VANCOUVER NANOMEDICINE DAY

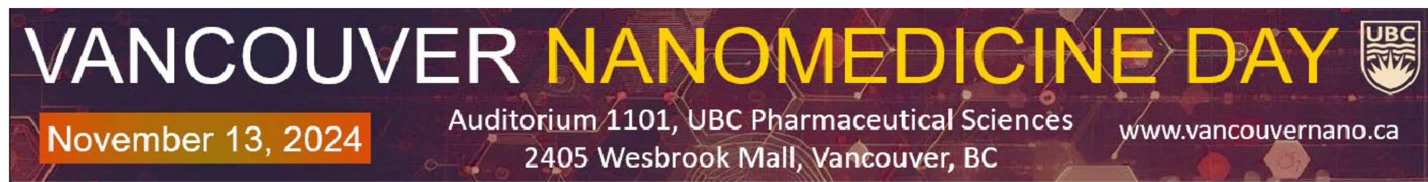
November 13, 2024

Auditorium 1101, UBC Pharmaceutical Sciences
2405 Wesbrook Mall, Vancouver, BC

www.vancouvernano.ca

All talks are in auditorium 1101 / overflow in auditorium 1201 / posters and breaks in lobby

	Session 1		Chair: Joel Finbloom
9:00 AM	Joel Finbloom / Urs Hafeli	Welcome to the 9th Vancouver Nanomedicine Day 2024	UBC Pharmaceutical Sciences
9:10 AM	Amani Hariri	Advancing DNA-Based Optical Sensors for Precision Diagnostics and Delivery	UBC Chemistry
9:45 AM	Cole DeForest	Environmentally Controlled Therapeutic Delivery from User-Programmable Hydrogel Biomaterials	University of Washington
10:20 AM	Mahdieh Shokrollahi Barough	Immunogels Can Shape the Local and Systemic Immune Response in Favor of Tumor Elimination	University of Victoria
10:35 AM	Tiffany Carlaw	CRISPR/Cas9 Base Editing Gene Therapy of Human Cystic Fibrosis Models	UBC Pharmaceutical Sciences
10:50 AM	Miffy Cheng	Liposomal Lipid Nanoparticles for Extrahepatic Delivery of mRNA	UBC Pharmaceutical Sciences
11:05 AM		Coffee Break	
	Session 2		Chair: Urs Hafeli
11:30 AM	Samir Mitragotri	A Hitchhiker's and Backpacker's Guide to Nanomedicine	Harvard University
12:05 PM	Sams Sadat	Lipid Nanoparticles for Pulmonary Delivery of mRNA	Cytiva
12:20 PM	Vanessa Chan	Targeted Delivery of TLR Agonists for the Enhancement of Cancer Immunotherapy	UBC Pharmaceutical Sciences
12:35 PM	XuXin Sun	A Novel Nanomedicine Based Combination Therapy for Lung Metastasis Melanoma	UBC and BCCRI
12:50 PM		Lunch Break	
	Session 3		Chair: Miffy Cheng
2:00 PM	Kim Woodrow	Design and Applications of Mucosal Delivery Systems	University of Washington
2:35 PM	Sriram Subramaniam	Accelerated Therapeutic Discovery with Cryo-EM and AI	Biochemistry, UBC
3:10 PM	Yihao Wang	Toward Ultra-Bright Luminescent Lanthanide Nanoparticles for Time-Gated Bioanalysis and Imaging	UBC Chemistry
3:25 PM	Nikki Salmond	EV-Associated Enolase1 as a Biomarker for the Diagnosis of Early-Stage Breast Cancer	UBC Pharmaceutical Sciences
3:50 PM	Erik Olsen	Simultaneous Confocal Fluorescence and Interferometric Scattering CLiC Microscopy for NP Analysis	UBC Michael Smith Laboratories
4:05 PM		Poster Session	
5:45 PM	Joel Finbloom	Poster Prizes and End of Nanomedicine Day	



Dear Participants,

It is our great pleasure to welcome you to **Vancouver Nanomedicine Day 2024**. It's truly an honour to organize this fantastic symposium for its 9th meeting, with over 250 people registered from across the Vancouver area and beyond. We are especially proud that this conference attracts a mix of academic and industry scientists to discuss their work and learn about new approaches to nanomedicine.

During Nanomedicine Day 2024, 14 talks and over 30 posters will highlight the discoveries and innovations in nanomedicine that are contributing to global progress in acute, chronic and orphan disease treatment and management. Nanomedicine has allowed us to deliver drugs directly to disease sites, to dramatically improve their efficacy and reduce their toxicity, and to enable gene therapies with the potential to treat most human diseases. Diagnostics and imaging agents based on nanotechnology will help us to detect disease earlier and to more accurately monitor the effectiveness of therapy. In all of these areas, revolutionary developments are ongoing, at enormous speed.

Please use this day not only to learn about new research, but also as a chance to network with each other in order to spark new ideas and foster collaborations between clinicians, basic researchers, engineers, trainees, research partners, life science, pharma, and biotechnology companies, and beyond.

Thank you for attending and we look forward to an exciting day of science!

Sincerely,

Joel Finbloom Urs Hafeli

Co-Chairs, Nanomedicine Day 2024
Faculty of Pharmaceutical Sciences, University of British Columbia
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About the Invited Speakers

Amani Hariri, University of British Columbia
Department of Chemistry

Dr. Amani Hariri is an assistant professor of chemistry at UBC. After finishing her undergraduate studies in Chemistry, Dr. Hariri moved to Montreal where she pursued a PhD in Materials Science at McGill University with a research focus on DNA characterization at the single molecule level using fluorescence methodologies. In 2017, she started her postdoctoral fellowship at Stanford University in the lab of Prof. Tom Soh where she also became an instructor, and was focused on the development of advanced biosensors for detecting biomolecules at low concentrations directly in complex environment with high sensitivity and specificity for the early detection and personalized treatment of diseases.

The Hariri lab focuses on developing DNA-based nanosystems for precision delivery and diagnostics. They will create a range of systems from simple sensors to more sophisticated and complex delivery vehicles that respond to specific chemical or biological triggers offering precise control over drug encapsulation and release.

Cole DeForest, University of Washington
Departments of Chemical Engineering and Bioengineering.

Dr. Cole A. DeForest is the Weyerhaeuser Endowed Associate Professor in the Departments of Chemical Engineering and Bioengineering, the Associate Chair and Graduate Program Director of Chemical Engineering, the Director of Education of the Molecular Engineering & Sciences Institute, as well as a core faculty member of the Institute for Stem Cell & Regenerative Medicine at the University of Washington (UW) where he began in 2014.

He received his B.S.E. degree from Princeton University in 2006, majoring in Chemical Engineering and minoring in Material Science Engineering and Bioengineering. He earned his Ph.D. degree under the guidance of Dr. Kristi Anseth from the University of Colorado in Chemical and Biological Engineering with an additional certificate in Molecular Biophysics. His postdoctoral research was performed with Dr. David Tirrell in the Divisions of Chemistry and Chemical Engineering at the California Institute of Technology (Caltech). He has published ~70 peer-reviewed articles, including as the corresponding author for those appearing in *Nature Materials*, *Nature Chemistry*, *Nature Chemical Engineering*, *Advanced Materials*, *JACS*, *PNAS*, *Science Advances*, *Nature Reviews Materials*, and *Nature Reviews Bioengineering*. Dr. DeForest has received numerous research awards and honors including the Society for Biomaterials' Young Investigator Award (2020), NIH Maximizing Investigators' Research Award (MIRA R35, 2020), NSF CAREER Award (2017), and many others. Notably, he has also been recognized for excellence in teaching and was awarded the UW Presidential Distinguished Teaching Award (2016), given annually to a single Assistant Professor across all of the UW. His research has been supported through fellowships and grants from the National Science Foundation, the National Institutes of Health, the Defense Advanced Research Projects Agency, and the US Department of Education.

About the Invited Speakers

Samir Mitragotri, Harvard University
Paulson School of Engineering and the Wyss Institute of Biologically Inspired Materials

Professor Mitragotri's research is focused on drug delivery. His research has advanced fundamental understanding of biological barriers and has led to the development of new materials as well as technologies for the treatment of various ailments including diabetes, cancer, skin diseases, multiple sclerosis, and infections, among others. Many of his inventions have advanced to clinical technologies. His research has led to a number of start-up companies. At the same time, the fundamental knowledge developed through his research has advanced the understanding of the biology of barriers in the human body. Prof. Mitragotri has pioneered novel technologies using ultrasound and ionic liquids to enable transdermal delivery of proteins, peptides, and siRNA. He has also developed novel technologies including ionic liquids for oral delivery of proteins such as insulin and other peptides. Prof. Mitragotri has invented systems that make use of synthetic carriers hitchhiking on natural cells such as red blood cells, macrophages, neutrophils, T cells, and NK cells for targeted delivery of drugs and cells.

Professor Mitragotri is the *Hiller Professor of Bioengineering* and *Hansjörg Wyss Professor of Biologically Inspired Engineering* at Harvard University. He has authored over 400 publications, is an inventor on over 225 issued/pending patents, and he has given over 500 invited lectures. He is an elected member of the National Academy of Engineering, National Academy of Medicine and National Academy of Inventors. He is also an elected fellow of AAAS, CRS, BMES, AIMBE, and AA PS. He received BS in Chemical Engineering from the Institute of Chemical Technology, India and PhD in Chemical Engineering from the Massachusetts Institute of Technology.

About the Invited Speakers

Kim Woodrow, University of Washington
Department of Bioengineering

Kim A. Woodrow is a Professor in the Department of Bioengineering at the University of Washington. She earned her MS and PhD degrees from Stanford in Chemical Engineering. From 2006-2009, Dr. Woodrow was a postdoctoral fellow in Biomedical Engineering at Yale University. Since joining UW Bioengineering in 2010, Dr. Woodrow's research interests have focused on applications at the intersection of engineering and mucosal biology, where her lab works on the design and synthesis of biomaterials for applications in mucosal infections and mucosal immunity. Dr. Woodrow is an NIH-funded investigator, the recipient of the Creative and Novel Ideas in HIV Research (CNIHR) award from the Office of AIDS Research, a recipient of grants from The Bill and Melinda Gates Foundation, and was awarded a NIH Director's New Innovator Award. She has served as a member of the NIH-NANO study section, and on the technical program committees for BMES and the international HIV R4P conference. She has been recognized with the University of Washington FACET Award, Undergraduate Research Mentor Award and as the Science in Medicine New New Investigator for the School of Medicine.

Dr. Woodrow's laboratory researches the design and applications of mucosal delivery systems. The Woodrow lab has engineered materials and devices for different mucosal compartments that address applications in HIV, contraception, and immunity. In one example, core-shell nanoparticles were developed for targeting and combination drug delivery to gut-homing T cells in the GALT for HIV treatment and cure. In a second example, an oral cavity microneedle patch was developed to investigate immunity to mucosal pathogens. In a third example, the Woodrow lab has developed a long-acting uterine system for applications in gynecological health.

Sriram Subramaniam, University of British Columbia
Department of Biochemistry and Molecular Biology

Dr. Subramaniam received his Ph.D. in Physical Chemistry from Stanford University and completed postdoctoral training in the Departments of Chemistry and Biology at the Massachusetts Institute of Technology. He began his independent research career at the Johns Hopkins School of Medicine, first as Assistant Professor, and then as Associate Professor before he moved to the National Cancer Institute, NIH as Senior Investigator and Chief of the Biophysics Section in the Laboratory of Cell Biology in the intramural program of the National Cancer Institute.

He has published over 177 peer-reviewed research publications, including journals such as Cell, Nature and Science. He has a career h-index of 58, and his publications have been cited over 12,340 times. His research contributions have been recognized by recent major awards and nominations including the National Cancer Institute Research Highlights Award (2013), Federal Technology Transfer Award (2015), NIH Director's Award for Scientific Excellence (2015), Orloff Award from NHLBI, NIH for outstanding scientific contribution (2016), election as Fellow of the Biophysical Society (2018), and nomination for the Breakthrough Prize in the Life Sciences.

Immunogel Containing Gemcitabine Can Shape the Local and Systemic Immune Response in Favor of Tumor Elimination

Mahdieh Shokrollahi Barough[1,2], Amir Seyfoori[2], Esfandyar Askari[2], Mehdi Mahdavi[3], Nazanin Mojtabavi[1], Mohsen Akbari[2,4]

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2. Laboratory for Innovations in Micro Engineering (LiME), Department of Mechanical Engineering, University of Victoria, Victoria, British Columbia, Canada
3. ATMP Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
4. Center for Advanced Materials and Related Technologies, University of Victoria, Victoria, British Columbia, Canada

Background: Injectable hydrogels with chemotherapy agents offer controlled release and targeted delivery in solid tumors. Neoadjuvant therapy in triple-negative breast cancers can utilize these hydrogels for tumor resection and immune system enhancement. Gemcitabine reduces regulatory T cells and MDSCs within tumors.

Methods: We developed an injectable hydrogel with gelatin, Laponite, and gemcitabine, and administered it subcutaneously to breast cancer animal models with 4T1 cells. This study comprised three animal experiments: a drug optimization pilot, an anti-tumor response formulation, and an immune response evaluation.

Results: Dose optimization indicated that 300 µg/ml gemcitabine killed tumor cells without harming immune cells and reduced regulatory T cells. The hydrogel with 6% gelatin and Laponite stopped tumor growth and healed some animals, making it ideal for immune response studies. Drug-loaded hydrogel increased CD8-positive lymphocytes and reduced regulatory T cells more than free-drug administration. Drug-free hydrogel had a limited synergistic anti-tumoral effect and increased helper T1-related genes. Over 80% of immune cells in drug-loaded hydrogels were CD3-positive.

Conclusion: Drug-loaded hydrogels shaped immune cells in lymph nodes and spleen, showing a systemic effect from local administration. The decrease in regulatory-T cells and increase in active CD107a-positive T cells in lymph nodes indicate immune activation against the tumor.

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CRISPR/Cas9 Base Editing Gene Therapy of Human Cystic Fibrosis Models Following Topical Application of Lung-Optimized Lipid Nanoparticles

Tiffany Carlaw[1], Belal Tafech[1], Gaurav Sadhnani[2], Jerry Leung[3], January Weiner[2], Kevin An[4], Anita Balász[5], Hendrik Fuchs[5], Dieter Beule[2], Marcus A. Mall[2,5], Jay Kulkarni[4], Pieter R. Cullis[3,4], Sarah Hedtrich[1,2,6,7]

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[7]Max-Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin 13125, Germany

Inherited lung diseases are a challenge for the development of gene therapy strategies. In particular the mucosal lining is a significant barrier for effective delivery via an inhalant route [1]. Recently, we have made significant strides to overcome this delivery barrier and are primed for the development of novel gene therapeutics [2, 3]. We selected seven adenine deaminase base editor amenable mutations causing cystic fibrosis, an inherited lung disease caused by a dysfunctional CFTR gene.

We designed and are currently screening CRISPR/Cas9 base editing strategies to correct each pathogenic mutation selected. Starting with $\textit{CFTR}^{R1162X}$ we obtained patient cell line (nasal epithelial cells) to use as a model to evaluate and optimize gene editing in primary cells. We then optimized our gene editing approach by testing novel base editor constructs, comparing modifications to the mRNA structure and modifications to sgRNA stability.

We screened various sgRNA designs, base editors and ratios of mRNA:sgRNA using Lipofectamine™ RNAiMAX to effectively repair our target mutation $\textit{CFTR}^{R1162X}$ in primary patient derived cells. Next, we sought to optimize our mRNA expression by comparing different untranslated regions (UTRs). We then compared existing and novel modifications to improve the stability of the sgRNA. Lastly, we encapsulated our RNA cargo in lipid nanoparticles (LNPs) and observed significant on-target base editing.

The next steps in this project will be to demonstrate significant restoration of CFTR function the gold standard Ussing Assay assessed after transmucosal delivery of our lead LNP formulation and optimized cargo in 3-dimentional transwell models of lung epithelium.

1. Boegh, M. and H.M. Nielsen, *Mucus as a barrier to drug delivery – understanding and mimicking the barrier properties.* Basic Clin Pharmacol Toxicol, 2015. **116**(3): p. 179-86.
2. Bolsoni, J., et al., *Lipid Nanoparticle-Mediated Hit-and-Run Approaches Yield Efficient and Safe In Situ Gene Editing in Human Skin.* ACS Nano, 2023. **17**(21): p. 22046-22059.
3. Tafech, B.C., T.; Leung, J.; Sadhnani, G.; Fuchs, H; An, K.; Kulkarni, J.; Cullis, P.; Hedtrich, S. , *Lipid Nanoparticle-Mediated Gene Editing Following Topical Application*, U.o.B. Columbia, Editor. 2024.

Liposomal Lipid Nanoparticles for Extrahepatic Delivery of mRNA

Miffy Hok Yan Cheng[1]

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Advancing the development of safe, highly effective lipid nanoparticles (LNP) for extrahepatic delivery is a continuous endeavour aimed at broadening the scope of commercial nucleic acid therapeutics. Current clinical LNP formulations are rapidly cleared from the circulation following intravenous (i.v.) administration and limits mRNA delivery to other tissues. To address this, we investigate the properties of LNP mRNA systems containing high proportions of bilayer forming lipids, using equimolar egg sphingomyelin and cholesterol as the bilayer-forming components. Our findings reveal that optimizing the ratio of bilayer forming lipids to ionizable lipids can induce liposomal morphology with a solid core suspended in an aqueous interior surrounded by a lipid bilayer. Notably, liposomal LNPs improved blood circulation and enhance protein expression in spleen, lymph nodes and pancreas compared to Onpattro like formulations. The prolonged blood circulation lifetime is attributed to reduced plasma protein adsorption. The transfection competency of liposomal LNP systems is attributed to export of the solid core from the LNP as the endosomal pH is lowered.

This research was supported by Canadian Institutes for Health Research.

Lipid Nanoparticles for Pulmonary Delivery of mRNA

Sams M. A. Sadat[1], Ruchi Sharma[1], Leanna Yee[1], Nikita Jain[1], Jay Paquette[1], and Anitha Thomas[1]

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Despite the recent successes of lipid nanoparticle (LNP)-mediated mRNA vaccines against SARS-CoV-2, inhalation-based gene therapy and mucosal immunization remain challenging with respect to treating various lung diseases. Systemic therapeutic applications of currently available lipid and non-lipidic nanoparticulate platforms have been investigated mostly for liver diseases because of their favorable accumulation tendency into the liver, which in due course restricts their access to other organs, including the lungs. Herein, we demonstrate the preclinical success for the direct intranasal (IN) delivery of mRNA-encapsulated LNPs towards lung-targeting applications.

Firefly luciferase (Fluc) enzyme-encoded mRNA was encapsulated in LNPs using NxGen microfluidic NanoAssemblr® technology platform. The lead IN solution compositions were mixed with the Fluc-mRNA-encapsulated LNPs at a 1:1 ratio and were assessed for *in vivo* luciferase expression in mouse lungs following a single IN administration of 0.1 mg/Kg dose of Fluc-mRNA. In addition, IN vaccine efficacy study for the IN solution was performed in female BALB/c mice receiving SARS-CoV-2-encoded self-amplifying mRNA (saRNA)-encapsulated LNPs at a prime and booster dose of 5 µg with 4-weeks apart. Immune responses were measured in the collected plasma serum of the vaccinated mice.

Our preclinical investigations exhibited significant *in vivo* luciferase expression in the lungs of female BALB/c mice receiving intranasally administered Fluc-mRNA LNPs mixed with IN solution. In the immunogenicity study, IN mRNA-LNP vaccination systemically induced SARS-CoV-2 spike protein-specific binding and neutralizing antibodies in vaccinated mice.

Our IN solution platform is capable of delivering nucleic acids-loaded nanoparticles into the lungs by retaining the Critical Quality Attributes (CQA) of the drug product. Thus, the future development of non-systemic nanoparticle-based delivery of nucleic acids for various lung diseases looks promising.

Targeted Delivery of TLR Agonists for the Enhancement of Cancer Immunotherapy

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TLR7/8 agonists like Resiquimod (R848) and Imiquimod (IMQ) are excellent immune stimulating agents, but they are rapidly absorbed, are generally insoluble, and can trigger cytokine storm if administered systemically. Treatment of solid cancers are often aggressive and result in a high rate of recurrence, therefore immunotherapy can be favoured for a lasting response. We demonstrate the targeted delivery of R848 and IMQ using lipid nanoparticles to treat peritoneal metastasis of colon cancer (PM), and liver cancer, respectively. For PM, sequential dosing of our formulation of R848 achieved a 60% cure rate, defined as survival past 160 days and no recurrence upon rechallenge. Tuning of the tumor microenvironment from immunosuppressive to immune active was also observed throughout the duration of treatment. Combination with anti-PD-1 antibody boosted cure rates to 90%. For liver cancer, our formulated IMQ decreased the size of tumor nodules on the liver and treated mice were protected against lung metastasis. RNA-seq revealed that administration of the formulated IMQ at a single dose resulted in an increased expression of genes related to innate immune expression in the tumour, whereas by the last dose, an increased infiltration of cytotoxic T cells could be observed, likely as a consequence of this early innate immune activation.

A novel nanomedicine based combination therapy for lung metastasis melanoma

XuXin Sun[1,2], Cat Zhang[1], Casmil Credo[4], and Marcel Bally[1,2,3], Anna Blakney[4]

[1]Experimental Therapeutics, BC Cancer Research Institute, [2]Faculty of Medicine, University of British Columbia (UBC), [3]Faculty of Pharmaceutical Sciences, UBC, [4] Michael Smith Laboratories, UBC

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Background: Melanoma is an aggressive skin cancer that often spreads to the lung at late stage. The melanoma tumor is often considered immunologically suppressive which lacks the infiltration of cells that exhibit antitumor activities.[1] Interleukin-12 (IL-12) based cytokine immunotherapy has shown some potential in treating melanoma but its use is associated with dose related toxicity [2]. There is an urgent need to improve this conventional cytokine therapy. We have used an IL-12 samRNA-LNP (self-amplifying messenger RNA-lipid nanoparticle) system to produce the IL-12, triggering a systematic immune response to combat the melanoma. Irinotecan (CPT-11) is a topoisomerase I inhibitor that is currently used in first and second-line chemotherapy treatments for multiple cancers. A novel liposomal formulation of CPT-11 (referred to as Irinosome High C) has been developed. This formulation relies on Cu(II) ions to complex CPT-11 and control its release from the liposome. More importantly, it is found that the Irinosome High C can "normalize" the tumor's vasculature, shifting the tumor microenvironment from immune-suppressive to immune-supportive. Therefore, it is proposed that the liposomal formulation may be ideally suited to be used in combination with immunotherapeutic such as the IL-12 samRNA-LNPs.

Method: samRNA-LNPs are administered intravenously in C57BL/6 mice to determine the safe and appropriate dose. Blood was withdrawn from the mice to also determine the time point which have elevated IL-12 levels in blood stream. Murine melanoma cancer cell line (B16F10) are inoculated intravenously into C57BL/6 mice to mimic lung metastases of the melanoma. samRNA-LNP and Irinosome High C are administered sequentially, and the survival data was based on clinical scores. Lungs from combination treated and control groups are extracted on Day 18 (approximately 3 days before endpoint for saline control group) and the total black nodules in the lung are counted. Splenocytes from all groups are also extracted to perform ex vivo co-culture assay with B16F10 cells to verify whether the combination therapy generate greater immune response against the cancer.

Results: Mice receiving combination therapy of Irinosome High-C and the IL-12 samRNA-LNP have longer survival compared to monotherapy groups or the control group. This group also had a lower tumor burden (judged by the total number of black nodules formed in the lung). Splenocyte co-culture assay with B16F10 cells demonstrate that IL-12 samRNA-LNP treated groups have higher IL-12 levels in the spleen and key anti-tumoral cytokines such as IL-2 and IFN-γ are also elevated.

Conclusion: This novel liposomal CPT-11 formulation Irinosome High C has great therapeutic potential to be used in combination with new saRNA-LNP IL-12 immunotherapies for melanoma. The new combination approach may offer a cheaper and potentially safer and more efficacious treatment plan. Future studies will be investigating the mechanism of how combination therapy improves local and systematic anti-tumoral immune response.

[1] Wang, L., Geng, H., Liu, Y., Liu, L., Chen, Y., Wu, F., Liu, Z., Ling, S., Wang, Y., & Zhou, L. (2023). Hot and cold tumors: Immunological features and the therapeutic strategies. *MedComm*, *4*(5), e343.

[2] Gao, W., Pan, J., & Pan, J. (2022). Antitumor Activities of Interleukin-12 in Melanoma. *Cancers*, *14*(22), 5592.

Toward Ultra-Bright Luminescent Lanthanide Nanoparticles for Time-Gated Bioanalysis and Imaging

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Time-gated imaging (TGI) is a very useful method for analyzing biological samples. Many cells, tissues, and bodily fluids exhibit background autofluorescence that hinders fluorescence-based measurements of biomarkers. TGI avoids this background by using lanthanide complexes and other emitters with long photoluminescence (PL) lifetimes. A delay or “time gate” between pulsed excitation and measurement of PL emission intensity allows the comparatively short-lived autofluorescence to decay to negligible levels while the lanthanide complex is still emitting. One challenge with this methodology is that it typically requires sophisticated and expensive laboratory instruments. We recently addressed this challenge with the development of a simple and low-cost smartphone-based device for TGI. A remaining challenge is that luminescent lanthanide complexes (LLCs) are not very bright.

To achieve higher brightness, we are developing novel polymer dot (Pdot) materials doped with a terbium (Tb) LLC. The host polymer in the Pdot nanoparticle is an acrylate with pendant carbazole fluorophores, which is blended with a polymer with pendant Tb LLCs. The carbazole has the required properties to act as a Förster resonance energy transfer (FRET) donor with the Tb LLC as the acceptor. With efficient energy transfer, the Tb LLC is able to borrow brightness from the very large molar absorption coefficient of the carbazole Pdot. This presentation will describe the physical characterization of these nanoparticles, spectroscopic characterization of the energy transfer and emission, optimization of nanoparticle composition, and utilizing and benchmarking the nanoparticles for human Erythropoietin (hEPO) detection by immunoassay and SK-BR3 human breast cancer cells immunolabelling and cellular imaging, including with a smartphone-based device for TGI. The novel Pdots will enable new types of TGI and more sensitive analyses. These advancements will provide more efficient, more accessible, and more inclusive methods for enhancing health care.

Extracellular Vesicle-Associated Enolase1 as a Biomarker for the Diagnosis of Early-Stage Breast Cancer.

Salmond N[1], Moravcova R[2], Tam K[1], Khanna K[1], Rogalski J[2], Lynn K[3], Brackstone M[4], Watson PH[5], Williams KC[1].

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[5]*Deeley Research Centre, BC Cancer Agency, University of British Columbia, Victoria, BC, Canada.*

The key to successful treatment and long-term remission of breast cancer is to diagnose it early. Approximately 80% of breast growths are benign, but current methodologies used for breast cancer diagnosis are unable to distinguish a benign versus cancerous mass, meaning that many unnecessary and invasive needle biopsies are carried out every year. We need a liquid blood biopsy – a routine blood test – to diagnose breast cancer tumors at the earliest stage. We propose that extracellular vesicles (EVs) -- circulating tumor nano-fragments -- show great promise for the development of new diagnostic procedures because the contents of EVs reflect those of the parental cancer cell. Size exclusion chromatography was used to isolate EVs from 77 early-stage breast cancer, 19 benign, and 20 healthy plasma samples. Peptide mapping identified EV-associated enolase 1 as a predictive early breast cancer biomarker, and the biomarker was validated in patient plasma using a high throughput and clinically relevant ELISA assay. Plasma enolase 1 was able to diagnose breast cancer patients at the earliest stage-I (AUC 0.7864. Sensitivity 80 % = specificity 72.73 %) and distinguish cancerous from benign breast growths. MDA-MB-231 breast cancer cells were shown to secrete both soluble and EV-associated enolase 1 into conditioned media. MDA-MB-231 cells were grown as a vascularized tumor on a chicken embryo model. The cell line-derived tumors secreted enolase 1 into the plasma of the chicken embryo. The data indicates that a simple blood test for enolase 1 could be used as a screening tool to identify individuals at high risk of breast cancer, enabling diagnosis at the very earliest stage. The test could also be used alongside imaging techniques to determine if someone has a benign growth or a cancerous growth to aid the clinician's decision as to whether a biopsy is necessary.

Simultaneous Confocal Fluorescence and Interferometric Scattering CLiC Microscopy for Detailed Size, Mass, and Loading Characterisation of Individual Suspended Nanoparticles

Eric Boateng*[1,2], Erik Olsén*[1], Albert Kamanzi[1,3], Yao Zhang[1,4], Bin Zhao[5], Ed Grant[2], Pieter R. Cullis[5], Sabrina Leslie[1,6]

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[3]ScopeSys Inc., Vancouver, BC, Canada
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[5]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada
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* These authors contributed equally to this work.
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The ever-improving sensitivities of both label-free optical microscopy and fluorescence microscopy techniques combined with their single particle resolution have made it such that optical microscopy techniques are now essential tools in characterizing the size, refractive index, and material composition of nanoparticle samples such as lipid nanoparticles (LNPs). However, the existing quantitative characterization methods struggle with simultaneous measurements of multiple fluorescence signals while also measuring the label-free scattering signal of individual suspended nanoparticles, which significantly limits the ability to correlate particle properties with dynamic function. Moreover, the few existing methods cannot be used to characterise nanoparticles both when in solution and when inside cells, which restricts their impact in nanomedicine development.

Here, by combing a confocal microscope suitable for live-cell imaging with convex lens-induced confinement (CLiC)[1], we show that confocal interferometric scattering (iSCAT), differential interference contrast (DIC), and multiple fluorescence signals can all simultaneously be recorded and used for detailed characterisation of suspended nanoparticles trapped inside microwells. We apply this novel multiparametric imaging platform to investigate a series of mRNA-LNP formulations of practical therapeutic interest, providing simultaneous size, refractive index, fluorescence markers, and cargo information of individual suspended nanoparticles under different solution conditions. For example, as a function of mRNA-LNP formulation, we investigate changes in size, refractive index, and mRNA payload, and as a function of solution pH, we investigate changes in size, refractive index, and brightness of a pH-reporting lipid nanoparticle. Combined, these achievements establish a quantitative foundation for simultaneous confocal-fluorescence and label-free characterisation of nanoparticles. Moreover, since the same optical setup can detect individual biological nanoparticles both when in solution and when inside cells, this method development is a key step towards time-resolved quantitative optical characterization of the journey of single mRNA-LNPs inside cells.

[1] Kamanzi, A., *et al.* Simultaneous, single-particle measurements of size and loading give insights into the structure of drug-delivery nanoparticles. *ACS Nano* **15**,19244-19255 (2021).

CANADA'S IMMUNO-ENGINEERING & BIOMANUFACTURING HUB

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NOVEMBER 2024

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OVERVIEW

Canada's Immuno-Engineering and Biomanufacturing Hub brings together a coalition of partners to accelerate the development and manufacturing of lifesaving medicine. Led by the UBC, CIEBH is pursuing an integrated program of biomedical research and talent development, along with building associated infrastructure to accelerate the development and manufacturing of immune-based therapeutics such as next-generation vaccines, antibodies, and cell and gene therapies. By establishing and aligning the **capabilities to discover, develop, manufacture and test homegrown vaccines and therapeutics in less than 100 days**, we are enhancing resilience and readiness to respond rapidly to future pandemics and other health threats.

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POSTER PRESENTATIONS (P1-P16)

VANCOUVER NANOMEDICINE DAY

November 13, 2024

Auditorium 1101, UBC Pharmaceutical Sciences
2405 Wesbrook Mall, Vancouver, BC

www.vancouvernano.ca

Poster Session is 4:05-5:45 in the UBC Pharmaceutical Sciences Lobby

P1	Shagun Kothari	Influence of Lipid-siRNA organisation on the dissociation of Porphyrin Lipid Nanoparticles	UBC Michael Smith Laboratories
P2	Eric Boateng	Intracellular Dynamics of LNP-mediated mRNA Delivery: Quantitative Sigle-Cell Profiling Toward Enhanced Therapeutics and Vaccines	UBC Michael Smith Laboratories
P3	Ashley Braun	Strategies for Producing Clinical and Commercial RNA-LNP Drug Products	Cytiva
P4	Noah Brittain	Biofilm-Inspired Peptide Nanofibre Hydrogels for Bacterial Encapsulation	UBC Pharmaceutical Sciences
P5	Carraugh C. Brouwer	Imaging individual bioaffinity interactions with a real-time, label-free immunoassay	UBC Chemistry
P6	G.H. Darwish	Supra-Quantum Dot assemblies and Immunoconjugates for Enhanced Intra- and Extra-cellular Imaging via Microscopy and Smartphone-based Detection	UBC Chemistry
P7	Tahirah DCosta Correia	Fabricating core-shell microcapsules for reliable and repeatable ultrasound triggered drug-release	McMaster University
P8	Vanessa Dos Passos Maio	Influence of Chronic Kidney Disease and Vascular Calcifications on the Fate of Targeted Polymer Nanomedicines	Laval Faculty of Pharmacy
P9	Maya Stibbards-Lyle	Single-particle CLiC size-and-payload analysis of mRNA-lipid-nanoparticles with applications to vaccines and genetic medicines	UBC Michael Smith Laboratories
P10	Giovanna Cassone Salata	Co-Loaded Nanoemulsion with Paclitaxel and P-Glycoprotein Inhibitor Suppresses Clonogenicity, Alters Cell Cycle Progression, and Triggers Apoptosis in Triple-Negative Breast Cancer Cells	University of São Paulo
P11	Nooshin Ghodsian	Comparison of plant based and animal based sources of squalene as adjuvant for injection applications	Evonik
P12	Sadie Graves	Single-particle CLiC quantification of mRNA-lipid nanoparticle size and RNA copy number without the use of labels in formulation	UBC Michael Smith Laboratories
P13	Victoria Hartman	Optimized prime: a transformative LNP composition for scalable RNA vaccines	Cytiva
P14	Ruchi Sharma	Effect of LNP components on mRNA mediated Protein Expression of Lipid Nanoparticles in Different Organs	Cytiva
P15	David Jung	From high throughput screening to commercial manufacturing of Lipid Nanoparticles	Evonik
P16	Jad Kaj	Utilizing the Aggregation Behavior of Fluorescent Dyes to Develop Novel Fluorescent Nano-assemblies	UBC Chemistry

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POSTER PRESENTATIONS (P17-P33)

VANCOUVER NANOMEDICINE DAY

November 13, 2024

Auditorium 1101, UBC Pharmaceutical Sciences
2405 Wesbrook Mall, Vancouver, BC

www.vancouvernano.ca

Poster Session is 4:05-5:45 in the UBC Pharmaceutical Sciences Lobby

P17	Seth Keenan	Prototype Smartphone-Based Device for Flow Cytometry	UBC Chemistry
P18	Albert Zehan Li	Investigating the Effect of Fluorophore-Quencher Modifications on the Performance of Optical Aptamer-Based DNA Sensors	UBC Chemistry
P19	Hannah Ly	Optimization of Multiplex CRISPR-Cas9 Editing of Human Primary T Cells Using Lipid Nanoparticles (LNPs) and Subsequent Off-Target Evaluation	Cytiva
P20	Maya Nathani-Sim	Improved Nucleic Acid Delivery by Microneedle Patch Vaccination	Univesity of Washington
P21	Kevin Fox	Implications of circulatory protein adhesion on lipid nanoparticle pharmacokinetics	UBC Biochemistry
P22	Mana Novin	Efficient Gene Editing in CD34+ Hematopoietic Stem and Progenitor Cells Using Non-Viral Lipid Nanoparticles	Cytiva
P23	Chinekwu Nwagwu	Stimuli-Responsive Nanocomplexes for Antibiotic and Enzyme Codelivery in Biofilm Infections	UBC Pharmaceutical Sciences
P24	Jakob Ostberg	Assessment of iron oxide magnetic nanoparticles for adipose-tissue targeted delivery of anti-diabetic agents: A proof-of-concept study using rosiglitazone	Univeristy of Northern British Columbia
P25	William Primrose	Controlling the Size of Glassy Organic Dots Exhibiting Thermally Activated Delayed Fluorescence for Bioimaging	UBC Chemistry
P26	Kelly Rees	Dextran-Encapsulated Nanoparticles and Super-Nanoparticle Assemblies for Application to Cellular Immunolabeling	UBC Chemistry
P27	Agnes Szwarczewski	Towards Intracellular Deployment of Concentric FRET Probes for Multiplexed Bioimaging and Sensing	UBC Chemistry
P28	Christine Traaseth	Development of Smartphone-based Devices for Nanomaterial-enabled Point-of-Care Assays	UBC Chemistry
P29	April St Pierre	Developing novel lipid nanoparticle formulations to enhance extrahepatic delivery	UBC Pharmaceutical Sciences
P30	Tinotenda Masvikeni	Red Emissive Organic Molecules Exhibiting Delayed Emission for Bioimaging	UBC Chemistry
P31	Roza Vaez Ghaemi	A smart material as a replacement for Descemet's membrane endothelium complex (DMEC)	UBC Chemical and Biological Engineering
P32	Yao Zhang	Developing Iron Oxide and Gold Loaded Lipid Nanoparticles for Triggered Release	UBC Biomedical Engineering
P33	Mehar Gayatri Namala	Gene editing in liver towards the treatment of life-threatening cardiovascular diseases: PoC for efficient in-vivo genome editing/base editing using Lipid Nanoparticle Library	Cytiva

CANADA'S IMMUNO-ENGINEERING & BIOMANUFACTURING HUB

Poster Presentations from Submitted Abstracts

P1

Influence of Lipid-siRNA organisation on the dissociation of Porphyrin Lipid Nanoparticles

Shagun Kothari[1,2], Yulin Mo[3], Albert Kamanzi[1,4], Juan Chen[5], Gang Zheng[3], Sabrina Leslie[1,6*]

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siRNA-Lipid nanoparticles (LNP) are a promising addition to the therapeutic toolbox for targeted gene therapies to treat diseases. LNPs protect the siRNA cargo from degradation and deliver it into cells, causing RNA interference at tumour site. However, degradation and inefficient endosomal escape of siRNA reduce their effectiveness. Additionally, the poorly understood structural organization of individual LNP components and the relationship between LNP structure and intracellular behavior, particularly before RNA cargo release, remains obscure. One approach to overcome this and to optimize these nanomedicines is to investigate light-triggered release of siRNA at the tumour site.

Herein, we report a strategy that uses Convex Lens-induced Confinement (CLiC) microscopy to visualise the dynamics of photo-activated release of siRNA cargo through single-particle imaging of trapped nanoparticles with different N/P ratios. We irradiated Porphyrin-LNPs, made by incorporating photosensitive lipids called porphyrin lipids, and the siRNA cargo tagged with Alexa 488 dye, with 647 nm and 488 nm wavelength lasers respectively. We applied CLiC two-colour imaging to simultaneously detect the release of the siRNA cargo and dissociation of the porphyrin-LNP. The results of these experiments elucidated that porphyrin-LNP maintained the physical properties of an LNP and generated reactive oxygen species, causing a release of the siRNA cargo. Moreover, our studies revealed that lipid-siRNA organisation significantly affects the performance of functional lipids in response to external stimuli like light, thereby affecting siRNA escape efficiency. We observed that increased siRNA loading impacts LNP dissociation; siRNA release increased with decrease in the N/P ratio.

Here, we established that by examining the photoactive kinetic and structural properties of these drug-delivery vehicles as a function of design parameters at the single-particle level, we gain new insights into lipid-siRNA organisation within LNPs and their release mechanisms. In turn, we will use these insights to inform the advancement of functional light-activatable LNP formulations.

P2

Intracellular Dynamics of LNP-mediated mRNA Delivery: Quantitative Sigle-Cell Profiling Toward Enhanced Therapeutics and Vaccines

Eric Boateng[1,2], Erik Olsen[1], Yao Zhang[1,3], Albert Kamanzi[2], Bin Zhao[4], Frances Lock[5], Alexa Smith[1], Charles Yan[6], Miffy Cheng[5], Edward Grant[2] Ronald Chan[1], Jenny Moon[1], Leonard Foster[1], Robin Coope[5], Pieter Cullis[4], Sabrina Leslie[1,6]

[1] Michael Smith Laboratories, [2] Department of Chemistry [3] School of Biomedical Engineering, [4] Department of Biochemistry & Molecular Biology, [5] BC Cancer Research Institute, [6] Department of Physics and Astronomy, University of British Columbia, Vancouver, British Columbia, Canada.
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Lipid nanoparticles LNPs, as the primary delivery vehicles for messenger RNA-based vaccines (e.g., COVID-19 vaccines), play a pivotal role in protecting mRNA cargo and facilitating its delivery into target cells [1,2]. However, the mechanisms governing LNP fusion, trafficking, and mRNA release within cells remain poorly understood, particularly at the single-cell level. By employing multimodal single-cell imaging and super-resolution microscopy methods, we aim to provide insights that relate the impact of LNP size, mRNA payload, and multi-step intracellular interactions to their clinical therapeutic outcomes. We hypothesize that variations in LNP biophysical properties such as size, structure, payload and dynamics, and mRNA release kinetics at the single-cell level can be studied and applied to better understand and optimize therapeutic efficacy.

Parallel work in our lab (being presented by Erik Olsen) has validated the Convex Lens-induced Confinement (CLiC) imaging platform together with interferometric scattering (iSCAT) and multichannel fluorescence microscopy for detailed single-particle mRNA-LNP characterization [3]. Here we apply the same microscope platform to image single cells for drug development, and demonstrate subcellular localization with an enhanced signal–to-noise-ratio and multichannel fluorescent signal detection sensitivity compared to conventional live-cell imaging. With this new platform, we generate super-resolution single-cell profiles detailing multiple single-LNP trajectories, including details of mRNA release and cell viability [4]. We dynamically track and quantify LNP size, number, and mRNA cargo co-localized with cellular organelles like lysosomes and endosomes in the cells, and correlate these properties for trajectories with different outcomes.

Over the long term, by exploring the complexities of LNP-mediated mRNA delivery at the single-cell level and correlating imaging data with genomics, animal study and clinical results, we aim to unveil key factors influencing mRNA therapeutic and vaccine effectiveness. The expected findings could improve delivery efficiency, revolutionize LNP formulation design, and expand treatment possibilities for challenging medical conditions.

[1] Pardi, N., et al. mRNA vaccines – a new era in vaccinology. *Nature Reviews Drug Delivery.* **17**(4) 261-279 (2018).
[2] Mo, Y. et al. Light-Activated siRNA Endosomal Release (LASER) by Porphyrin Lipid Nanoparticles. *ACS Nano*. **17**(5) 4688-4703 (2023).
[3] Leslie, R.S., Fields, A.P., and Cohen, A.E. Convex lens-induced confinement for imaging single molecules. *Analytical Chemistry*. **82**(14): p. 6224 (2010).
[4] Johanna M. J., et al. Cellular and delivery barriers to lipid nanoparticle mediated delivery of RNA to the cytosol. *bioRxiv* (2024). https://doi.org/10.1101/2024.05.31.596627

P3

Strategies for Producing Clinical and Commercial RNA-LNP Drug Products

Ian Johnston, Ashley Braun, Robert Young, Maria Kerin, Deepkanwal Singh, Braeden MacDougall, Brian Ma, Anastasia Lazic, Leanna Yee, Curtis Robin, Felix Yuen, Pierrot Harvie, Suraj Abraham, Samuel Clarke

Affiliation: Cytiva, Vancouver, Canada

Presenting Author: Ashley Braun

The RNA-lipid nanoparticle (LNP) vaccines for the SARS-CoV-2 pandemic highlight the impact of genomic medicines deployed at scale. Despite growing momentum, developing RNA-LNPs still faces significant manufacturing challenges. The mixing process to encapsulate RNA within LNPs is among the most difficult unit operations to scale-up to commercial throughput rates and batch sizes, while maintaining critical quality attributes (CQAs) such as size and biological potency.

We have developed the new NanoAssemblr commercial formulation system (CFS) for commercial-scale production of RNA-LNPs. This system utilizes the same NxGen mixing technology across the complete NanoAssembr suite of instrumentation to enable rapid scale-up and production. To demonstrate the scale-up capability of the suite, we produced and characterized a SARS-CoV-2 saRNA-LNP vaccine candidate under increasing flow rates and batch volumes to mimic the drug development process from discovery through to commercial production. The formulation flow rate was stepwise increased from 12 mL/min using the NanoAssemblr Ignite+ through to 800 mL/min using the new NanoAssemblr CFS. The saRNA-LNPs were all of similar quality with sizes ranging from 61 – 83 nm, PDI < 0.18 and RNA encapsulation efficiency > 94%. ANOVA testing showed no significant differences in size, PDI, or encapsulation efficiency when comparing instruments or mixers. *In vitro* potency assays showed similar dose response curves between samples and consistent EC_{50} values between 1.3 to 3.6 ng/mL. Finally, *in vivo* immunization studies showed a robust SARS-CoV-2 specific IgG response in all instrument-mixer combinations with titers varying between 1.94×10^5 to 2.83×10^6 ng/mL.

This work demonstrates that production of nucleic acid-LNPs with consistent CQAs can be achieved on a wide range of scales using NanoAssemblr instruments and NxGen mixers. The NanoAssemblr CFS can prepare RNA-LNPs at the scale and throughput needed to meet commercial manufacturing goals for range of applications including vaccines, in vivo therapeutics, and ex vivo cell therapies.

P4

Biofilm-Inspired Peptide Nanofibre Hydrogels for Bacterial Encapsulation

Presenter: Noah Brittain

Noah Brittain[1], Joel Finbloom[1]

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Background: Most bacteria live in fibrous matrixes called biofilms. Biofilms comprise biological polymers that provide nanoscale structural support and regulate beneficial bacterial behaviour. We are interested in using this beneficial bacteria-nanomaterial interaction to harness bacteria for human benefit. We will take inspiration from the protein fibres found within natural biofilms to create self-assembling oligopeptide nanofibres. These nanofibres are modularly assembled oligopeptides that self-assemble into fibres whose properties we can finely adjust by changing the peptide sequence of the starting oligopeptides. In addition, introducing peptides into the nanofibre with chemically reactive centres can create inter-fibre crosslinks to form a hydrogel. In this study, we aim to study how altering the material properties of these nanofibres and their hydrogel form changes their interaction with bacteria, allowing us to understand better how we can encapsulate bacteria in nanostructured systems.

Methods: Oligopeptides for nanofibre formation were formed using solid-phase peptide synthesis and then self-assembled into nanofibers, which was confirmed using electron microscopy, circular dichroism, the fluorescent beta-sheet indicator thioflavin T, and zeta potential. The fibres were then incubated with the model bacteria *Escherichia Coli*, and biocompatibility was assessed using fluorescent metabolic assays. Finally, we used thiol-maleimide cross-linking chemistry to form hydrogels and accessed bacterial distribution through confocal microscopy.

Results: We show that we can form nanofibers with different material/chemical properties, which changes their biocompatibility with *E. coli*. We then formed hydrogels that encapsulate *E. coli*, enabling us to change their 3-dimensional behaviour.

Conclusions: We have shown that bacteria can be encapsulated within peptide nanofibre hydrogels and that changes in peptide nanofibre chemistry can change bacterial behaviour. These findings will help to inform how material properties can be altered to change the encapsulation of bacteria for the many applications of bacteria in modern society.

P5

Imaging individual bioaffinity interactions with a real-time, label-free immunoassay

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Immunoassays rely on the highly specific interactions of antibodies and antigens to detect low concentrations of analytes in samples, making them highly useful in diagnostic tests and for biochemical research. However, traditional immunoassays such as enzyme-linked immunosorbent assay (ELISA) rely on the use of labels for detection, which can increase the complexity of sample preparation and the number of steps required to complete the assay. We present a novel label-free immunoassay based on interferometric scattering mass photometry (iSCAT). iSCAT detects single proteins in solution in real time by measuring the interference between scattered light from proteins adsorbing to an antibody-functionalized coverslip and a reference reflection from the coverslip. In addition to analyte detection from antibody-antigen interactions, iSCAT affords mass resolution of protein biomarkers, providing a further layer of analysis. The small sample volumes and low concentrations required for an iSCAT immunoassay are highly economical, and the shortened workflow delivers results in half the time of a typical ELISA. A label-free immunoassay for human IgM determines the concentration of IgM as low as 0.25 nM, and control experiments validate the specificity of the assay.

P6

Supra-Quantum Dot assemblies and Immunoconjugates for Enhanced Intra- and Extra-cellular Imaging via Microscopy and Smartphone-based Detection

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Abstract: The development of ultrabright labels for bioanalysis and imaging continues to grow and benefit applications that require highly sensitive detection (e.g. assays of low-abundance biomarkers, single-molecule tracking, or the use of smartphone-based devices for point-of-care diagnostics). One approach to developing ultrabright fluorescent materials is incorporating multiple copies of a bright luminescent material into a larger supra-nanoparticle assembly (supra-NP).

Here, we present a facile method for the preparation and immunoconjugation of supra-quantum dot assemblies (supra-QDs), where multiple QDs are arranged around a central silica nanoparticle. The physical and optical properties of single- and multi-color supra-NPs were characterized at the ensemble and single-particle levels. Notably, the fluorescence properties of QDs were preserved upon assembly into supra-NP, and single supra-NP were an order of magnitude brighter than individual QDs and nonblinking. The assembly of supra-QDs with mixtures of red, green, and blue colours of QD enabled access to a library of colours from the RGB space for multiplexed detection, including colours that are difficult to obtain from individual QDs.

Immunoconjugation of supra-QDs via affinity-based tetrameric antibody complexes (TACs) and via direct covalent conjugation enabled selective fluorescent labelling of extracellular (e.g. HER-2 antigens expressed on the surface of breast cancer cells) and intracellular biomarkers (e.g. aggregated nuclear protein biomarker for neurodegenerative disease). The enhanced brightness of the supra-NPs enabled measurements on multiple platforms: a research-grade fluorescence microscope, a smartphone-based imaging platform, and a smartphone-based flow cytometer.

Overall, supra-NPs are promising materials for enhanced bioanalysis and imaging—especially with devices that utilize a smartphone camera.

P7

Fabricating core-shell microcapsules for reliable and repeatable ultrasound triggered drug-release

Tahirah DCosta Correia[1], Kayla Baker[2], Dr. Todd Hoare[3].

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2. Department of Materials Science and Engineering, McMaster University, Hamilton, ON.
3. Department of Chemical Engineering, McMaster University, Hamilton, ON.

(dcostact@mcmaster.ca, bakerk9@mcmaster.ca, hoaretr@mcmaster.ca)

Abstract:

A major challenge in drug delivery systems is the lack of control over the drug once it has been administered. Considerable studies have been conducted in the fields of targeted and controlled drug delivery. Addressing these challenges, this project aims to fabricate core-shell microcapsules for ultrasound-triggered drug release. In this study, we particularly explored the potential of microcapsules produced via electrospraying, their size, optimal storage conditions and drug release characteristics.

The proposed design includes a poly(lactic-co-glycolic acid) microcapsule shell phase with a core that contains target drug, and a shell in which silica corks of different porosity are embedded. The microcapsules are fabricated via electrospraying and possess either porous or solid sillica corks in the shell.

These corks allow for pulsatile and one-time drug release respectively on application of ultrasound. Upon ultrasound exposure, embedded silica corks are dislodged, creating pores for drug release. Without ultrasound, the microcapsules retain their contents, enabling ultrasound triggered on-demand release.

Microcapsules containing porous silica corks show highest baseline drug release on subjection to ultrasound, followed by microcapsules containing solid silica corks. The instantaneous rates as calculated, also showed highest value in porous silica throughout the studies. These results thus provide insight into the on-demand drug release capability of these microcapsules.

While both porous and solid silica microcapsules provide increased drug release under ultrasound, the tunability of the corks allows for controlling the release kinetics of drug release. The system offers multiple advantages and possesses potential applications in pulsatile insulin delivery for diabetes treatment and targeted delivery for diseases like cancer and bone infections, as well as intermittent local dosing for conditions like osteoarthritis pain relief.

P8

Influence of Chronic Kidney Disease and Vascular Calcifications on the Fate of Targeted Polymer Nanomedicines

Vanessa Dos Passos Maio[1], Fabrice Mac-Way[2] and Nicolas Bertrand[1]

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INTRODUCTION: Mineral and bone metabolism disorders are complications of chronic kidney disease (CKD) that lead to vascular calcification.[1] Vascular calcifications are hydroxyapatite deposits that worsen cardiovascular diseases and increase mortality rates in CKD patients.[2] Currently, no specific treatment exists to prevent or reverse vascular calcification.[3] OBJECTIVES: Evaluate whether reduced kidney function can influence the distribution and excretion of polymeric nanoparticles with a surface ligand to target vascular calcifications. Compare the pharmacokinetic profile of encapsulated and free vitamin K in rats. METHODOLOGY: A polymer with tetracycline at the chain end was synthesized and mixed with ^{14}C-PLGA to prepare targeted nanoparticles (Tet-NP) via nanoprecipitation. Tet-NP with varying amounts of surface tetracycline were incubated with hydroxyapatite particles. To determine pharmacokinetics and distribution profiles, Tet-NP were injected into a CKD and vascular calcification rat model. Blood samples and organs were analyzed by scintillation. As a model drug, vitamin K, encapsulated or free, was injected into CKD rats. Blood concentrations of vitamin K were analyzed by high-performance liquid chromatography. RESULTS: Tet-NP with higher tetracycline surface content showed greater affinity for hydroxyapatite. As expected, the groups with reduced renal mass showed a significant increase in serum creatinine values. No difference in the circulation of nanoparticles was observed between healthy rats, animals with CKD, and animals with CKD and calcification. In CKD rats, Tet-NP increased the area under the curve of vitamin K by, approximately, 10-fold compared to the tested commercial formulation. CONCLUSIONS: The pharmacokinetics and distribution profiles of Tet-NP in rats were not significantly affected by reduced renal function or the presence of vascular calcifications. Encapsulation of vitamin K in nanoparticles improved its pharmacokinetic profile. This information will help pursue the development of nanomedicines to treat vascular calcification.

[1] Ren, S-C *et al.* Vascular Calcification in CKD: An Update and Perspective. *A&D*.**13**, 673-697 (2022).
[2] Gregg, L P and S S Hedayati. Management of Traditional Cardiovascular Risk Factors in CKD: What Are the Data? AJKD **72**, 728-744 (2018).
[3] Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl.* **7**, 1-59 (2017)

P9

Single-particle CLiC size-and-payload analysis of mRNA-lipid-nanoparticles with applications to vaccines and genetic medicines

Maya Stibbards-Lyle[1], Albert Kamanzi[1], Ariadne Tuckmantel Bido[1], Yao Zhang[1,2], Martin Jasinski[1], Benjamin Wang[3], Michael Venier-Karzis[3], Romain Berti[1,3], Yifei Gu[1], Shagun Kothari[1,4], Alexa Smith[1], Jenny Zhu[1], Miffy Cheng[3], Pieter R. Cullis[3], Sabrina R. Leslie[1,6*]

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[6]Department of Physics, University of British Columbia, Vancouver, Canada
*Corresponding author: Sabrina Leslie, Sabrina.leslie@msl.ubc.ca

Background

Lipid nanoparticles (LNPs) are a promising solution for delivery of a wide range of medicines and vaccines. Optimizing their design depends on being able to understand and predict biophysical and therapeutic properties, which are influenced by their formulation and manufacturing parameters. However, existing tools often rely on average measurements of a heterogeneous population, which obscure key information about different subpopulations. Introducing quantitative tools which can analyze and understand the properties LNPs at a single-particle level and link these to functional and biological properties has the potential to improve clinical outcomes.

Methods

In this work, we develop and apply an in-solution method for characterizing nanoparticles with single-particle resolution. We use convex lens-induced confinement (CLiC) microscopy to isolate and quantify the diffusive trajectories and fluorescent intensities of individual mRNA-lipid nanoparticles trapped in microwells. We apply our method to investigate the size and mRNA payload distributions of lipid nanoparticle (LNP) vehicles containing mRNA, as a function of lipid formulation (focusing on Onpattro, Moderna, and Pfizer formulations) as well as solution pH and manufacturing parameters.

Results

Using two-colour imaging, we investigate the number and properties of particles which are loaded versus unloaded with mRNA, and report the loading efficiency for four classes of formulations. By examining quantitative intensity and size measurements at the single-particle level using CLiC microscopy, and acquiring complementary cryoEM images, we gain insights into LNP structure and the distribution of mRNA through the particle. Finally, these measurements have elucidated unique subpopulations across multiple formulations of mRNA-LNPs. Beyond structural studies, we present an outlook to CLiC investigations of mRNA-LNP dynamics, including interparticle fusion, drug-release as well as protein-nanoparticle interaction kinetics.

Conclusion

CLiC microscopy enables high-throughput, quantitative, in-solution measurements of mRNA LNPs as a function of design parameters, contributing new biophysical insights and help bring next-generation nanomedicines to fruition.

P10

Co-Loaded Nanoemulsion with Paclitaxel and P-Glycoprotein Inhibitor Suppresses Clonogenicity, Alters Cell Cycle Progression, and Triggers Apoptosis in Triple-Negative Breast Cancer Cells

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Ductal carcinoma *in situ* (DCIS) accounts for 25% of breast cancer cases and can become invasive, especially in patients with the rare triple-negative form. Current treatments involve aggressive interventions, highlighting the need for effective, localized therapies. This study developed tributyrin and hyaluronic acid nanoemulsions (NE) for intraductal co-delivery of paclitaxel (P), a cytotoxic drug susceptible to P-glycoprotein (P-gp) efflux, and elacridar (E), a P-gp inhibitor. The resulting nanoemulsion, obtained by sonication, exhibited a hydrodynamic droplet size of 165±15 nm and Zeta potential ranging from -32 to -42 mV. Viability assays on triple-negative breast cancer cells (MDA-MB-231) showed that treatment for 48 hours with NE-P+E reduced the IC50 values by 36.7 and 2.9-fold compared to P+E solution and NE-P, respectively. A clonogenic assay with IC15 treatment and a 6-hour exposure demonstrated that while the unloaded NE did not hinder colony formation, groups containing P, in solution or incorporated into the NE, significantly reduced clonogenicity by more than 70.5% ($p < 0.0001$, ANOVA-Tukey). The nanocarrier also induced G2/M cell cycle arrest with IC30 treatment and significantly increased the population in sub-G1 (indicative of cell death) by 5.8-fold ($p < 0.0001$, ANOVA-Tukey) compared to the untreated control. Annexin V and Propidium Iodide indicated that the primary cell death pathway induced by P was apoptosis (over 30% of the population), and encapsulation of P and E in the NE did not alter this mechanism (30.6% apoptosis and 1.1% necrosis). Western blot analysis revealed that NE P+E downregulated the anti-apoptotic marker BCL-2 by 2.5-fold and upregulated pro-apoptotic proteins such as yH2AX and PARP-1 by 17.5-fold and 4.8-fold, respectively, compared to the untreated control. These findings indicate that the NE loaded with both drugs reduces the tumorigenicity and clonogenicity of triple-negative breast cancer cells, thereby lowering the probability of cancer recurrence and potentially improving patient outcomes.

P11

Comparison of plant based and animal based sources of squalene as adjuvant for injection applications

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Squalene is an organic compound that is used in many applications such as vaccine adjuvants. In vaccine adjuvants, an emulsion containing squalene (animal-based, derived from shark liver oil) is being used in influenza vaccines in over 30 countries. Given current industry push to go for high purity, sustainable and non-animal derived source for squalene, plant derived squalene would be a better alternative to formulators developing vaccines. Plant derived squalene would have better quality, i.e. low impurities such as mercury, that is often associated with marine animals. This study compared the physiochemical and in-vitro toxicity of emulsions formulated with different sources of squalene with a standard commercial product. Plant derived squalene (PhytoSquene®) and animal derived squalene were separately formulated as an oil-in-water emulsion. The compositions were evaluated for Z-average particle size and Poly dispersibility index (PDI) using dynamic light scattering, pH, osmotic pressure and viscosity. The formulations were also compared with standard formulation available on market for research purpose. MTS assay and cell uptake measurements, using Jurkat cells, were done to compare in-vitro toxicity of the two emulsions formulations along with commercial product. The results indicated that the sizes and zeta potential of the emulsion prepared with PhytoSquene® was comparable to the emulsion prepared with animal derived squalene and the commercially product. Furthermore, the emulsion properties such as appearance, osmotic pressure, viscosity and pH were also similar and compared well with commercial product. The results of the in-vitro study indicated that both non-animal-derived (PhytoSquene®) and shark-derived squalene adjuvant formulations were not toxic to cells and were taken up by the cells equally. Overall, the results of this study demonstrated that plant derived squalene, like PhytoSquene®, as an effective and sustainable source to replace animal derived squalene in adjuvant emulsions.

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P12

Single-particle CLiC quantification of mRNA-lipid nanoparticle size and RNA copy number without the use of labels in formulation

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Advancements in the field of lipid nanoparticles (LNPs) are essential for the practical application of mRNA-based nanomedicines, such as in the implementation of the SARS-CoV-2 vaccine. However, the ongoing development of mRNA–LNP formulations remains limited by inadequate characterization of key properties like size and RNA copy number, both of which directly impact therapeutic efficacy. Typical bulk methods, which report population averages of characteristics such as size and RNA payload, fail to capture heterogeneity present in mRNA–LNP mixtures, where subpopulations may vary significantly in size and RNA content. Furthermore, many metrics of mRNA–LNPs rely on the addition of covalent labels to mRNA, labels which are expensive and absent from RNA cargo found in clinical formulations.

Using Convex Lens-induced Confinement (CLiC) microscopy, we have addressed these shortcomings by developing a method in which mixtures of mRNA–LNPs can be characterized at the single-particle level without the use of covalently labelled mRNA. LNPs are first confined in microwells to measure their diffusive trajectories; microfluidic reagent exchange is then used to release encapsulated mRNA molecules from confined LNPs, where they can be individually fluorescently labelled and imaged. Compared to covalent mRNA labelling, which introduces electrostatic interactions not present in clinical preparations, this technique also enables higher signal-to-noise ratio of fluorescent mRNA for more accurate determination of per-particle mRNA copy number.

Using this novel approach, we report on the heterogeneity of mRNA copy number in clinically relevant formulations and investigate the relationship between LNP size/diffusivity and mRNA payload. Furthermore, we apply this technique to study how manufacturing parameters (e.g., formulation buffers, including sodium acetate or sodium citrate) influence biophysical properties of mRNA–LNP formulations. This cost-effective method of quantifying individual mRNA–LNP payload can be applied to diverse emerging mRNA–LNP formulations to support their advanced characterization and optimization for practical use.

P13

Optimized prime: a transformative LNP composition for scalable RNA vaccines

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Lipid nanoparticle-encapsulated RNA (RNA-LNP) vaccines have played a pivotal role in the global response to the COVID-19 pandemic. With proven safety and efficacy, RNA-LNPs are now at various stages of development against a variety of other infectious diseases. However, limited access to potent ionizable lipids, a key component in LNPs, and the expertise required to formulate and scale LNPs present real barriers to entry in this field. To overcome these challenges, we developed and optimized a novel ionizable lipid and a corresponding LNP composition for the delivery of RNA in vaccine applications. Using a scalable mixing platform, we formulated vaccine-candidate LNPs with both mRNA and self-amplifying RNA and investigated their physicochemical attributes and in vivo immunogenicity.

The novel LNP composition, combined with a scalable mixing process, resulted in high-quality RNA-LNPs with reproducible particle sizes of 60 to 90 nm, polydispersity indices < 0.2, and RNA encapsulation of > 80%. When stored in cryopreservation buffer at -80°C for 6 weeks (wk), we observed no detrimental effects on physicochemical attributes and *in vitro* potency. We completed *in vivo* immunogenicity studies using SARS-CoV-2 self-amplifying RNA and haemagglutinin mRNA-LNPs. A strong immune response was mounted against both antigens, where we observed antigen-specific IgG titers of 10^6 ng/mL, and IFNγ responses > 100-fold higher than naïve mice. Finally, we found that RNA-LNPs prepared on a commercially relevant scale were comparable to those prepared at the benchtop scale with respect to physicochemical attributes and immunogenicity.

We envisage this new LNP composition will be used in a wide range of preclinical drug development activities including antigen screening and *in vivo* proof-of-concept studies. In addition, we have derisked the scale-up of this composition and the mixing process, showcasing how these technologies can be used to accelerate drug development timelines.

P14

Effect of LNP components on mRNA mediated Protein Expression of Lipid Nanoparticles in Different Organs

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Introduction:

In recent years, RNA therapeutics have received tremendous attention. To fully realize the potential of RNA therapeutics, lipid nanoparticles (LNPs) have been developed as an efficient delivery system. While LNPs have been widely used for delivery of RNA therapeutics into liver, non-hepatic delivery remains highly challenging. To better understand the mRNA expression of LNPs in different organs, we have evaluated the translation of luciferase encoded mRNA using various LNP systems.

Objectives:

To evaluate the effect of various parameters of compositions constituting LNPs for protein expression in different organs.

Methods:

LNP formulations were manufactured using NanoAssemblr® Ignite with different compositions with Luciferase encoded mRNA. Size and PDI were determined using Dynamic Light Scattering. The encapsulation efficiency was determined using the Ribogreen™ assay. The pKa of mRNA LNPs were measured by TNS assay. Luciferase expression as total flux in various organs were evaluated *in vivo* and *ex vivo* using IVIS imaging. Briefly, CD-1 mice were dosed with luciferase encode mRNA LNP intravenously at a 0.1 mg/kg dose. Four hours later mice were injected with D-luciferin and imaged using IVIS. After euthanasia, different organs were dissected for *ex-vivo* luminescence imaging using IVIS.

Results:

Size of the LNP was reported to be between 60-150 nm with PDI<0.2. The experimental pKa of different ionizable lipids were between 4.9 to 7.4. All LNPs tested demonstrated high luciferase protein expression in the liver.

Conclusions:

We have seen that various components in the LNP compositions have implications with respect to protein expression in liver, spleen, and other organs. These parameters affect the biodistribution and translation of the lipid nanoparticles.

P15

From high throughput screening to commercial manufacturing of Lipid Nanoparticles

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Knauer's NanoProducer system was instrumental in the manufacturing of the Pfizer/BioNTech's Comirnaty COVID-19 vaccine during the COVID-19 pandemic. Since then, the research and development of nucleic acid-based lipid nanoparticles (LNPs) has quickly expanded as the potential of nucleic acid based LNPs was proven with the development of the COVID-19 vaccines from both Pfizer/BioNTech and Moderna. Nucleic acid LNPs are generally composed of 4 to 5 lipids at varying ratios. Alternating the ratio of the lipids and the types of lipids have been shown to significantly impact the delivery and performance of the LNPs. Buffer composition has also been shown to significantly influence the physiochemical properties, performance, and stability of LNPs. Due to all the potential variables when developing new LNPs, high throughput screening is necessary to quickly formulate and identify lead candidates. One key factor in selecting a high throughput screening system is the ability to scale from the high throughput system to systems suitable to product batch volumes required for clinical phases and commercial production. Knauer recently launched the NanoScaler Pro to support high throughput screening of LNPs. The NanoScaler Pro utilizes the same impingement mixing technology that is utilized in the NanoProducer system. In this study we assessed the overall capabilities of the NanoScaler Pro to screen different factors that influence LNPs and the ability to scale up and down processes between the NanoScaler Pro and the NanoProducer. The result of our study suggests that NanoScaler Pro is capable of quickly screening the different factors needed to identify new LNP candidates and that we were able to successfully scale the conditions between the NanoScaler Pro and the NanoProducer. Thus, making the NanoScaler Pro a suitable high throughput system for screening LNPs that can be scaled to commercial product in the future.

P16

Utilizing the Aggregation Behavior of Fluorescent Dyes to Develop Novel Fluorescent Nano-assemblies

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The favourable photoluminescence (PL) characteristics of quantum dots (QDs) have made them very popular materials of bioimaging and diagnostics,[1] but their potential environmental impact limit their scope of application at scale.[2,3] Greener nanomaterials with similar PL properties to QDs (*e.g.* bright, spectrally narrow emission) are of significant interest. In this work, we are developing a novel type of fluorescent nanomaterial that utilizes special self-assembled superstructures of the organic fluorescent dye 1,1′-disulfobutyl-3,3′-diethyl-5,5′,6,6′-tetrachlorobenzimidazolyl-carbocyanine (TDBC), called "J-aggregates," to obtain functional PL properties similar to those of QDs, but with better sustainability and lower environmental impact. The J-aggregates are templated onto silica nanoparticles (SiNPs) and in a sandwich structure between layers of polyethyleneimine (PEI) and 1-Bromooctadecane-functionalized dextran (dex-OCTD). The final J-aggregate-based nano-assemblies, which have a hydrodynamic size of 100-130 nm, were characterized at the ensemble and single-particle levels. The assemblies were also successfully conjugated with tetrameric antibody complexes (TACs) for specific immunolabeling of SK-BR3 cells. Overall, J-aggregate based assemblies are promising as an alternative fluorescent nanomaterial for bioanalysis. We are now working to broaden the effective excitation spectrum for the aggregates via Förster resonance energy transfer (FRET) with a co-assembled sulfo-cyanine3 dye. We anticipate that this class of fluorescent nanoparticle will be highly useful for multiplexed imaging and for portable smartphone-based diagnostic technologies.

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P17

Prototype Smartphone-Based Device for Flow Cytometry

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Many areas of research and healthcare require the ability to detect, enumerate, and type different cells. Flow cytometry is one of the most widely used methods for solving this problem, but the instruments are very large and expensive, and are typically limited to specialized laboratories in wealthy and populous centres. This lack of access to a gold-standard method for cellular analysis is a major barrier to equity in healthcare. One possible method for reducing the cost of flow cytometry is the use of a smartphone-based device. With their global proliferation and built-in optoelectronic components, smartphones have excellent potential for use as detectors and platform devices to increase the accessibility of cellular and other laboratory analyses.

Our group has recently developed a prototype smartphone-based device for flow cytometry. This device uses the smartphone camera to track immunofluorescently-labeled cells as they pass through a flow channel [1]. Here, we describe development of the next generation of this device. Features of the device include a violet laser for the excitation of multi-colour fluorescence from super-QD immunoconjugates, a capillary flow channel for higher signal-to-background ratios, and improved magnification for sizing of cells and their nuclei. Nuclear and membrane staining on the same cells is quantitatively resolved in measurements. Potential applications of this device include the identification of cancerous cells from urine or blood samples and a complete blood cell count, the latter of which is the most frequent diagnostic test in the world. The technology developed through this research will help improve accessibility, equity and inclusion for advanced diagnostic health care in rural, remote, and other resource-limited communities.

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P18

Investigating the Effect of Fluorophore-Quencher Modifications on the Performance of Optical Aptamer-Based DNA Sensors

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Aptamers are a tremendously sensitive and specific nucleic acid affinity reagent, and combined with their high modularity, are a powerful biosensing tool for precision medicine. Aptamers are adapted into optical sensors by adding optical reporter molecules. These modifications are commonly selected to complement the sensor application and/or optimize their photophysical compatibility with each other (i.e. fluorophore-quencher pairs). The problem is that aptamer biosensing capability is easily compromised by structural alterations, which these reporter molecules and their bioconjugation precisely introduce. While this is not surprising, there is a lack of comprehensive studies on the structural (and potentially photophysical) impact of optical reporter modifications on sensor performance.

Here we evaluate the effect of fluorophore-quencher modifications on aptamer affinity in terms of their 1) position and 2) identity. We found that within a double-stranded (duplex-bubble) DNA system, when the reporter pair is positioned distanced from the aptamer binding region, at one of two positions, the complete abolishment of binding capability is achieved. Interestingly, this loss of binding function was aptamer species-dependent; the dopamine aptamer lost binding at one position and was functional at the other position, however for the serotonin aptamer, the opposite was true. We also demonstrate that the specific combination of a fluorophore-quencher pair can significantly shift the effective affinity of a sensor. When the reporter pair is positioned terminally in an aptamer molecular beacon structure, we found that the FAM-dabcyl combination had a K_d of 3.0 μM, however changing the combination to Cy3-BHQ-2 and Cy3-dabcyl increased the K_d by one and two orders of magnitude respectively (38.4 and 299.5 μM).

In this research, we show that it is vital to consider the position and identity of optical reporters during the design of aptamer-based sensors to optimize their performance and ultimately to advance their application within nanomedicine.

P19

Optimization of Multiplex CRISPR-Cas9 Editing of Human Primary T Cells Using Lipid Nanoparticles (LNPs) and Subsequent Off-Target Evaluation

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CRISPR/Cas9 has emerged as a dynamic tool for gene editing of human primary T cells. Previously, we demonstrated a novel lipid nanoparticle (LNP) reagent for engineering of gene-edited CAR T cells with high cell viabilities and potency, even after multiple genetic manipulations. Here, we extend this work by optimizing multi-gene knockouts and assessing potential off-target editing effects in T cells, with RNAs delivered either by electroporation or LNPs. Further, through extensive T cell media evaluation, we show the critical nature of cell culture conditions for efficient LNP-mediated transfections.

TCR or CD52 targeted Cas9 mRNA-LNP addition or electroporation yielded high single and double knockout efficiencies. We tested a range of sgRNA targets, wild-type and high-fidelity Cas9 mRNAs, and determined off-target editing. We achieved 90% ±2.6% double edited T cells (TCR–/CD52–). Similar results were obtained when comparing different LNP batch sizes (micro to milligram RNA) and cell culture vessels (0.1 to 45 million cells), demonstrating scalability of both LNPs and cell treatment. Editing efficiency and potency was affected by the type of culture media. LNP delivery to T cells for gene editing was optimized in two commercially available T cell culture media yielding similar EC_{50} values (0.3 to 0.5 µg/million cells).

The results from this study further support the utility RNA-LNPs for the genetic engineering of primary T cells. The simple and gentle nature of LNP cell treatment allows for multiple genetic engineering steps for simultaneous expression and deletion of proteins for future cell therapies. These LNPs can be easily manufactured from small-scale screening of RNA libraries to rapid scale-up for clinical translation.

P20

Improved Nucleic Acid Delivery by Microneedle Patch Vaccination

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Dissolving microneedle vaccine patches address limitations of traditional intramuscular vaccine delivery due to their potential for self-administration and prolonged stability of vaccine components, reducing issues of cold-chain storage and inequitable vaccine access highlighted during the COVID-19 pandemic [1]-[3]. We have previously developed a dissolving integrated fiber microneedle (iFMD) patch for delivery of protein antigens to induce mucosal immune responses and improve vaccine accessibility [4]. Here, we sought to deliver plasmid DNA (pDNA) via iFMDs and explore the effect of parameters including nucleic acid carrier and microneedle polymer composition on nucleic acid delivery.

The iFMDs consist of electrospun PLGA fibers backfilled with polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) (**Fig 1a**). GFP pDNA was complexed with Lipofectamine 3000 or linear poly(ethyleneimine) (PEI), and complexes were emulsified into polymer solutions. pDNA-loaded microneedle patches were successfully fabricated with low molecular weight (LMW) polymer (31kDa PVA, 50 kDa PVP) and high molecular weight (HMW) polymer (105 kDa PVA, 360 kDa PVP) backfill. iFMDs with lower molecular weight backfill demonstrated a 3-fold improvement in *in vitro* transfection efficiency compared to those with a higher molecular weight backfill. Lower molecular weight iFMDs with PEI as the nucleic acid carrier demonstrated a significant improvement in transfection efficiency of at least 37-fold over other formulations (**Fig 1b, 1c**). Higher transfection efficiency with lower molecular weight polymer backfill may be due to lower viscosity of polymer solutions impacting pDNA stability and improving diffusion of pDNA complexes. Overall, these results demonstrate the significance of dissolving microneedle formulation in plasmid DNA delivery and motivate further *in vivo* testing and development of the vaccine platform.

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P21

Implications of circulatory protein adhesion on lipid nanoparticle pharmacokinetics

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Lipid nanoparticles (LNPs) have revolutionized the field of drug delivery by enabling the encapsulation of therapeutic ribonucleic acid (RNA). Initial development of LNPs have produced a pharmacokinetic profile which targets the liver with rapid blood clearance following IV injection, earning LNP-RNA its first clinical approval in 2018 for the treatment of hereditary amyloidosis [1]. To unlock their full potential in additional indications, it's imperative for LNPs to have increased biodistribution and circulation times. Further investigation of Onpattro, the gold standard LNP formulation, has implicated circulatory protein adhesion as the primary factor in their liver targeting and rapid blood clearance. In particular, the circulatory protein ApoE has been shown to adsorb to the LNP's surface and deliver them to the liver for blood clearance via uptake by LDL receptors[1]. Recent development efforts have sought to shift biodistribution away from the liver by engineering LNPs to preferentially bind particular circulatory proteins over ApoE [2]. In contrast, this work aims to investigate the impact of LNPs with reduced circulatory protein adhesion on their biodistribution and biological half-life. By presenting data from in vivo radiolabeling and in vitro mass spec proteomics, we show that LNPs with reduced protein adhesion will exhibit a pharmacokinetic profile with increased circulation times and extra-hepatic delivery. We anticipate this discovery will lead to the rational design of LNPs towards the goal of a selective organ targeting drug delivery platform.

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P22

Efficient Gene Editing in CD34+ Hematopoietic Stem and Progenitor Cells Using Non-Viral Lipid Nanoparticles

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CRISPR-Cas9 gene knockouts in CD34+ hematopoietic stem and progenitor (HSPC) cells is emerging as a transformative tool for the correction of mutations that cause genetic blood diseases [1, 2]. In previous work, we used a novel lipid nanoparticle (LNP) reagent for the multiplex gene editing of CAR T cells, yielding high viability and efficient editing [3]. In this work, we explore the use of LNPs for the efficient CRISPR-Cas9 editing of CD34+ HSPCs, highlighting the vast potential of LNPs for pioneering gene therapeutics.

Direct LNP addition to CD34+ HSPCs achieved an average of 84 ± 6% of CD33 and 81 ± 2% of CD45 knockout efficiencies in six separate experiments. Post-treatment, the cells retained an average of 95 ± 3% viability. The LNP-treated HSPCs showed strong cell proliferation, maintaining >90% proliferation relative to untreated controls. The CFU assays indicated unchanged lineage formation in both RNA-LNP and empty LNP controls. Additionally, the study successfully scaled up LNP production from research to pre-clinical scales using a novel mixing technology.

This study demonstrates the potential of LNP-mediated CRISPR-Cas9 mRNA delivery as a promising approach for gene editing in HSPCs. The simple and gentle nature of LNP cell treatment allows for multiple genetic alterations for simultaneous expression and deletion of proteins paving the way for innovative gene therapies. The scalability of LNPs, enabled by novel mixing technology, permits efficient RNA library screening at small scales, and facilitates swift transition to large-scale clinical applications.

The results from this study further support the utility RNA-LNPs for the genetic engineering of HSPCs. The simple and gentle nature of LNP cell treatment allows for multiple genetic engineering steps for simultaneous expression and deletion of proteins for future cell therapies. These LNPs can be easily manufactured from small-scale screening of RNA libraries to rapid scale-up for clinical translation.

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P23

Stimuli-Responsive Nanocomplexes for Antibiotic and Enzyme Codelivery in Biofilm Infections

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Biofilms contribute to antibiotic resistance by creating complex barriers that protect bacteria from the action of antimicrobial agents and the host immune system thus making infections harder to treat. The extracellular polymeric substances (EPS) within biofilms trap antibiotics and reduce their efficacy, allowing bacteria to thrive and develop resistance [1]. In this study, we develop nanocomplexes (NCs) for the codelivery of antibiotics and EPS-degrading enzymes such as deoxyribonuclease (DNase). These nanocomplexes (NCs) were created through electrostatic interactions between positive and negative polymers and loaded with the antibiotic tobramycin as well as an EPS degrading enzyme (DNase). We had previously demonstrated that polyelectrolyte NCs can be used to deliver tobramycin to treat *P. aeruginosa* biofilm infections [2]. Since many bacterial infections are associated with acidic environments [1], the study focuses on developing pH-responsive NCs that are designed to navigate biofilm environments and release their cargo at lower pH levels. This approach aims to improve drug penetration, retention, and efficacy against persistent biofilm-associated infections. This approach not only enhances antibiotic efficacy but also targets bacterial biofilms, a major factor in antibiotic resistance [3]. We hypothesize that this bioinspired, stimulus-responsive method of antibiotic delivery will reduce adverse effects and improve therapeutic effectiveness.

The nanocomplexes (NCs) were formulated by combining the tobramycin and DNase with polycationic polymers and polyanionic polymers in varying ratios. The effect of buffer compositions, pH, and incubation time on the nanocomplexes was evaluated. In addition, other physicochemical parameters of the NCs such as size, PDI, surface charge, encapsulation efficiency as well as in vitro drug release were also evaluated. Tobramycin-loaded nanocomplexes with a particle size of about 250 nm and low PDI (approximately 0.2) were produced, with remarkable drug loading (>80 %), and pH-responsive swelling from approximately 250 nm to > 2 um. The polymer ratios used played a crucial role in determining the characteristics of the NCs. The buffer type, incubation duration, and pH also affected the properties of the NCs.

So far, the data suggests that the NCs possess promising features that could be explored in the design of more effective delivery systems for antimicrobial agents. However, further assessments such as microscopic examinations to understand particle swelling behavior are required to optimize these nanocomplexes.

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P24

Assessment of iron oxide magnetic nanoparticles for adipose-tissue targeted delivery of anti-diabetic agents: A proof-of-concept study using rosiglitazone

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Increased obesity prevalence has amplified diabetes, which affects over 537 million adults worldwide. Systemically administered drugs to treat diabetes display off-target adverse effects, demonstrating the need for tissue-targeted therapeutics. Adipose tissue is a key regulator of nutrient metabolism. In obesity, adipose tissue becomes dysfunctional with large, fibrotic adipocytes secreting pro-inflammatory cytokines that promote insulin resistance. Restoration of adipose tissue function is a key mechanism by which the thiazolidinediones (TZDs), potent anti-diabetic drugs, improve insulin sensitivity via actions on peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor expressed in adipose tissue. However, clinical use of TZDs demonstrated off-target effects on kidney and bone, worsening cardiovascular and bone health. Here, we used the therapeutic properties of TZDs to assess iron oxide magnetic nanoparticles (MNPs) as drug carriers for adipose tissue-targeted therapy for insulin resistance. The TZD, rosiglitazone was bound to alendronic acid-coated MNPs (rosi-MNPs) and concentrated in subcutaneous adipose tissue using an implanted magnet. To assess effects of adipose tissue-accumulated rosi-MNPs on insulin sensitivity, diet-induced obese mice (male, C57Bl/6) were treated for 18d with rosi-MNPs (1.5mg rosi/kg/d bound to 151.40μg ± 63.67μg MNPs (mean ± SD), sq), systemic rosiglitazone (1.5mg/kg/d, og), or vehicle (corn oil, og). We found that rosi-MNPs improved insulin sensitivity similar to systemic rosiglitazone compared to obese controls. PPARγ target gene expression analysis showed rosi-MNPs induced changes in the targeted adipose depot but not the untargeted depot, indicating specificity. Rosi-MNPs enhanced the generation of small, expandable adipocytes, a hallmark of insulin sensitivity. No changes in PPARγ target gene expression were seen in kidneys post-treatment with rosi-MNPs or systemic rosiglitazone, therefore a longer dosing period is required for assessment of adverse effects. This work identifies adipose tissue as an important target for anti-diabetic therapies and reports on the benefits and drawbacks of using MNPs as a tool for drug delivery.

P25

Controlling the Size of Glassy Organic Dots Exhibiting Thermally Activated Delayed Fluorescence for Bioimaging

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Luminescent glassy organic dots (g-Odots) are emerging as promising nanoparticles for bioimaging, exhibiting excellent brightness and photostability attributed to their glassy, rigid matrix.[1,2] Emission colour tuning in g-Odots is also easily accessible, since different hydrophobic fluorophores can be encapsulated within the glassy matrix of the particle. g-Odots are particularly useful when combined with dyes exhibiting phosphorescence or thermally activated delayed fluorescence (TADF), facilitating time-gated fluorescence imaging while the glassy core shields the emitters from oxygen. Despite their potential usefulness, strategies for controlling size in g-Odots remain unexplored – a critical parameter affecting cellular uptake and metabolism. Here, we investigate methods to control g-Odot size while retaining the glassy interior of the nanoparticles. Annealing of the glassy host material was explored as a post-synthetic step to promote the glass transition of the particles. Host-to-surfactant ratios were also investigated as a simple route to tunable particle sizes. Centrifugation and size exclusion chromatography were also examined as methods to fractionate g-Odots by size after synthesis. While established for other nanoparticle types, these methods have yet to be explored for g-Odots. Our findings provide strategies to control the size of g-Odots, which we anticipate will broaden their applicability in bioimaging applications.[3] These probes may also prove useful for applications other than cellular imaging where bright, robust particles are needed, such as imaging of vascular structure or lateral flow assays for point-of-care diagnostics.

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P26

Dextran-Encapsulated Nanoparticles and Super-Nanoparticle Assemblies for Application to Cellular Immunolabeling

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The small size and properties of nanoparticles (NPs) such as semiconductor quantum dots (QDs) and iron oxide magnetic NPs (MNPs) are of great interest for applications in bioanalysis and imaging.[1,2] Properties such as photoluminescence (PL) and superparamagnetism are size-dependent and therefore cannot be amplified by simply synthesizing larger particles. Combining many NPs into a colloidal assembly or "super-NP" that retains a nanoscale size while amplifying (*e.g.*, higher brightness of PL) or combining the properties of the single NPs (*e.g.*, fluorescent-magnetic composites) is beneficial in certain applications.

Here, amphiphilic dextran is used in a simple and rapid emulsion-based approach for the preparation of super-NP assemblies from a variety of hydrophobic materials, including three different QD compositions (CdSe/CdS/ZnS, InP/ZnS, and Si), MNPs, composites of QDs and MNPs, and composites of QDs or MNPs with conjugated polymers. Applicability to non-NP and soft materials was demonstrated through the preparation of conjugated polymer nanoparticles (CPNs) using amphiphilic dextran. The physical and optical properties of the super-NPs and CPNs were characterized at the ensemble and single-particle level. The super-NPs contained many hydrophobic NPs and the super-QDs were orders of magnitude brighter than individual QDs. The dextran-functionalized super-NPs and CPNs had good colloidal stability over many months, low non-specific binding, and enabled specific cellular immunolabelling using tetrameric antibody complexes (TACs). The super-QDs provided enhanced labeling brightness and contrast ratios compared to single QDs, the super-MNPs enabled magnetic pull-down of labelled cells, and composite materials had both capabilities. Overall, emulsification with amphiphilic dextran is a versatile method for the preparation of CPNs and super-NPs for applications in bioanalysis and imaging. There is excellent potential to use this method with many other NPs and polymer materials, and to adopt a variety of bioconjugation strategies moving forward.

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P27

Towards Intracellular Deployment of Concentric FRET Probes for Multiplexed Bioimaging and Sensing

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Förster resonance energy transfer (FRET)-based probes are powerful tools for bioanalysis. Concentric FRET (cFRET) is a design strategy that enables a single probe to detect multiple biomolecular targets simultaneously through a network of energy transfer pathways [1]. Herein, multiple copies of multiple dye acceptors are conjugated to a central quantum dot (QD) donor through biomolecular linkers (e.g., peptides). Presently, the multiplexed sensing capabilities of cFRET have been limited to *in vitro* detection [2]. cFRET probes are promising tools for studying important cellular processes involving multiple biomarkers and multi-step pathways; however, translating cFRET detection from test tubes to the intracellular environment introduces several new levels of complexity.

Here, we present our efforts at advancing cFRET towards intracellular imaging and sensing, by first exploring intracellular FRET. Using microinjection as a direct cellular delivery pathway, the cytosolic delivery, localization, and stability of the photoluminescence (PL) from QDs and dye-labelled peptides in lung cancer cells were studied, further leading to tests with microinjections of QD-peptide-dye conjugates as FRET probes. Temporal changes in the cytosolic QD and dye PL signals were investigated via fluorescence microscopy, where the results suggested further optimization of the probes to resist non-specific intracellular degradation.

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P28

Development of Smartphone-based Devices for Nanomaterial-enabled Point-of-Care Assays

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Smartphones are being widely adapted for point-of-care testing (POCT) due to their global ubiquity and on-board technologies. Smartphones, when paired with advanced luminescent materials like quantum dots (QDs) and polymer dots (Pdots), have sufficient sensitivity to become a viable alternative for many analyses with sophisticated laboratory instruments. To this end, we have developed a laser-scanning smartphone imaging platform (LS-SIP) and a total internal reflection fluorescence (TIRF)-SIP. The LS-SIP is ideal for readout of analytical tests like microwell plates, lateral flow immunoassays (LFIAs), and microfluidic or lab-on-a chip (LOCs) systems. This multi-purpose readout device helps avoid the possible problem of clinics accumulating a myriad of different devices for POCT. TIRF-SIP is ideal for minimization of user steps, and unprocessed biofluid samples, which requires that background from the sample matrix does not interfere with biomarker detection. TIRF has been proposed for POCTs but still utilizes a lab-based TIRF microscope that is incompatible with true POCT.

The LS-SIP and TIRF-SIP are composed of 3D-printed parts, simple optics, and a laser diode. The TIRF-SIP contains a dove-prism that TIRs the laser onto the sample platform. The TIRF-SIP was benchmarked using an immobilization assay of Dex-QDs to ConA. The LS-SIP has a DC motor, that scans a line-shaped laser beam over the sample areas that were designed to accommodate both large and small assays. The analytical performance of the device was evaluated using QDs, dyes and Pdots. Proof-of-concept assays were tested with LFIAs, and microwell plate formats. The LS-SIP is capable of multicolor imaging with picomolar limits of detection.

Overall, this research is a step toward improving diagnostic capability and accessibility—and thus equity and inclusion in health care—in resource-limited settings such as rural and remote communities in Canada and worldwide.

P29

Developing novel lipid nanoparticle formulations to enhance extrahepatic delivery

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Lipid nanoparticles (LNP) are a technology used in multiple approved drugs, as well as in many clinical and pre-clinical research projects. Encapsulation within LNP allows molecules such as mRNA to be effectively used as therapeutics in-vivo. Current standard LNP formulations, similar to those used in the Comirnaty and Spikevax vaccines and the RNAi therapeutic Onpattro, consist of an ionizable lipid, a phospholipid, cholesterol, and a PEG-lipid[1]. These formulations, however, present some significant limitations. Firstly, this formulation accumulates mainly within the liver[1], which is a hurdle to the development of LNP-based therapeutics for non-liver diseases. Secondly, standard LNP formulations have been found to result in very low endosome escape after uptake by cells, which means that only about 2% of the encapsulated RNA from the drug reaches the cytosol intact and able to be translated[2]. Finally, safety concerns have been raised due to the potential immunogenicity of PEG-lipids, particularly for applications that may require repeat dosing[3].

We seek to develop novel LNP formulations that can improve upon these limitations of current LNPs, by incorporating non-PEGylated replacements for the PEG lipids within LNP. The structure of these non-pegylated lipids contain moieties which may interact with the cell surface in a different manner than current, PEG-containing LNP formulations, which we hypothesize will contribute to improved delivery to extrahepatic cell-types. As well, these formulations will be able to incorporate NS polymers, another novel lipid component developed in the Li lab which we have shown to increase LNP endosome escape, and consequently improve transfection efficiency.

Current research progress focuses on the development and characterisation of these novel LNP formulations. A range of formulations are produced and tested for size, zeta potential, and encapsulation efficiency. From this range of formulations, in-vitro and in-vivo transfection efficiency can be tested to determine optimal lipid compositions.

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P30

Red Emissive Organic Molecules Exhibiting Delayed Emission for Bioimaging

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Fluorescence bioimaging is an indispensable tool in disease diagnosis and in understanding cellular processes. Thermally activated delayed fluorescence (TADF) and room temperature phosphorescence (RTP) emitters are increasingly gaining interest in bioimaging applications due to their longer luminescence lifetimes. Specifically, red to near-infrared (NIR) emitters are of great interest as they emit within the biological transparency window of 650 nm - 1350 nm wherein red light can penetrate deeper into tissue and can offer better resolution against cellular autofluorescence. Herein reported are, a TADF emitter, **NAI-Q-MeOTPA,** and an RTP emitter, **NAI-Py-MeOTPA,** which incorporate the strong electron-donating group 4,4′-dimethoxytriphenylamine and strong acceptor cores quinoxaline-naphthalimide and pyrido[2,3-*b*]pyrazine-naphthalimide, respectively. Delayed emission was observed for both luminophores with the emission maxima of **NAI-Q-MeOTPA** and **NAI-Py-MeOTPA** as 746 nm and 749 nm in toluene, respectively. When encapsulated into glassy organic dots (g-Odots), the luminophores maintained NIR emission with delayed lifetimes. The **NAI-Q-MeOTPA** and **NAI-Py-MeOTPA** g-odots were probed for bioimaging in HeLa cells in which g-Odot encapsulation was observed. Comparable SBR and SNR values were observed due to the cellular autofluorescence attenuation in the 630 – 740 nm imaging window used.

P31

A smart material as a replacement for Descemet's membrane endothelium complex (DMEC)

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One of the top five causes of human visual deterioration is corneal damage. Despite its high success in restoring vision, corneal transplantation (keratoplasty) is limited by the scarcity of corneal donors and the accessibility of eye banking facilities. Consequently, there is a demand for a smart, synthetic material that could function as a healthy cornea and restore vision.

Herein, an innovative, smart material that could be used as a replacement for Descemet's membrane endothelium complex (DMEC) is presented. The prototype implant consisted of a base membrane made of poly(vinyl alcohol) (PVA) that was embedded with iron oxide nanoparticle enriched pNIPAM-based hydrogel components. The incorporation of magnetite iron oxide nanoparticles in the hydrogel aimed at elevating its temperature above the lower critical solution temperature (LCST) through exposure to an alternating magnetic field (AMF). It was hypothesized that the shrinkage of hydrogel fragments would lead to pore formation and enable water passage through the membrane. Upon removal of the AMF, the hydrogels would return to their original size, resulting in closed pores. The obtained PVA/pNIPAM membranes were characterized by FTIR, DSC, UV-Vis-NIR spectroscopy, swelling, tensile, contact angle measurements, SEM, and optical microscopy. The responsiveness of the pNIPAM components was evaluated by thermography and optical microscopy.

Optical microscopy analysis demonstrated a significant and reversible change in the size of the pNIPAM fragments embedded in the PVA membrane when exposed to the AMF. Despite not achieving the desired control over water transmission, the observed decrease in size of the pNIPAM-based fragments highlighted the potential of the PVA/pNIPAM composite membranes as a smart replacement for DMEC. Nevertheless, further research is required to address challenges such as reducing the water permeability of the base membrane, ensuring the creation of pores suitable for water transport, and improving the visible light transmittance of this smart system.

P32

Developing Iron Oxide and Gold Loaded Lipid Nanoparticles for Triggered Release

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Lipid nanoparticles (LNPs) loaded with chemotherapeutics or mRNA are the gold standard of therapeutic delivery. Despite their clinical success, a major limitation of current LNP technologies is their lack of targeting and organ specificity. Therefore, we believe that next-generation, stimuli-responsive LNPs should incorporate an extra triggering/targeting agent in addition to the therapeutic agent to confer localized organ-specific targeting. This can lead to a reduction in unwanted toxicity and an overall improvement in clinical outcomes.

In this work, we have developed LNPs loaded with a therapeutic agent (Doxorubicin) and a number of trigger/target agents: iron oxide nanoparticles (IONP) and gold nanoparticles (GNP). IONP could act as an agent for magnetic targeting with a strong magnetic field gradient, or for hyperthermic or mechanic delivery using an oscillating magnetic field. GNP could act as an agent for various plasmonic effects using a pulsed laser in resonance with the GNP surface plasmon frequency.

Co-encapsulation of both the drug and the metallic nanoparticles (NPs) inside LNPs is significantly challenging. To achieve this, we have developed an LNP system that is comprised of two compartments: an aqueous compartment for the therapeutic agent, and a glyceride oil compartment for the metallic NPs. The metallic NPs are first synthesized, followed by a surface modification procedure. This modification results in hydrophobic particles that preferentially accumulate in the oil compartment of LNPs, and guarantees their dispersibility in ethanol; a requirement to be compatible with our scalable, rapid mixing, T-tube formulation technique. We have demonstrated that the metallic NP-loaded, dual-compartment LNPs can be subsequently loaded with Doxorubicin using the standard ammonium sulfate gradient approach, resulting in Doxorubicin crystals in the aqueous compartment of LNPs. We have comprehensively tested a series of variables to optimize formulations leading to the best-in-class hybrid LNP systems containing both the therapeutic and triggering agents. Interestingly, our optimized formulations containing GNP demonstrate successful triggered release in the presence of pulsed laser irradiation.

Finally, we have conducted preliminary *in vivo* studies to validate pharmacokinetic and pharmacodynamic properties. Such hybrid systems could have the potential to revolutionize the LNP-therapeutic delivery landscape.

P33

Gene editing in liver towards the treatment of life-threatening cardiovascular diseases: PoC for efficient in-vivo genome editing/base editing using Lipid Nanoparticle Library

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Introduction:

The transformative potential of CRISPR–Cas9 gene-editing technologies, including CRISPR-Cas9 base editors (ABE, CBE), in modifying human genes has paved new path for genomic medicines. In this study, we present the development of novel ionizable lipids as functional excipients for designing vehicles for delivering CRISPR-Cas9.

Methods:

Guide RNA (sgRNA) and messenger RNA (Cas9 or ABE 8.8m) were co-formulated in LNPs using NanoAssemblr® Ignite. Size and PDI were determined using Dynamic Light Scattering. The encapsulation efficiency was determined using the Ribogreen™ assay. Cas9-TTR LNPs and ABE 8.8m-PCSK9 LNPs were administered intravenously into CD-1 and C57BL/6 mice, respectively, at 2-3 mg/kg dose. Serum was collected 7 days post injection and analyzed for *TTR* and *PCSK9* levels through ELISA. Further, we have done NGS sequencing to evaluate editing levels.

Results:

Size of the LNPs were reported to be between 50-140 nm with PDI<0.2. PNI LNPs showed significant editing of the mouse transthyretin (*TTR*) gene and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene in the mouse liver, resulting in approximately 75% and 80% reductions in serum protein levels, respectively, following a single administration of 2mg/Kg LNPs. It has been shown earlier that ABE-cas9 show off-target editing even with-in the targeted locus. Novel LNPs showed precise base editing of PCSK9 locus without having off targeting effects.

Conclusions:

We achieved clinically relevant levels of in-vivo genome editing of TTR and PCSK9 targets as examples. These findings highlight the potential of the PNI-lipid library as a highly effective and promising platform for genome editing.

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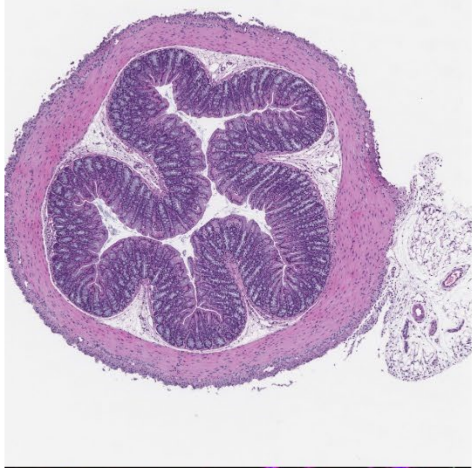

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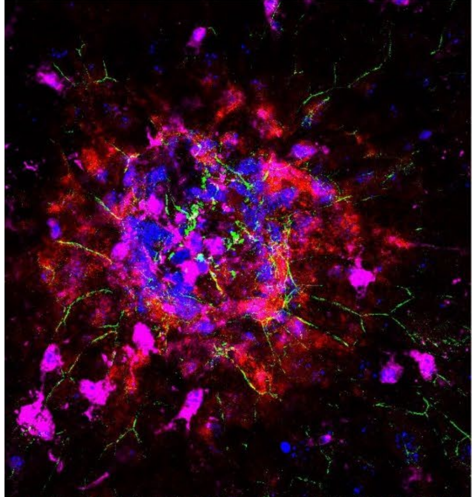

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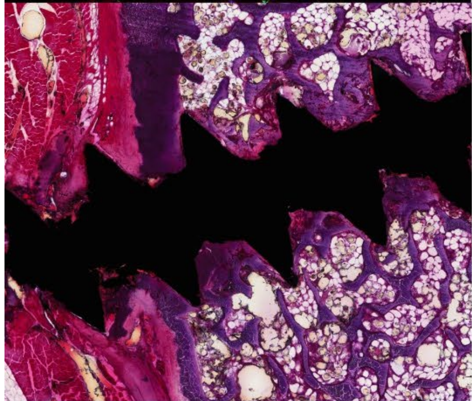

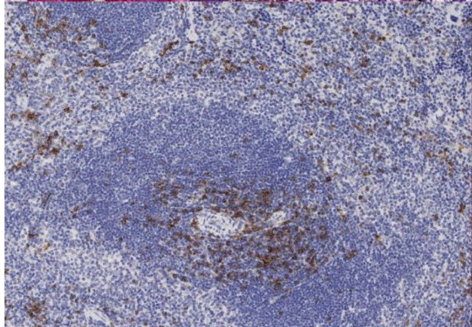

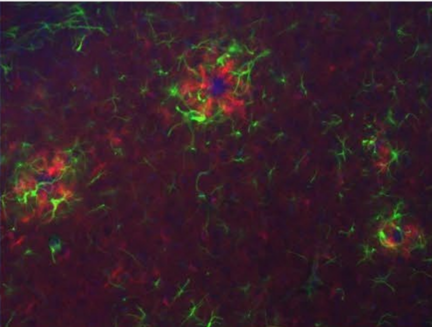

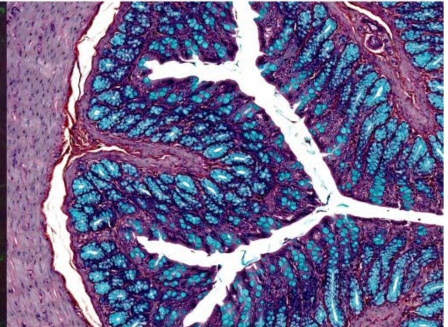

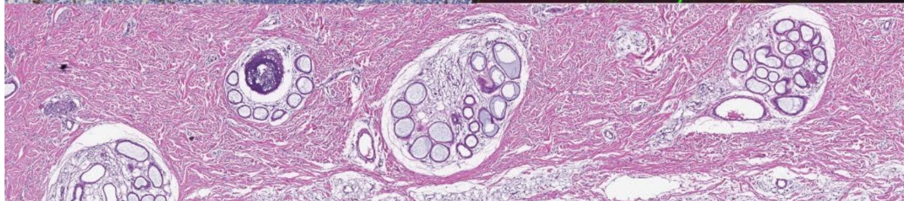

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- A purpose-built, 43,000 sq ft. production facility for GMP-grade* enzyme manufacturing
- Direct access to our OEM & Customized Solutions Team who is committed to your growth and success

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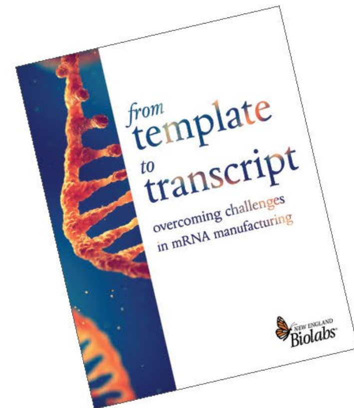

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"GMP-grade" is a branding term NEB uses to describe products manufactured or finished at NEB's Rowley facility. The Rowley facility was designed to manufacture products under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Products manufactured at NEB's Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.