in weight loss in clinical trials, they lack targeted delivery and fail to promote adipocyte thermogenesis. Urolithin A (UA), a metabolite derived from ellagic acid, is recognized for its ability to stimulate thermogenesis and induce white adipocyte browning, making it a promising anti-obesity candidate. However, its poor solubility and lack of targeted delivery have limited its clinical application.

Lipid-dendrimer-hybrid nanoparticles (LDHNs) address these limitations by combining the advantages of dendrimers with the biocompatibility of lipids. Furthermore, LDHNs show promise for oral administration, enhancing patient compliance and convenience. In addition, traditional drug evaluation methods (animal studies/monolayer cell cultures) present limitations in accurately predicting drug pharmacodynamics and physiology.

This research aims to develop LDHNs to enhance the targeted delivery of UA and promote adipocyte thermogenesis, alongside the development of a microfluidic multi-organ-on-a-chip model for preclinical drug evaluation using lithography techniques.

Methods: UA will be encapsulated within LDHNs via sonication and microfluidic methods optimized for particle size, stability, and controlled drug release. Adipose-targeting peptides will be conjugated to the lipid components to enhance uptake by adipocytes, thereby promoting thermogenesis. Efficacy will be evaluated in obese mice and through a multi-organ microfluidic chip system, providing an ethical and predictive alternative to traditional animal testing.

Results: The results indicated that dendrimers significantly increased the aqueous solubility of UA. Compared to dendrimers alone, LDHNs exhibited a more uniform size distribution (dendrimers: >500 nm; LDHNs: <200 nm) and reduced surface charge. The

encapsulation efficiency of UA in LDHNs reached 60%, and the formulation remained stable for at least seven days under physiological conditions (37°C in PBS).

Conclusion: The use of UA as an anti-obesity drug addresses the current lack of medications that directly target adipocytes and promote thermogenesis. Simultaneously, the development of LDHNs as a novel drug delivery system offers researchers an innovative alternative for targeted delivery and controlled release.

Synthesis of amphiphilic PLA block copolymers with variable hydrophilic blocks by sequential ring opening and RAFT polymerization

Jonathan Buiel[1], Wojciech Raj[1], Jean-Michel Rabanel[2], Hu Zhang[1], Daria Boffito[3], Xavier Banquy[1]

[1]Faculté de Pharmacie, Université de Montréal, [2]School of Pharmaceutical Sciences, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada, [3]Département de Génie Chimique, École Polytechnique Montréal

Purpose. Polymeric nanoparticles (PNPs) have been extensively investigated for their potential to improve drug pharmacokinetics, including bioavailability and biodistribution. Despite the abundance of fundamental research and clinical trials, PNPs have failed to reach the market. This failure is largely attributed to the non-specific distribution of PNPs, wherein >95% of an intravenous administration does not reach the pathological site.[1] This non-specific distribution of PNPs is regulated by their surface composition and opsonization. To prevent opsonization and thus increase half-life, anti-fouling hydrophilic polymers, such as PEG, must be incorporated at the surface of PNPs.[2] However, PEG has been ineffective in directing the distribution of PNPs to any specific organ, despite increasing their half-life.

Methods. We report the synthesis of polymers, which were either obtained by either (a)sequential ring-opening polymerization of lactide onto a dual initiator and chain transfer agent, followed by RAFT polymerization of various methacrylates, or (b) post-ring-opening polymerization modification of polylactic acid with an atom transfer radical polymerization (ATRP) initiator and subsequent ATRP of methacrylates.

Results. To test the biodistribution properties of PNPs with variable (non-PEG) anti-fouling surfaces, we have synthesized roughly 15 amphiphilic PLA block copolymers with a variable hydrophilic block, either in nature or size. PLA block copolymers include hydrophilic blocks that are either uncharged, zwitterionic, positive, or negative. Reaction conditions were optimized to ensure compatibility with solvents that dissolve the PLA macroinitiator, the monomer, and the resulting amphiphilic block copolymer. All polymers were synthesized on a scale exceeding 1 g, with polydispersity indexes ranging from 1.05 to 1.25, and were characterized using ^{1}H NMR and size exclusion chromatography (SEC).

Conclusion. The diversity in the hydrophilic blocks—ranging from uncharged to zwitterionic, positively charged, or negatively charged—will enable us to systematically investigate the role of surface charge on anti-fouling behavior and in vivo biodistribution upon polymer self-assembly into nanoparticles. We anticipate significant variability in biodistribution profiles, though the extent of this variability remains to be determined. If successful, these nanocarriers could be tailored for specific organ targeting, offering a platform for encapsulating small-molecule drugs and enhancing therapeutic outcomes.

Theranostic Gold Nanoparticles for Prostate Cancer Radiotherapy: PAE-Mediated Delivery and Clinical Pilot Study

Mohamed Aborig[1], Charly McKenna[2], Ernest Osei[3], Anthony Mutsaers[2], Shawn Wettig[1]

[1]University of Waterloo - School of Pharmacy, Waterloo, Ontario, [2]Ontario Veterinary College - University of Guelph, Guelph, Ontario, [3]WRHN Cancer Centre- Waterloo Regional Health Network, Waterloo, Ontario

Purpose: Radiation treatment of prostate cancer remains limited by radiotherapy-induced toxicity to adjacent organs. To address this, we developed two theranostic gold nanoparticles (Curc-GNPs and EGCG-GNPs) that combine gold's photoelectric effect with the anticancer properties of phytochemicals curcumin and EGCG. Additionally, we introduce a novel technique for local delivery of NPs directly to canine prostate via prostatic arterial embolization (PAE) in 3 healthy lab beagles and 1 clinical case.

Methods: GNPs were characterized using UV-vis, FTIR, DLS, TEM, CT imaging, and DPPH assays. In-vitro studies included: Cytotoxicity: XTT assays (PC3 cells; 24-72h) Uptake: ICP-MS Au quantification (10/20h) Radiosensitization: Clonogenic assays for enhancement ratio (SER) using 6 MV X-rays

In-vivo mouse studies assessed biodistribution (IP injection; Au levels at days 1-56). Canine trials involved:

Beagle 1: Intra-arterial (IA) injection of1.5mg/mL EGCG-GNP into right lobe

Beagle 2: PAE alone. 300-500 µm embosphere beads into right lobe

Beagle 3: IA injection of 1.5mg/mL EGCG-GNP in both lobes + PAE into right lobe

Clinical case: 12-year-old Boston Terrier with prostatic urethral carcinoma received 1.5mg/mL EGCG-GNP+10mg/mL carboplatin via IA injection.

Results: EGCG & Curc-GNPs were spherical with core sizes of 10-15 nm, Water-immersed tubes exhibited 58 HU/mg CT contrast. EGCG-GNP demonstrated superior cytotoxicity ($p<0.01$ vs Curc-GNP) and higher uptake in PC3 cells for non-serum supplemented medium. EGCG-GNP demonstrated a higher SER of 1.96 vs 1.82 for Curc-GNPs ($p<0.05$). Mouse studies revealed hepatic/splenic accumulation with progressive clearance (-0.31%Au/ day), and no signs of histopathological toxicity. PAE delivery in beagles was well tolerated with only one adverse event being transient bowel discomfort. In-vivo contrast enhancement was faint or nonexistent for 1.5mg/mL EGCG-GNP. While 3.0mg/mL showed higher contrast (117 HU) in-situ, vascular dispersion was limited. The clinical case developed fatal sepsis/cardiac arrest post EGCG-GNP +carboplatin coadministration.

Conclusions: PAE-mediated delivery of nanoparticles to canine prostates is feasible and well-tolerated in healthy models. While 3.0mg/mL EGCG-GNP achieved diagnostic-grade contrast, viscosity and dispersion were challenges. Therapeutic combinations of EGCG-GNP with carboplatin require careful toxicity evaluation. Future work should optimize nanoparticle formulations and explore contrast-enhancing adjuvants for clinical translation.

Ultraporous Metabolic Hydrogels: a Key Innovation in the Oral Treatment of Phenylketonuria

Sofie Dhondt[1], Catherine Nadeau[1], Philippe Delbreil[1], Xavier Banquy[1], Davide Brambilla[1]

[1]Université de Montréal, Montreal, QC, Canada

Purpose: Phenylketonuria (PKU) is the most common inherited disorder of amino acid metabolism. The condition can be caused by various mutations in the phenylalanine hydroxylase gene or the gene encoding its BH4-cofactor. The resulting impaired breakdown of phenylalanine (Phe) causes its plasma concentration to rise to toxic levels. If left untreated, severe developmental delays and behavioral, psychiatric and motor problems are inevitable. Even with early diagnosis lifelong treatment is required to mitigate PKU-related symptoms. Yet, current therapies are limited in scope and practicality.[1] To address this, we developed an ultraporous hydrogel (TGels) for oral delivery and gastro-intestinal protection of phenylalanine ammonia lyase (PAL). Not only offers oral administration a safer and more comfortable treatment, but enzyme substitution therapy is also suitable for all genetic variants causing PKU. Additionally, it benefits from a dual Phe-lowering action by targeting both food-derived Phe and systemic Phe that is available in the intestine via enterorecirculation.[2]

Methods: TGels are synthesized by mixing porous vaterite microspheres (templates) and prepolymer, followed by UV-induced crosslinking, freeze drying and acid template removal. PAL is loaded into the TGels by absorption. Swelling in the intestinal lumen does not aim to release PAL but creates a protective enzyme-rich micro-environment in which Phe and the enzymatic product can diffuse freely. Our assessment consists of 3 major steps: *in vitro* characterization and optimization of the TGel matrix to minimize release, *in vitro* optimization of the PAL loading to maximize activity and *in vivo* evaluation of the selected PAL-TGel.

Results: TGels demonstrated 50% enzyme retention after 6 hours in simulated intestinal fluid. Optimization of the PAL-mixture led to maximized loading capacity and preserved enzymatic activity while minimizing disruption to digestive enzymes. *In vivo* studies confirmed TGel transit in the canine small intestine, with potential for effective Phe reduction.

Conclusion: PAL-loaded TGels represent a promising innovation for oral PKU management, offering a non-invasive, effective alternative to current therapies. By enabling dietary freedom and improving adherence, TGels could significantly enhance patient quality of life. Additionally, TGels hold potential for broader applications for gastrointestinal-targeted therapies.

Unraveling Variability in Melatonin Products Using a PBPK Modeling Approach to Dissolution and Pharmacokinetics

Shengnan Zhao[1, 2], Malaz Yousef[1, 2], Jieyu Zuo[1, 2], Neal M. Davies[1, 2], Raimar Löbenberg[1, 2]

[1]University of Alberta, Edmonton, Alberta, Canada, [2]Pharmacy and Pharmaceutical Science, Edmonton, Alberta, Canada

Purpose: Melatonin is widely used to manage insomnia, regulate circadian rhythms, and address other sleep-related disorders. However, its regulatory classification varies across regions, with products categorized as dietary supplements, over-the-counter (OTC) medications, or prescription drugs. These differences result in variations in marketed doses and dosage forms. This study aimed to evaluate dissolution characteristics of commercial melatonin products from different regions and investigate the impact of formulation differences on pharmacokinetics using physiologically based pharmacokinetic (PBPK) modeling.

Methods: Melatonin products from various countries were selected and analyzed for dissolution behavior to assess formulation differences. A PBPK model was developed to predict plasma concentration-time profiles based on the dissolution data of the tested products. Additionally, an in vitro-in vivo correlation (IVIVC) analysis was conducted to determine the critical dissolution criteria required to achieve bioequivalence (BE).

Results: The pharmacokinetic simulations revealed substantial variability among the tested formulations. Among the 16 immediate-release (IR) products, C_{max} varied by 15.91-fold, while $AUC_{0\text{-}inf}$ exhibited a 9.20-fold difference. IVIVC analysis indicated that for IR formulations, achieving bioequivalence requires a dissolution rate of at least 85% within 15 minutes.

Conclusion: These findings underscore the significant variability in melatonin product quality and pharmacokinetic profiles, highlighting the urgent need for harmonized regulatory guidelines, stricter quality control measures, and standardized dissolution testing protocols. Implementing such measures would enhance the consistency, safety, and therapeutic efficacy of melatonin products worldwide.

Vibrations as a Tool for Modulating Surface Interaction Forces between supported lipid bilayers

Nahid Hassanpour[1], Chang-Sheng Wang[1], Daria Camila Boffito[2], Xavier Banquy[1]

[1]University of Montreal, [2]Polytechnique Montréal, Montreal, Canada

Purpose. Understanding and controlling surface interactions are essential for numerous biomedical applications, including drug delivery, biosensing, and tissue engineering. In this study, we explore the potential of low-amplitude vibrations (< 10nm) as a non-invasive and tunable tool for manipulating surface interaction forces on model-supported lipid bilayers.

Methods. The Langmuir-Blodgett technique deposited lipid bilayers composed of DSPE-DPPC onto mica substrates. These bilayer-coated surfaces were subsequently transferred to a Surface Forces Apparatus (SFA) equipped with a vibration module, allowing for precise measurements of the interaction forces between the lipid bilayers under controlled vibrational conditions.

Results. Our results show that vibrations can effectively modulate surface forces, transitioning from adhesive to repulsive behavior with changes in both amplitude and frequency. Strong adhesion was observed between the surfaces at lower vibration frequencies and amplitudes. However, as vibration frequency increased, the adhesive forces weakened, giving rise to repulsive forces. This shift demonstrates vibration's potential as a dynamic tool for regulating lipid bilayer interactions.

Conclusion. These findings introduce vibrations as a novel and non-invasive strategy for fine-tuning surface interactions at the molecular level. This approach holds significant promise for advancing biomedical technologies that require precise control of surface forces, such as enhancing nanoparticle circulation times in drug delivery or improving biosensor designs.

APPEASED : Assessing Pharmacokinetics and Pharmacodynamics of daily Enteric- coated Aspirin in patients with StablE Diabetes

Florence Lipp[1, 2], Cassandra Kezouh[1, 2], Catherine Roy[1, 2], Marie-Ève Newberry[1, 2], Samara Bloom[1, 2], Melanie Welman[2], Guillaume Marquis-Gravel[1, 2], Marie Lordkipanidzé[1, 2]

[1]Université de Montréal, [2]Montreal Heart Institute, Montreal, QC, Canada

Purpose. Diabetes is a significant cardiovascular risk factor, increasing 2- to 4-fold the risk of myocardial infarction or death. Aspirin is recommended to reduce thrombotic risk in patients with cardiovascular disease. However, its effectiveness in primary cardiovascular prevention for diabetic patients remains uncertain. The APPEASED trial aims to assess the pharmacokinetic and pharmacodynamic profiles of response to aspirin in patients with diabetes, and to evaluate the feasibility of conducting a larger-scale trial investigating the potential benefits of alternative aspirin dosing regimens.

Methods. Diabetic subjects with no documented cardiovascular disease were eligible. Platelet activity was measured on day 0, before any aspirin intake, and on day 7 after six days of daily intake of 81 mg enteric-coated aspirin. During the second visit, blood samples was collected before and after ingestion of the last tablet (24 hours and 2 hours post-dose). Platelet aggregation was assessed with light transmission aggregometry (LTA) in response to arachidonic acid (AA). Thromboxane B2 (TxB2) levels were measured by ELISA, and COX-1 acetylation was evaluated by western blotting. Feasibility parameters included rates of recruitment, completion of study visits and adhesion to all doses.

Results. Ten participants (20%) showed insufficient inhibition of platelet function in response to AA 24 hours after aspirin intake. However, 2 hours after intake, all subjects displayed adequate inhibition. Aspirin reduced TxB2 levels and acetylated COX-1, confirming its pharmacological efficacy. Recruitment rates were low, suggesting a multi-centre study would be required, but other feasibility parameters were met for a larger trial.

Conclusion. One fifth of diabetic patients were non-responders to aspirin 24 hours after the last dose, while 0% were non-responders 2 hours after intake, pointing at pharmacodynamics rather than pharmacokinetic causes of poor response. This variability in responses suggests an alternative mechanism of platelet activation in a high proportion of diabetic patients on aspirin.

Dermal Drug Distribution in Normal and Psoriatic Skin Assessed in Silico via PBPK Modeling and Simulations

Jasmina Novakovic[1], William W van Osdol[1], Jessica Spires[1], Maxime Le Merdy[1], Eleftheria Tsakalozou[2], Priyanka Ghosh[2], Viera Lukacova[1]

[1]Simulations Plus Inc, Toronto, ON, Canada, [2]Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, USA

Purpose. For locally acting topical dermatological drug products, measurement of drug concentrations at the effect site in diseased skin is challenging. Dermal physiologically based pharmacokinetic (PBPK) models can describe drug partitioning into skin layers. Our objective was to predict dermis drug concentrations in normal and diseased skin using Transdermal Compartmental Absorption & Transit (TCAT ™) Model in GastroPlus®. For this purpose, we modeled clobetasol propionate (CP) amounts in the dermis obtained via dermal open-flow microperfusion upon Dermovate cream topical application on non-lesional and lesional psoriatic skin [1].

Methods. First, we developed the dermal PBPK model for CP considering physicochemical and biopharmaceutical properties of the cream in non-lesional skin assuming normal skin physiology [2]. The model was subsequently adapted to predict CP dermis concentrations in lesional psoriatic skin. To account for psoriasis pathology, the default values representing normal/non-lesional skin for epidermal layer thickness, dermal blood flow, and stratum corneum diffusivity were increased 4-fold, 5-fold, and 2-fold, respectively, based on information collected from literature [3 - 8]. Parameter sensitivity analysis (PSA) was performed for individual parameters, or their combinations.

Results. The depth of the microperfusion probe corresponds to dermal sub-layer 14 in the TCAT model. The observed CP concentrations in non-lesional skin are overlaid with model-projected unbound concentrations in sub-layers 12, 14 and 16 in Figure 1(A). The observed CP concentrations in lesional psoriatic skin are overlaid with the projected CP unbound concentrations in sub-layer 14 for combinations of parameter values from PSA in Figure 1(B).

Conclusion. The results indicate (a) the model's ability to reflect the reported CP concentrations, and the (b) appropriateness of the implemented changes in psoriatic skin. Using CP as an example, the TCAT ™ Model projected dermis concentrations in non-lesional and psoriatic skin. Further model validation is warranted.

This project was supported by US FDA and HHS under U01 FD006526 and U01 FD007320 ($1M, 100% funded by FDA/HHS). The views expressed here do not reflect official policies of FDA or HHS, nor does any mention of trade names imply endorsement by the U.S. Government.

Effects of music elements on the activities of cytochrome P450 1A1 in Sprague Dawley rats

Ali Shalaby[1], Jinal Adhiya[1], Alaa Al-Dajani[1], Heidi Ahonen[2], Ayman El-Kadi[1], Tony Kiang[1]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, [2]Faculty of Music, Music Therapy Department, Wilfrid Laurier University, Waterloo, ON

Purpose:The cytochrome P450 (CYP) 1A1 enzyme plays a crucial role in drug metabolism (e.g., bronchodilators, antipsychotics), biosynthesis, and the activation/detoxification of carcinogens. This research aims to investigate the effects of music, a ubiquitous external stimulus, on CYP1A1 activities in male and female Sprague-Dawley (SD) rats.

Methods:Male and female SD rats (6-8 weeks old; 200-300 g; N=8 per group, with equal representation of each sex) were exposed to composed music (fast tempo, irregular rhythm, and atonal harmony [FT IR AH]) or the control (no music) for 24 h in a soundproof room. CYP1A1 activity was quantified in vitro using hepatic microsomes with 7-ethoxyresorufin (0-16 µM; 0.2 mg/mL protein) under initial velocity conditions, with resorufin production measured via fluorescence detection (excitation: 550 nm, emission: 585 nm). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (N=2, with equal representation of each sex) (20 µg/kg, IP) served as a positive control to validate CYP1A1 activity. Enzyme kinetics were analyzed using GraphPad Prism 10.4.1 with substrate inhibition model fitting.

Results:CYP1A1 activity is expressed as mean ± SEM (pmol/mg protein/min). Rodents exposed to TCDD demonstrated a statistically significant increase in CYP1A1 activity (116.5) compared to the control group (2.5±0.3) (Figure 1a). Our enzyme kinetic modeling revealed that FT IR AH music-exposed animals exhibited significantly higher V_{max} (2.50 ± 0.36 pmol/mg/min, $p < 0.05$) and intrinsic clearance (6.57 ± 0.72 µL/mg/min, $p < 0.05$) values compared to control animals (0.79 ± 0.18 pmol/mg/min and 2.09 ± 0.31 µL/mg/min, respectively), as determined by substrate inhibition fitting for resorufin formation. However, no significant difference was observed in K_m (Figure 1b). Detailed breakdowns of sex-dependency are presented in Figures 1C-F. Within the control group, intrinsic clearance was significantly higher in female rodents (2.71 ± 0.30 µL/mg/min, $p < 0.05$) compared to males (1.47 ± 0.34 µL/mg/min), suggesting a potential sex-related difference in metabolic activities.

Conclusion:Given the crucial role of CYP1A1 in drug metabolism and toxicology, our findings suggest that music could serve as a novel external stimulus for modulating CYP1A1 enzyme activity, potentially altering its therapeutic effects, toxic responses, or drug interactions.

Limitations of Allometric Scaling in Drug Development: Mechanistic Insights Beyond Body Weight

Marlon III Cadauan Mallillin[1, 2], Raimar Loebenberg[1], Neal Davies[1]

[1]University of Alberta, Edmonton, AB, Canada [2]University of Santo Tomas, Manila, Philippines

Purpose. This study examines the limitations of traditional allometric scaling in drug development, highlighting scenarios where body weight-based extrapolation fails to accurately predict human pharmacokinetics (PK) and pharmacodynamics (PD). It explores the necessity of integrating physiologically based pharmacokinetic (PBPK) modelling and biomarker-driven approaches to enhance drug safety and efficacy.

Methods. A structured review of the literature was undertaken using PubMed, EMBASE, and CINAHL to analyze the historical applications, successes, and failures of allometric scaling in drug development. Case studies of high-profile clinical failures, including TGN1412 and troglitazone, were reviewed to identify key factors contributing to interspecies scaling discrepancies. Additionally, a search for allometric scaling and PBPK software was conducted to assess the available computational tools used in drug development for dose extrapolation and risk mitigation.

Results. Traditional allometric scaling effectively predicts human doses for well-characterized small molecules but struggles with complex biologics, immunotherapies, and compounds with species-specific metabolism. The structured literature review confirmed that notable failures, such as TGN1412's severe cytokine response and troglitazone's unforeseen hepatotoxicity, underscore the inadequacy of mg/kg scaling in capturing interspecies differences in absorption, distribution, metabolism, and excretion (ADME). A review of computational tools identified various software platforms for allometric scaling and PBPK modelling, highlighting their potential to refine dose predictions. Studies further emphasized that supplementing allometric scaling with PBPK simulations and early human biomarker assessments improves prediction accuracy and reduces clinical risks.

Conclusion. While allometric scaling remains a valuable tool in drug development, its limitations necessitate a more mechanistic approach for complex therapeutics. Integrating PBPK modelling and human biomarker data enhances the reliability of dose extrapolation, improving safety and efficacy outcomes. The use of advanced computational tools in allometric scaling and PBPK modelling offers a strategic advantage in reducing clinical trial failures and facilitating the development of novel therapeutics.

jpps Journal of Pharmacy and Pharmaceutical Sciences

The formation of enantiomers of epoxyeicosatrienoic acids, and expressions of the involved metabolic enzymes, in male and female rats fed standard or high fat diets.

Amel Hamza, Dion r. Brocks, Ayman O.S. El-Kadi

University of Alberta, Edmonton, Canada

Purpose: Cytochrome P450 (CYP) derived epoxyeicosatrienoic acid (EETs) are bioactive lipids with enantiomeric forms with biological functions. Obesity can alter the CYP (2J and 2C23) involved in their formation and elimination (soluble epoxide hydrolase, sEH). Here the effects of sex and diet-induced obesity were examined on hepatic CYP expression, and the formation of the four regioisomeric EET enantiomers.

Methods: Sprague-Dawley rats (n=5/sex/diet) were fed either standard or high-fat diet for 14 weeks. Liver was collected and microsomal protein isolated. Expression of mRNA (real time PCR) and microsomal protein (Western blot) of the enzymes was measured. After spiking microsomes with arachidonic acid (1 mM), enantiomers of S/R or R/S 5,6-, 8,9-, 11,12-, and 14,15-EET were analyzed using LC-MS/MS. To assess the effects of sex and diet, two away ANOVA was used ($p<0.05$).

Results: CYP2C23 protein and mRNA levels significantly decreased in both males and females given high-fat diet. For CYP2J there was no significant change for protein, but in males mRNA was increased. Interestingly, hepatic sEH protein expression was significantly increased in obese male rats but remained unchanged in females; no difference was seen in mRNA. Female rats had decreased formation rates of 5R/6S-, 8S/9R-, 11S/12R-, and 14S/15R-EET, compared to males given the same diet; the HFD did not change formation rates. For 14R/15S-EET, HFD caused a decreased formation rate in males but not females, with no sex differences being apparent within the same diet. There was no effect of sex or diet in formation rates of 5S/6R- or 11S/12R-EET. The data suggests sex-related differences in the expressions of the CYP involved in EET formation. We were able to compare within the same sex, the effect of diet. Only for 14R/15S-EET, which is thought to be formed by CYP2J, was HFD observed to cause a decrease in its formation rate in males.

Conclusion: There were a number of sex-differences in EET formation. The HFD caused a decrease in CYP2C23 expression, although in EET formation rates its effect were restricted to 14R/15S-EET formation in males.