VANCOUVER NANOMEDICINE DAY 2019

Thursday, September 12
The Nest, UBC

8:30		**Registration**
9:00	Urs Hafeli	**Welcome**
9:10	Michel Meunier	*Plasmonics for Diagnostics and Therapy in Nanomedicine*
9:25	Wilson Poon	*Effect of Removing Kupffer Cells on Nanoparticle Tumour Delivery & Elimination*
9:40	**Shana Kelley**	**Nanoparticle-Mediated Rare Cell Analysis: A New Tool for Liquid Biopsy** **INVITED TALK**
10:20		**Break**
10:40	Colin Blackadar	*Pharmacokinetics of Native and Formulated Innate Defense Regulator Peptides*
10:55	Cathy Yan	*A Clew Into Improving Targeted Drug Delivery Through pH-Dependent DNA Origami*
11:10	Ada Leung	*The Metaplex Technology: Use of Coordination Chemistry & Nanotechnology to Develop Poorly Soluble Metal-Binding Small Molecules*
11:25	**Tayyaba Hasan**	**Photodynamic Therapy with Nanomedicines** **INVITED TALK**
12:05		**Lunch & Poster Session**
1:30	**Ralph Mason**	**Photoacoustic Imaging: a Timely Tool for Drug Discovery & Assessment of Tumor Pathophysiology** **INVITED TALK**
2:10	Philip Cohen	*Re-188 Nano-Colloid Brachytherapy for Non-Melanoma Skin Cancer*
2:15	Lisa Silverman	*Co-Encapsulation of Curcumin with SN-38 Leads to Increased Encapsulation Efficiency in PCL-b-PEO Polymeric Nanoparticles*
2:20	Dayana Campanelli de Morais	*Effects of Cellulose Nanocrystal on Physical Properties of a Dental Adhesive Resin Blend*
2:25	Waleed Mohammed-Saeid	*Poly(Acrylic Acid) Grafted Cellulose Nanocrystals (CNC-PAA) as Novel Nano-Hydrogels for Local Delivery of Platinum Based Chemotherapeurtics in Oral Cancer*
2:30	Nikki Salmond	*Development of a Liquid Blood Biopsy Test for Breast Cancer Using Nanoscale Flow Cytometry*
2:35	Courtney van Ballegooie	*Enhancing the Modification of Protein Nanoparticles with X-Rays & Gold: Potential for Triggered Drug Release*
2:40	Bruce Verchere	*A Lipid Nanoparticle Approach for Targeting the NLRP3 Inflammasome in Tissue-Resident Macrophages in Diabetes*
2:45		**Break & Poster Session**
3:30	**Pieter Cullis**	**Design of Lipid Nanoparticle Delivery Systems That Enable Gene Therapies** **INVITED TALK**
4:10	Ndeye Khady Thiombane	*Single-Cell Analysis for Drug Development Using Convex Lens-Induced Confinement (CLiC) Imaging*
4:25	Zeynab Nosrati	*Targeting of Inflamed Joints in Rheumatoid Arthritis*
4:40	Sarah Hedtrich	*Nanomedicine in Skin Research - Where Are We at?*
4:55	Anne Nguyen	*Self-Assembling Polymer-Drug Nanoparticles: A Platform Technology for Nanomedicine Optimization*
5:10		**Closing (Pieter Cullis / Urs Hafeli)**
5:20		Group Photo outside the Nest
5:40		Bus departs for Westin Bay Marina (for Pacific cruise)
7:00		"Magic Spirit" departs from Coal Harbour at Westin Bay Marina (1601 Bayshore Drive)

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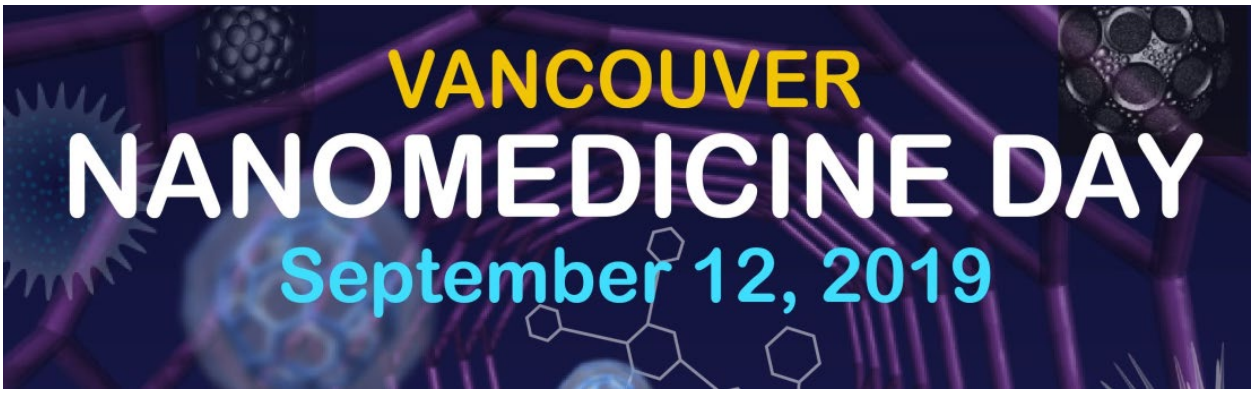

Dear Participants,

It is my great pleasure to welcome you to the 5th Vancouver Nanomedicine Day. It's truly an honour to be able to attract 350 renowned Canadian and international experts from across the field of nanomedicine.

During Nanomedicine Day, 20 talks and 47 posters will highlight the discoveries and innovations in nanomedicines that are contributing to global progress in acute, chronic and orphan disease treatment and management. Nanomedicines have allowed us to deliver drugs directly to disease sites, to dramatically improve their efficacy and reduce their toxicity, and to enable gene therapies employing RNA and DNA 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.

You will have unique networking opportunities with clinician-scientists, basic researchers, trainees, research partners, life science and startup biotechnology companies, board members and globally recognized researchers and innovators from UBC and NMIN (Nanomedicines Innovation Network).

But it does not end there. As research meetings should also be fun, we have arranged an evening boat trip and hope that you will be able to join us. It's an extra opportunity to discuss collaborations and network with other participants while enjoying beautiful views of Vancouver and Canada's Pacific coast and watching the sunset.

Thank you all for coming and I hope to see you next year again.

For the organizing committee,

Prof. Urs Hafeli, Faculty of Pharmaceutical Sciences,
University of British Colombia, urs.hafeli@ubc.ca

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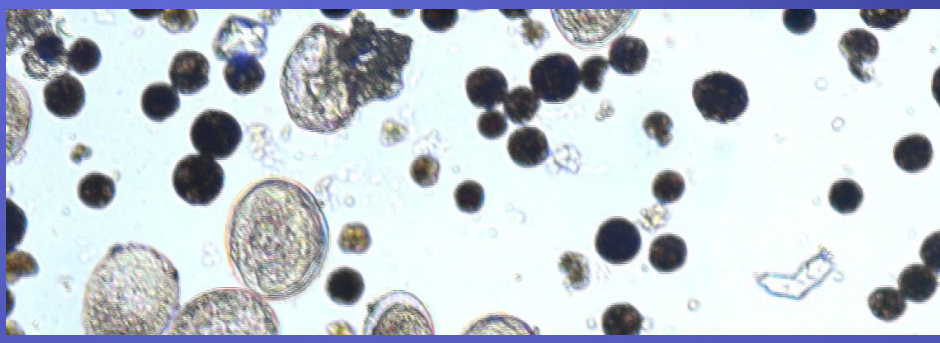
TALKS



Talk # 1

Plasmonics for diagnostics and therapy in nanomedicine

Michel Meunier

Professor of Engineering Physics and Biomedical Engineering
Department of Engineering Physics, Polytechnique Montreal
(michel.meunier@polymtl.ca)

Plasmonic nanoparticles such as gold, silver or their alloys are interesting nanomaterials for their applications in nanomedicine. These nanoparticles present specific optical scattering and absorption properties that can be used for therapy and diagnostics in nanomedicine. In this presentation, I will briefly present recent developments in this field at Polytechnique.

Targeted Laser Gene Therapy: A new method for delivering exogenous biomolecules into targeted cells using a femtosecond laser and plasmonic nanoparticles has been developed (1). The technique of laser nanosurgery has been used to perform gene transfection in living cells (2,3), neuron stimulation (4) and delivery of biomolecules in vivo for ophthalmic applications (5). More specifically, the proposed new technique addresses the current void in efficient, cell-specific, retinal drug delivery systems. Here we show that we can selectively optoporate retinal cells *in vivo* using a 800nm fs laser irradiation and functionalized gold nanoparticles (AuNPs). Following intravitreal injection and incubation, irradiation resulted in siRNA uptake by retinal cells. Importantly, neither AuNP intravitreal injection nor irradiation resulted in retina cells death. This non-invasive and non-viral gene therapy tool may provide a safe, cost effective approach to selectively target retinal cells (6).

Laser nanotherapy of eye cancer: Retinoblastoma is a rare form of cancer of the retina mostly occurring in the eye of young children. We successfully show that gold plasmonic nanoparticle-mediated laser hyperthermia is a potential efficient therapy to kill the cancerous cells (7).

Quantitative multiplexed cytology analysis: An innovative experimental setup for quantitative multiplexed cytology analysis based on the application of spectrally distinctive plasmonic NPs biomarkers has been developed. Proprietary alloy nanoparticles have been synthesized (8,9) and used to perform multiplexed 3D imaging of cells and tissues. Accurate differentiation of different NPs in a highly scattering cellular environment such as cytopathological samples is ensured by a specifically designed hyperspectral imaging system. Enhanced NPs contrast and 3D spatial resolution in the cells-NPs complex are achieved by using reflected light microscopy method using high numerical aperture imaging objective (10). Functionalized spectrally distinctive NPs can provide a new type of specific markers with better stability and multiplexing ability for a reliable diagnosis for pathology.

Conclusion: Our plasmonic based techniques show promise of innovative tools for basic research in biology and medicine as well as effective alternative technologies that could be adapted to the therapeutic and diagnostic tools of the clinic.

References (from Meunier's laboratory)

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(3) E. Bergeron et al, Nanoscale, 7, 17836 (2015)
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(9) D. Rioux, M Meunier, US Patent App. 10/239,122, (2019)
(10) S. Patskovsky et al, J. Biophotonics, 8, 401-407 (2015)

Talk # 2

Effect of removing Kupffer cells on nanoparticle tumour delivery and elimination

Wilson Poon[1,2*], Anthony J. Tavares[1,2], Yi-Nan Zhang[1,2], Ben Ouyang[1,2,3], Benjamin R. Kingston[1,2], Jamie L. Y. Wu[1,2], Qin Dai[1,2], Rickvinder Besla[4], Ding Ding[1,2], Angela Li[4], Juan Chen[5], Stefan Wilhelm[6,7], Gang Zheng[5,8], Clinton Robbins[4,9], Warren C. W. Chan[1,2,10,11,12]

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The mononuclear phagocyte system (MPS) of the liver (i.e. Kupffer cells) has been identified as the biggest barrier for nanoparticle delivery as it can sequester up to 99% of the injected nanoparticle dose [1]. We examined this barrier's impact on delivery. We removed the liver Kupffer cells *in vivo* using clodronate liposomes and observed that nanoparticle tumour delivery could be increased up to 150×, depending on particle design, chemical composition, degree of Kupffer cell depletion, and tumor type [2]. Furthermore, nanoparticle elimination from the body is important for decreasing unintended off-target accumulation and toxicity. Here we discovered that removal of Kupffer cells also increased fecal elimination by more than 10×. This was because Kupffer cell removal increased nanoparticle interaction with liver sinusoidal endothelial cells (LSECs). These LSECs impacted hepatobiliary elimination by allowing nanoparticles smaller than their fenestrae diameter (~100 nm) to fall through for increased access to the space of Disse and hepatocytes [3]. Together, these studies define concepts and strategies that can improve nanoparticle delivery and control nanoparticle fate to reduce unwanted bioaccumulation. This is critical to engineering medically useful and translatable nanotechnologies.

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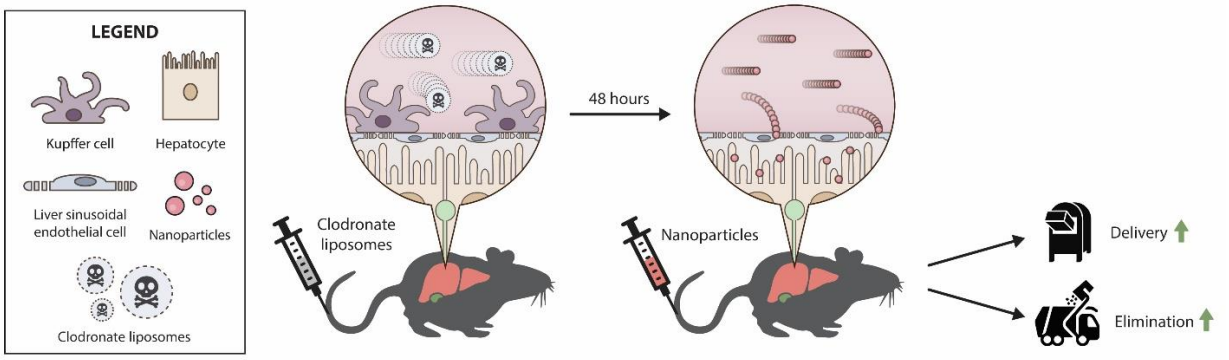
[2] Tavares et al, Effect of Removing Kupffer Cells on Nanoparticle Tumor Delivery. Proc. Natl. Acad. Sci. U. S. A. 2017, **114**, E10871 - E10880.
[3] Poon et al, Elimination Pathways of Nanoparticles. ACS Nano 2019, **13**, 5785-5798.



Invited Talk # 3

Nanoparticle-Mediated Profiling of Rare Cells for Liquid Biopsy

Shana Kelley
University of Toronto, Toronto, Ontario M5S 3M2, Canada

The analysis of heterogeneous ensembles of rare cells requires single-cell resolution to allow phenotypic and genotypic information to be collected accurately. We developed a new approach, magnetic ranking cytometry, that uses the loading of individual cells with functionalized magnetic nanoparticles as a means to report on biomarker expression at the single cell level. This approach can be used to profile circulating tumor cells in blood and provides a high-information content liquid biopsy in a single measurement. It profiles both protein (*Nature Nanotechnology*, 2017) and nucleic acid (*Nature Chemistry*, 2018) analytes at the single cell level.

Pharmacokinetics of Native and Formulated Innate Defense Regulator Peptides

Tullio V. F. Esposito[1,2], Colin Blackadar[1], Daniel Pletzer[3], Cristina Rodriguez-Rodriguez[1,4,5], Evan Haney[3], Robert E. W. Hancock[3], Katayoun Saatchi[1]* and Urs O. Häfeli[1]*

[1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada; [2]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [3]Centre for Microbial Disease and Immunity Research, Department of Microbiology and Immunology, Faculty of Science, University of British Columbia, Vancouver, Canada; [4]Department of Physics and Astronomy, Faculty of Science, University of British Columbia, Vancouver, Canada; [5]Centre for Comparative Medicine, University of British Columbia, Vancouver, Canada; *Corresponding Authors: kathy.saatchi@ubc.ca and urs.hafeli@ubc.ca

Host defense peptides (HDPs) are short, cationic, amphipathic peptides with antimicrobial, anti-inflammatory, and immunomodulatory activity. Unfortunately, poor physiochemical properties and biocompatibility have limited their efficacy in treating systemic inflammation and infections. There is also a significant lack of data on their pharmacokinetics and biodistribution, particularly on how they are affected by dose and route of administration.

To address this, we performed the first detailed pharmacokinetic assessment in mice of a lead HDP, innate defense regulator 1002 (IDR1002), via subcutaneous, intraperitoneal, intratracheal, and intravenous administration using nuclear imaging techniques. We also assessed how IDR1002 responds to changes in dose by administering it at concentrations ranging between 2.5 and 40 mg/kg.

We found that IDR1002 has an impractically narrow therapeutic window. At low intravenous doses it was rapidly cleared, predominantly by the kidneys; the peptide had a plasma half-life of only 1.2 minutes at 2.5 mg/kg. At higher intravenous doses, IDR1002 precipitated into large enough particles to be immediately lethal. For other routes of administration, IDR1002 displayed similar behaviour with rapid absorption at lower doses and formation of local depots at higher doses. No dose or route tested provided a plasma half-life suitable for therapeutic use. Therefore, formulations that improve the solubility and blood plasma half-life of IDR1002 are needed for further translation of this promising peptide.

We recently re-formulated IDR1002 by conjugating it to a nanoscale carrier via a cleavable linkage. This construct was designed to hold the peptide in the blood and release it from that site gradually over time. A SPECT/CT pharmacokinetic study found this to be true, with the half life of IDR1002 increased to just over 6 hours. Furthermore, the constructs were non-toxic and did not precipitate upon dose escalation, significantly improving the biocompatibility and therapeutic window of IDR1002. Efficacy studies with an invasive *Pseudomonas aeruginosa* strain, PA14, are currently underway.

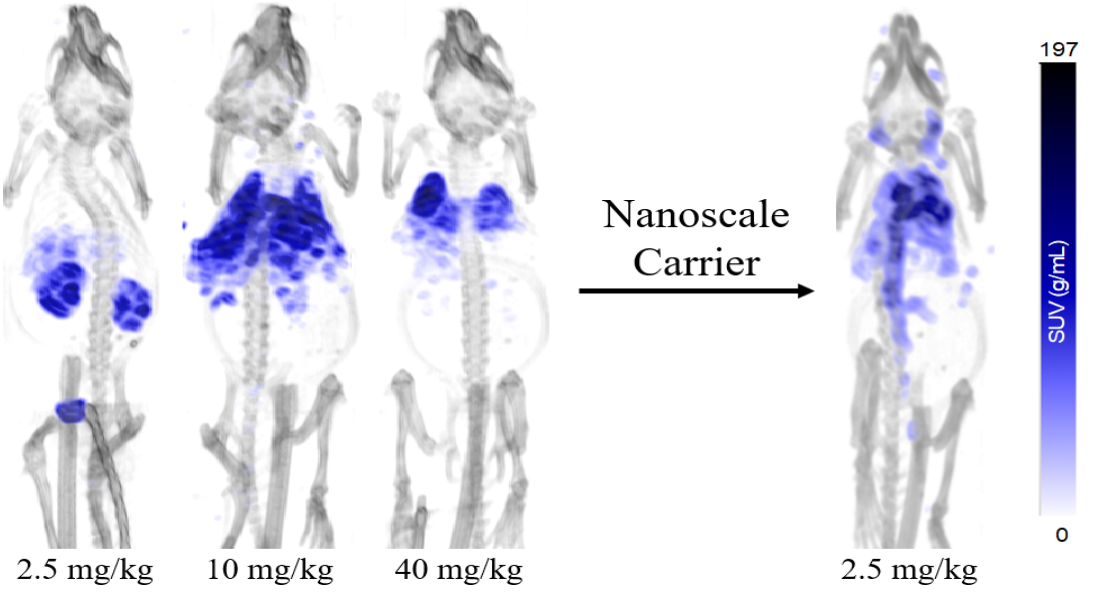


Figure 1. SPECT/CT renderings of intravenously administered IDR1002 before and after the re-formulation. All scans are at 0 hours post-injection. The renderings on the left show that IDR1002 is rapidly filtered by the kidneys at 2.5 mg/kg and that it embolized in the lung capillary bed at the higher doses. The rendering on the right shows our re-formulation in the bloodstream.

Talk # 5

A Clew Into Improving Targeted Drug Delivery Through pH-Dependent DNA Origami

Cathy Yan[1,2,*], Kendrew Wong[1,2,*], Melanie Law[1,2]

[1]BIOMOD, Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC,

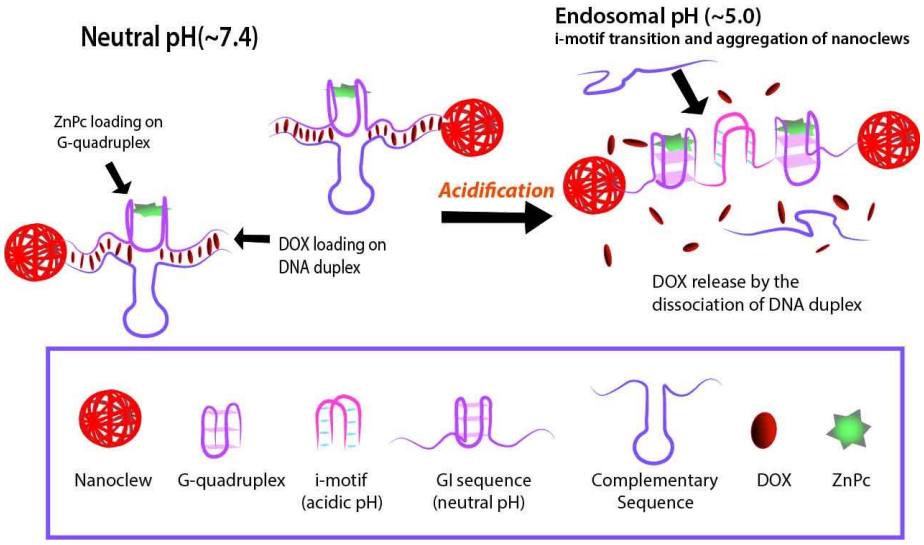
[2]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC

*These authors contributed equally to this work

Hodgkin's lymphoma arises from abnormally large and multinucleated B cells that eventually form tumours. The ability of Hodgkin's lymphoma to spread to any tissue in the body via the lymphatic system [1] poses serious challenges in treating the disease. Due to the drawbacks of conventional chemotherapy [2], alternative methods of drug delivery are required. Using DNA origami, we created a biocompatible nanostructure that mitigates the damage caused by off-target effects [3] and executes dual combinatorial therapy.

The device consists of two major components - a DNA duplex composed of a G-quadruplex and i-motif (GI) sequence encapsulating the drug, and the delivery vehicle known as a nanoclew. Atomic force microscopy and laser scanning confocal fluorescence microscopy were used to confirm nanoclew assembly. Aptamers encoded in the nanoclew are specific to Hodgkin's lymphoma and allow for the device to be endocytosed. The lower pH caused by endosomal acidification induces a conformational change of the GI sequence, leading to the release of its complementary sequence along with doxorubicin. Circular dichroism confirmed that the GI sequence both maintained its folding integrity and properly underwent conformational change when exposed to a decrease in pH. Incorporation of zinc phthalocyanine into the G-quadruplex generated reactive oxygen species under laser radiation, to which cancer cells are vulnerable [4]. Binding, internalization, and efficiency in killing lymphoma cell lines (HDLM-2, KM-H2) will be shown through flow cytometry, confocal microscopy and Trypan Blue assays respectively.

Our design of a combinatorial therapy, composed of chemotherapy and photodynamic therapy, works to more effectively treat Hodgkin's lymphoma through improved specificity compared to conventional means.



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[2]Treating Classic Hodgkin Lymphoma, by Stage. (n.d.). Retrieved May 08, 2019, from https://www.cancer.org/cancer/hodgkin-lymphoma/treating/by-stage.html

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The Metaplex Technology: Use of coordination chemistry and nanotechnology to develop poorly soluble metal-binding small molecules

Ada W. Y. Leung[1,2,3], Kent T.J. Chen[2,4], Michael J. Abrams[1], and Marcel B. Bally[1,2,4,5,6]

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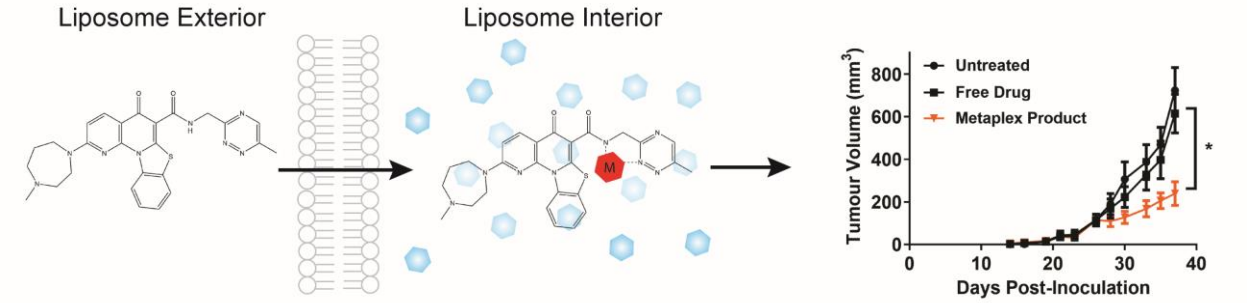
[6]Pathology & Laboratory Medicine, University of British Columbia, Canada

Nearly 70% of all small molecule drug candidates are discarded and not pursued for pharmaceutical development because of poor aqueous solubility (<1 mg/mL). It is well-known that this problem can be addressed through reformulation strategies. Yet, there is still a plethora of compounds that cannot be efficiently formulated using conventional methods. Cuprous' Metaplex technology is a platform that utilizes coordination chemistry and liposomal formulations to encapsulate small, typically slightly soluble to practically insoluble, molecules. In many cases, encapsulation efficiency of >98% can be achieved within minutes following addition of selected compounds to pre-formed liposomes. Depending on the drug of interest, the apparent solubility can be increased over 500-fold, taking a compound that has a maximum solubility of 0.02 mg/mL to over 10 mg/mL. Cuprous has validated the approach using over 20 investigational compounds and approved drugs. Selected Metaplex formulations have been assessed *in vivo* and where possible the activity has been compared to the free compound. These Metaplex products are safe to administer parenterally and orally. In the former example, the Metaplex formulation extends the compound's circulation life time, reduces toxicity, and improves therapeutic activity: advantages that are typical of many liposomal formulations. Cuprous also has examples where formulations assessed were of therapeutically active compounds that are insoluble and have never been administered parenterally before now. Altogether, the Metaplex technology is applicable to small molecules capable of forming coordination complexes and Cuprous is applying this technology to enable pharmaceutical development of anticancer agents, antimicrobials, and immune enhancers. This asset is expected to attract industrial partners and will be used to produce best-in-class nanomedicines for a broad range of disease indications.



Invited Talk # 7

Optically Activated Nanomedicines: Photodynamic Priming as a Tool

Tayyaba Hasan, PhD
Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA; Division of Health Sciences and Technology, Harvard University and Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Optical activation of materials leads to thermal, photochemical and radiative processes which can be captured for response-based therapeutic design. The ability to use light as a reagent to control drug release further allows for the fabrication of light controllable intelligent multiagent constructs that attack multiple pathways making the nanomedicines more effective against cancer. Combination therapy is a fairly well accepted standard for cancer treatment and management of other diseases. Typically, these are administered separately with their own pharmacokinetics, hitting targets at different times which reduces the synergism potential. Nanomedicines, to some extent can overcome this limitation by delivering the multiple agents to the target site at the same time provided there is synergism in any aspect of the agents. Photodynamic therapy (PDT) is a photochemistry-based process that is approved for several clinical applications world-wide. It involves the exposure of light activatable molecules to appropriate wavelengths that leads to the generation of active molecular species that is responsible for targeted death. There are many unique attributes to this process. Because of the requirement of light and the photosensitizer being present at the same place at the same time there is an additional level of selectivity. Neither light alone nor the photosensitizer have an effect on target cells by themselves. In addition to the direct cytotoxic effect, the photodynamic activation primes the microenvironment in a process call *P*hoto*D*ynamic *P*riming (PDP) to enable a more potent response to conventional treatments so the PDP becomes an enabler of other treatments, particularly when administered in a Nanoplatform. Strategies for syntheses and applications in biology and medicine will be discussed. The essentials of the platform are presented in the conceptual Figure 1 below.

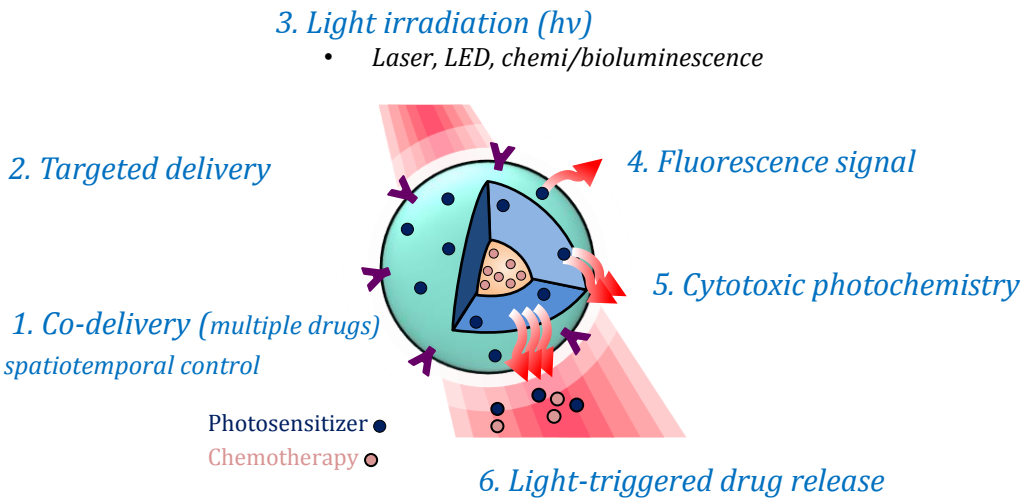


Figure 1. Optically Activated Nanoplatform for Photodynamic Therapy:
1. Co-delivery of multiple agents designed for light-based spatiotemporal release of each agent
2. Tissue/cell/organelle-specific Targeted Delivery
3. Light triggered controlled release
4. Fluorescence for image guided treatment design on-line. Visualization of localization
5. Cytotoxic photochemistry, priming of target for multiagent enablement. Release of reactive molecular species that cause cell death and impact target for subsequent treatments
6. Light triggered release of conventional drug to the primed target

Invited Talk # 8

Photoacoustic Imaging: a Timely Tool for Drug Discovery and Assessment of Tumor Pathophysiology

Devin O'Kelly[1], James W. Campbell III[1], Jeni L. Gerberich[1], Li Liu[1], Kevin G. Pinney[2], Mary Lynn Trawick[2], David J. Chaplin[2], Payal Kapur[3], James Brugarolas[4], Ralph P. Mason[*1],

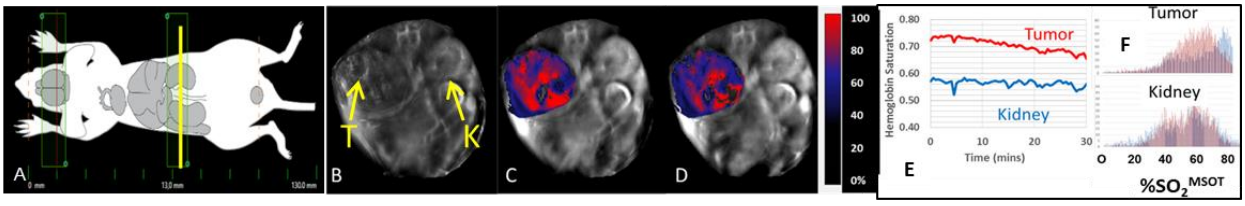
Departments of [1]Radiology, [3]Anatomical Pathology, [4]Hematology Oncology, UT Southwestern Medical Center, Dallas, TX; [2]Chemistry and Biochemistry, Baylor University, Waco, TX

(Ralph.Mason@UTSouthwestern.edu)

Photoacoustic imaging (PAI) has recently become commercially available as a tool for pre-clinical research and has demonstrated use in clinical trials. PAI offers unique, non-invasive insight into living systems based on pulsed light inducing local thermal expansion in a material, in turn generating acoustic perturbations detectable as ultrasound. Multispectral optoacoustic tomography (MSOT) uses excitation at specific wavelengths and spectral unmixing based on the spectral characteristics of individual molecules to reveal chromophore distributions; typical spatial resolution is about 150 μm, to a depth of several centimeters, with sub-second temporal resolution [1]. Several natural endogenous tissue components are readily visualized, including oxy- and deoxy-hemoglobin. MSOT appears particularly suitable for evaluating sequelae of vascular disrupting agents (VDAs) [2]. As shown below, MSOT reveals vascular heterogeneity in tumors and dynamic observation showed progressive hypoxiation over 30 mins following administration of the classic first-generation VDA combretastatin (CA4P). Histograms of vascular oxygenation showed distinct hypoxiation and continual monitoring of [HbO_2] showed the selective onset of hypoxia in the tumor, while the contralateral kidney remained unaffected.

Many exogenous materials provide strong optoacoustic signals, including various fluorescent dyes such as indocyanine green and IRDye 800CW. Nanoparticles may be loaded with dye, but plasmonically active substrates such as gold and silver provide particularly strong inherent signal. Such agents may act as reporter tags or be tailored for sensitivity to specific environmental stimuli, revealing tissue properties such as pH and enzyme expression [3].

MSOT is particularly effective at revealing changes in tissue pathophysiology such as changes in vasculature accompanying tumor development and response to therapy. MSOT can track pharmacokinetics and biodistribution of infused materials as well as the consequent pharmacodynamic response to an intervention. This review will provide perspective on the capabilities and applications of MSOT with vignettes from our research and the literature.



Photoacoustic assessment of acute vascular disruption in tumor. A) Cartoon showing location of axial imaging plane in mouse; **B**) Distinct anatomy is apparent in the transaxial slice MSOT image showing orthotopic XP373 renal cell carcinoma (T) and contralateral control kidney (K). **C**) Maps of relative tumor vascular oxygenation overlaid on B; **D**) Regional hypoxiation was seen in the tumor vasculature following CA4P; **E**) Dynamic changes were observed over 30 mins following CA4P (120 mg/kg, IP), while contralateral normal kidney showed no change, **F**) histograms, verifying selective activity against the tumor (blue baseline, red post CA4P).

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Supported in part by NIH P30 CA142543, P50 CA196516, S10 OD018094 and CPRIT.

Talk # 9

Re-188 COLLOID BRACHYTHERAPY FOR NON-MELANOMA SKIN CANCER

Philip Cohen [1], Tassia Godoy [2], Shannon Brown[3], Cesidio Cipriani[4]

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Non melanoma skin cancers, either squamous cell or basal cells are among the most common types of skin cancer, with the cancers so ubiquitous they are often not reported. Multiple therapies exist for treating skin cancers, and many, such as local excision or chemical or thermal therapies are acceptable if scarring is not a concern. However in many individuals the cancers are multiple, or located on particularly vulnerable or inaccessible parts of the face such as lips, eyelids, ears, nose.

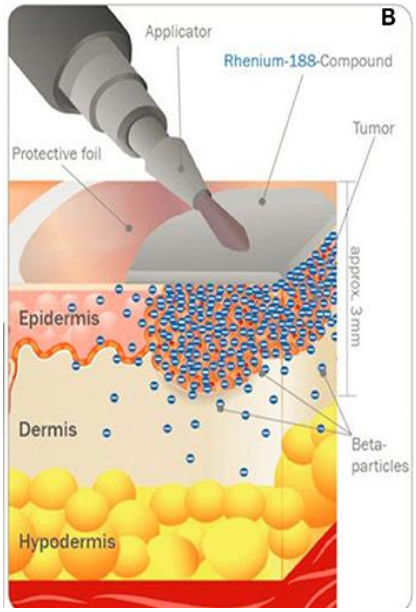
A particularly original and attractive treatment modality using ^{188}Re-particulates is the use of ^{188}Re-colloids within a brachytherapy device for skin cancer treatment. Radioactive patches made of nitrocellulose filter paper loaded with ^{188}Re-tin colloids were developed by Jeong et al.). This method was successfully used in patients with keloids, a benign dermal fibro proliferative tumor, and non-melanoma skin cancers [1]

Fifty-three patients with histologically confirmed basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) were treated. Three months later, complete healing was obtained in 100% of the treated patients; even after a single application in 82% of the cases. After a mean follow-up of 51 months, no clinical relapses were observed in the treated patients, and histological examination confirmed complete tumor regression. The inert matrix containing the ^{188}Re is able to adapt to every skin surface without contamination, imparting an accurate distribution of dose and sparing the healthy tissue.[2]

This therapy is now being introduced commercially in several centers in Canada, Australia, Germany, and South Africa. ^{188}Re colloid brachytherapy use will likely become more available as a result, and it's use likely extended to other therapeutic applications

1. Shukla J, Mittal BR. ^{188}Re Tailor Made Skin Patch for the treatment of skin cancers and keloid: overview and technical considerations. Int J Nucl Med Res. (2017) 107–113.

2. Lapareur et al Rheunium-188 Labeled Radiopharmaceuticals: Current Clinical Applications in Oncology and Promising Persp ectives



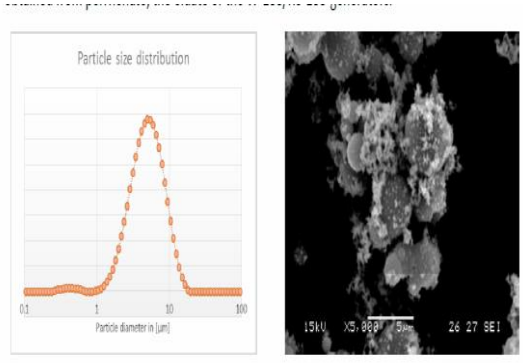
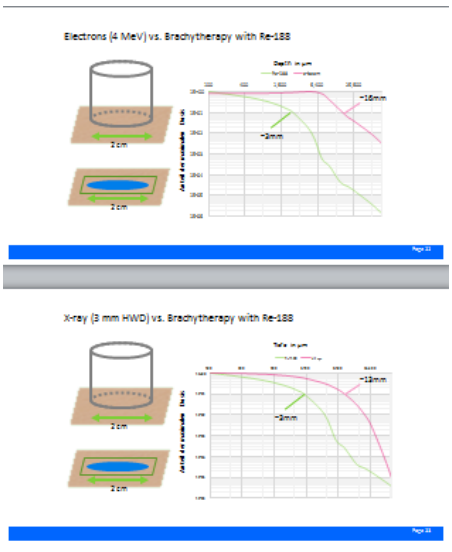


Figure 2: Left, particle size distribution of dirhenium-heptasulfide from our production. Right, scanning electron microscope image of dirhenium-heptasulfide particles (round).



Co-encapsulation of Curcumin with SN-38 leads to Increased Encapsulation Efficiency of SN-38 in PCL-b-PEO Polymeric Nanoparticles

Lisa Silverman, and Matthew G Moffit

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Irinotecan (CPT11), is converted to 7-ethyl-10-hydroxycamptothecin (SN-38), in the body, but only 2-8% of the CPT-11 administered to a patient is converted to SN-38, which is 100-1000 times more potent. Clinical use of SN-38 is limited because it is virtually insoluble in water and is unstable above pH 6, making it a prime candidate for encapsulation. One problem is that it is very difficult to encapsulate SN-38, with encapsulation efficiencies commonly below 10 percent.

By combining SN-38 with another drug, we could potentially increase its efficacy and encapsulation efficiency. Using a liquid-gas two-phase microfluidic reactor, we have manufactured particles co-encapsulating SN-38 and curcumin.

Particles without curcumin had an approximately 6.6% encapsulation efficiency of SN-38, while particles with an optimal combination of curcumin and SN-38 had an encapsulation efficiency of approximately 14%, while particle size remained constant at approximately 50nm.

These results represent a new option to effectively increase the encapsulation efficiency of SN-38 in polymeric nanoparticles.

[1]Y Cao, L Silverman, C Lu, R Hof, J Wulff, and M Moffitt. *Molec. Pharm.* **2019**,*16*, 96. [2] A Bains, Y Cao, S Kly, J Wulff, and M Moffitt. *Molec. Pharm.* **2017**,*14*, 2595.

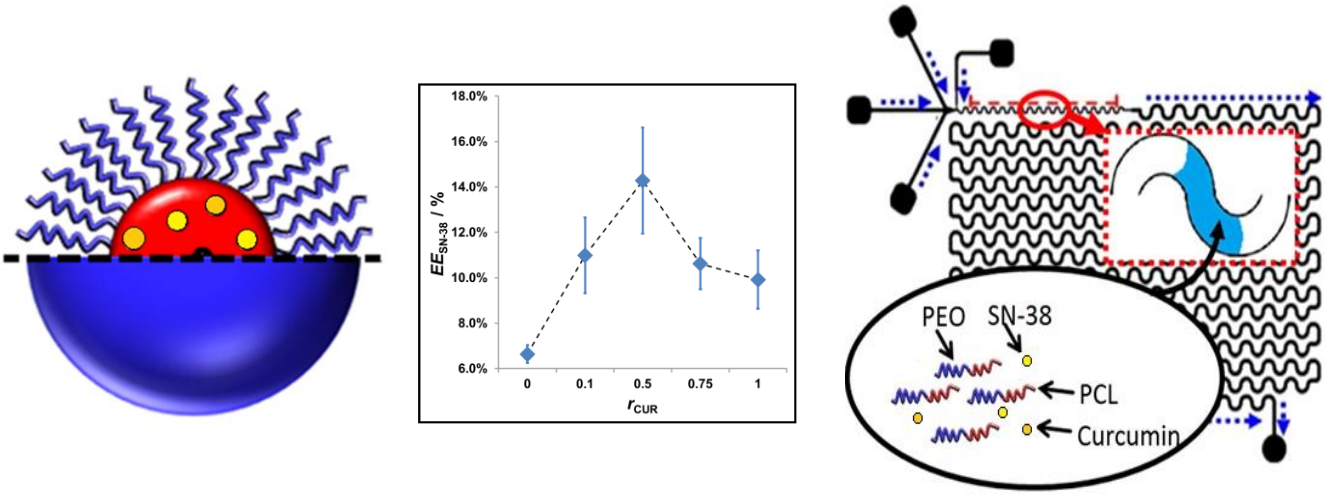

Figure 1: Microfluidic manufacture of polymeric particles co-loaded with curcumin and SN-38 increases encapsulation efficiency of SN-38.

Effects of cellulose nanocrystal on physical properties of a dental adhesive resin blend

De Morais DC*[1], Palma-Dibb RG[1,2], Dietrich C[1], Thanh-Dinh N[3], MacLachlan MJ[3], Carvalho RM[1], Manso AP[4]

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Objectives: To test the effect of cellulose nanocrystal (CNC) on physical properties of an experimental adhesive resin. **Methods:** Adhesive resin blend (50%BisEMA; 30%TeEGDMA; 14%HEMA; 4%ethanol; 2%photoinitiators) was loaded with non-treated or treated (-NT or -T) CNC at 0.5%, 1.0%, 1.5% or 3.0% by weight (n=10). Mixtures were stirred for 24-hour followed by 24-hour vacuum degassing. Control group was produced with resin blend only. The properties investigated were **flexural strength (FS**) (ISO4049) stored for 24 hours, **dry (FSd**) or in **artificial saliva(FSs**), at 37°C; **water sorption (WS**) and **solubility (S**); **degree of conversion (DC**); **contact angle (CA**); and **thermogravimetric analysis (TGA)**. Data were analyzed individually in two-way ANOVA tests, using material (-NT or -T) and CNC percentages as the two factors. Post hoc multiple comparisons were performed using Tukey's test, at α=5%. **Results:** For the **FSd** results, all concentrations of CNC-NT and 0.5% of CNC-T were similar to control group. For **FSs** results, CNC-NT at 0.5% and 1% showed lower FS compared to control ($p<0.05$) (Table 1). Moreover, for **WS** results, CNC-T and -NT at 3% showed statistically higher values than control. **Solubility(S)** results for CNC-NT, were all similar to control; however, for CNC-T at 0.5% and 1.5%, S was higher than control. In **DC**, all treated (-T) presented a decrease, regardless of the concentration. For **CA** all CNC %s, in both conditions, were similar to the control group. In **TGA**, no significant differences were observed to treated vs. non-treated CNC. **Conclusions:** Depending on the concentration and treatment, CNC can be used into a resin blend without significant change of its physical properties. **Acknowledgements:** Start-up funds UBC (RMC, APM); FAPESP, Brazil (2017/19229-9 to RGP-D)

Table 1: Result and comparisons among the tested conditions, (-NT/ -T) and the CNC concentrations (%), for each statistical analysis.

		Control	CNC 0.5%	CNC 1.0%	CNC 1.5%	CNC 3.0%
Flexural strength Saliva (FSs) (MPa)	CNC-NT	47.4(7.4)^{A}	36.4(2.6)^{bBC}	33.6(5.2)^{bC}	42.7(4.8)^{aAB}	44.6(4.8)^{aA}
	CNC-T		43.2(7.4)^{aA}	42.8(6.5)^{aA}	44.0(4.3)^{aA}	42.6(4.0)^{aA}
Flexural strength Dry (FSd) (MPa)	CNC-NT	76.5(5.0)^{A}	71.1(5.8)^{bA}	75.0(8.0)^{aA}	68.1(8.3)^{aA}	74.2(4.7)^{aA}
	CNC-T		82.8(9.6)^{aA}	67.0(3.8)^{bB}	65.8(9.0)^{aB}	71.8(4.8)^{bB}
Water Sorption (WS) (µg/mm^3)	CNC-NT	76.5(5.0)^{A}	59.2(12.0)^{aC}	60.8(7.3)^{aC}	58.0(9.8)^{aC}	80.2(7.9)^{aA}
	CNC-T		67.2(9.5)^{bB}	64.3(10.7)^{aBC}	68.5(11.4)^{aBC}	80.6(19.7)^{aA}
Solubility (S) (µg/mm^3)	CNC-NT	13.6(11.3)^{C}	20.9(20.5)^{aC}	23.1(9.9)^{aC}	17.2(6.4)^{aC}	19.3(8.0)^{aC}
	CNC-T		35.6(15.4)^{bA}	21.3(9.4)^{aBC}	33.5(16.9)^{aAB}	20.8(10.4)^{aBC}
Degree of Conversion (DC) (%)	CNC-NT	51.15(0.55)^{ABC}	53.08(2.40)^{aAB}	56.43(1.81)^{aA}	45.67(6.37)^{aBC}	44.21(2.55)^{aC}
	CNC-T		37.07(1.87)^{bD}	38.70(4.65)^{bD}	38.29(3.29)^{bD}	38.82(3.55)^{aD}
Contact Angle (CA) (°)	CNC-NT	17.76(7.38)^{ABC}	23.4(7.39)^{aA}	19.27(6.97)^{aA}	20.20(6.05)^{aA}	19.94(5.51)^{aA}
	CNC-T		25.43(10.20)^{aA}	23.38(5.99)^{aAB}	17.08(4.76)^{aBC}	13.45(2.03)^{bC}

Data is presented by means (SD). Capital letters compare -NT and -T, lower case letters compare CNC concentrations.

In vitro and *ex vivo* characterization of poly(acrylic acid) grafted Cellulose Nanocrystals (CNC-PAA) as novel nano-hydrogels for local delivery of platinum-based chemotherapeutics in oral Cancer

Waleed Mohammed-Saeid[1,2], Mohammad Reza Vakili[1*], Julian Ethier-Hopwood[1], Behzad Ahvazi[3], Afsaneh Lavasanifar[1*]
1 Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada, afsaneh@ualberta.ca, vakili@ualbertaz.ca
2 College of Pharmacy, Taibah University, Medina, Saudi Arabia
3 InnoTech Alberta, Edmonton, AB, Canada

Oral squamous cell carcinoma (OSCC) is the most prevalent and dangerous form of oral cancers with an average 5-year survival rate of 57%. Surgery and radiation therapy accompanied by chemotherapy are treatment options for OSCC. Platinum(Pt)-based antineoplastic agents, such as cisplatin and carboplatin, are commonly used via systemic administration for the clinical management of OSCC. The intrinsic toxicity of these agents following systemic administration, especially the nephro- and neurotoxicity, limits their clinical use. Local delivery of such chemotherapeutic agents to the oral tumor can assist in reducing the side effects of therapy while providing higher concentration of the drug at the tumor site, when compared to systemic administration.

The objective of this research was to evaluate the potential application of hydrogels based on a newly generated derivatives of cellulose nanocrystals (CNC), i.e., poly(acrylic acid) grafted CNC (CNC-PAA), for local delivery of platinum-based antineoplastic agents in OSCC. Cisplatin formulations of CNC-PAA were evaluated for their cytotoxicity against two human OSCC cell lines, HSC-3 and OSC-19, making comparisons with the free drug. *In vitro* drug release and *ex vivo* penetration of platinum from the hydrogel films in comparison to free drug was also studied via dialysis method and Franz diffusion cells using porcine buccal tissue, respectively. In addition, a newly designed apparatus was used to evaluate the ex vivo muco-retentive behavior of the developed nanogels and incorporated platinum drug by measuring the kinetics of gel/platinum wash out from the surface of porcine buccal tissue by simulated saliva fluid.

Our results showed a lower *in vitro* cytotoxicity for the CNC-PAA formulation of cisplatin compared to free drug (2-3 fold increase in IC_{50}), owing to a slower drug release from this formulation. Only 30% of incorporated cisplatin was released from CNC-PAA-Pt complexes after 2 hr compared to > 80 % release of free cisplatin, at same time point. The results from muco-retentive apparatus confirmed the advantage of PAA grafted CNC compared to PAA or CNC alone in the formation of muco-retentive nano-gels. The findings demonstrated the potential applicability of the CNC-PAA as a safe and efficient nano-gel carrier for Pt-based chemotherapeutic in oral cancer therapy

Funding: Alberta innovates Biosolutions

Talk # 13

Development of a liquid blood biopsy test for breast cancer using nanoscale flow cytometry

Nikki Salmond & Karla Williams

Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver

Breast cancer (BCa) is the second most common cancer amongst women in Canada and there is a need for improved non-invasive methods for diagnosis and for prognostication of metastasis. Lipid bilayer enclosed nano-sized extracellular vesicles (EVs: 50 – 200 nm) are released by all cell types and carry information about the cell of origin in protein signatures. Consequently, EVs reflect the health or disease status of the original cell from which they derived. EVs reside in blood plasma, meaning EV composition can be analyzed from liquid blood biopsies. As EVs are difficult to isolate, a high throughput methodology that can rapidly analyze EVs in complex biological fluids is in high demand. Here we use an emerging technology -- nanoscale flow cytometry -- to detect and quantitate breast derived EVs in plasma from subjects who are healthy, have benign breast disease, ductal carcinoma in situ (DCIS) or BCa. Using the EV marker CD9, we identified EVs in whole plasma without isolation or pre-enrichment for EVs. Using multi-color nanoscale flow cytometry we were able to detect populations of EVs from different cells of origin including breast (Mammaglobin-A positive) and platelet (CD41 positive) derived EVs. Breast derived EVs were subsequently purified from plasma by size exclusion chromatography were characterized by nanoscale flow cytometry, Western blot, nanoparticle tracking analysis and electron microscopy. Overall, nanoscale flow cytometry shows great promise for the analysis of breast derived EVs in complex biological samples and when used in conjunction with a cancer specific marker, hormone marker or marker associated with metastasis, the technology could be used for diagnosis and prognostication of individual cancer patients.

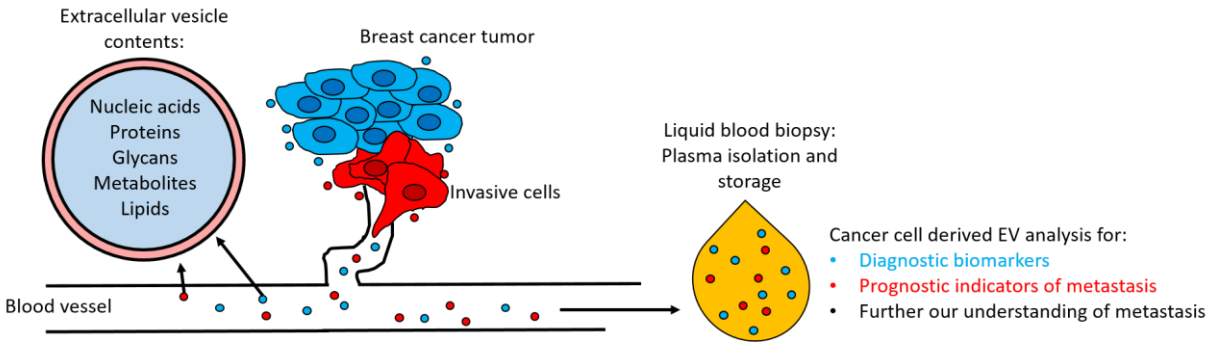

Figure 1: Diagram depicting the usefulness of BCa derived EVs in liquid blood biopsies. BCa cells exhibit unique protein expression characteristics as compared to neighboring healthy cells. EVs released by BCa cells into the blood stream reflect the protein expression changes of the cell of origin and could be used in a liquid blood biopsy to diagnose the presence of a BCa tumor, or to ascertain the aggressiveness of a BCa and prognosticate the likelihood of metastasis.

Enhancing the Modification of Protein Nanoparticles with X-Rays and Gold: Potential for triggered drug release

Courtney van Ballegooie[1,4*], Alice Man[1,4], Alessia Pallaoro[2,4], Byron Gates[2], Cornelia Hoehr[5], Donald Yapp[3,4]

[1]Faculty of Medicine, University of British Columbia; [2]Department of Chemistry, Simon Fraser University; [3]Faculty of Pharmaceutical Sciences, University of British Columbia; [4]Experimental Therapeutics, BC Cancer; [5]Life Sciences, TRIUMF

Zein is a class of prolamin proteins found in the endosperm of corn and is an attractive drug carrier for several reasons: its (1) biocompatibility, (2) renewable and abundant source, and (3) its amphiphilic nature. The amphiphilic nature of Zein allows the encapsulation of hydrophobic drugs and formation of nanoparticles (NPs) [1]. Our group has previously used microfluidics to produce these NPs in a robust and reproducible manner [2]. We are now examining the use of X-rays as an external trigger to release drugs from Zein NPs. The radiolysis of water by X-rays generates reactive oxygen species (ROS) which, under certain conditions, is enhanced by the presence of gold NPs. ROS are known to react with surrounding materials, such as DNA, proteins, and lipids, to degrade and destabilize their structure [3]. The destabilization of Zein NPs in this way could potentially be used to release drug on-demand with the application of X-rays. We present here, the design of Zein NPs that are destabilised by exposure to X-rays. We also show data indicating how the total X-ray dose, dosing schedule, and presence of gold NPs, impact the structure of Zein NPs. Overall, the presence of gold NPs and increase in applied total dose lead to a greater degree of protein modification in the Zein NPs. Interestingly, the dosing schedule, whether the dose was applied at a single time point or as smaller doses at multiple time points, was found to have no impact on the degree of modification of Zein NPs.

[1] Y Luo (2014) J. Appl. Polym. Sci. **131**, 1-12; [2] C van Ballegooie (2019) Processes **7**, 290; [3] C van Ballegooie (2019) Pharmaceutics **11**, 125

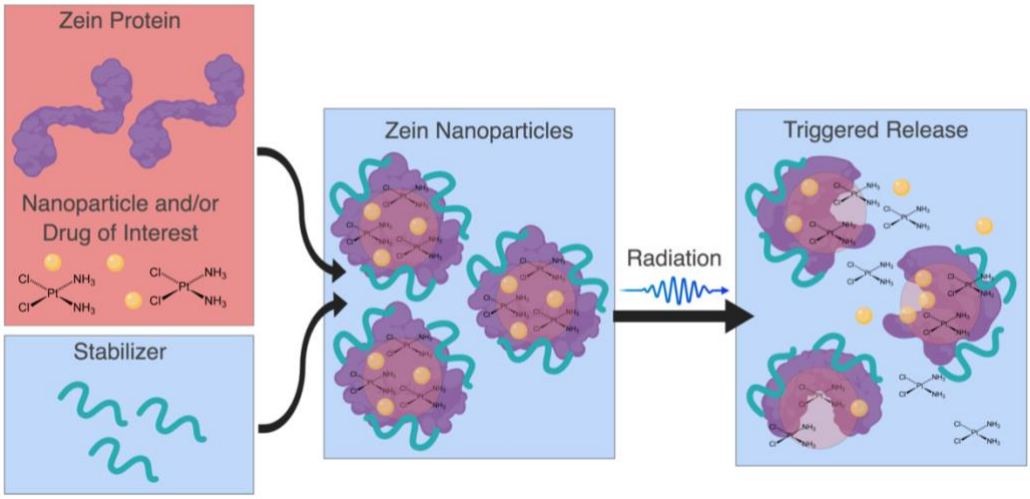

Figure 1: Schematic Representing the Formation and Radiation Induced Triggered Release of Zein NPs

Talk # 15

A lipid nanoparticle approach for targeting the NLRP3 inflammasome in tissue-resident macrophages in diabetes.

Heather Denroche[1,4], Alex Hsu[1], Sam Chen[4], Joshua Zaifman[2,4], Ying Tam[4], Chris Tam[4], Marco Ciufolini[2,4], Pieter R. Cullis[3,4], C. Bruce Verchere*[1,4]

[1]Department of Surgery, University of British Columbia, Canada, bverchere@bcchr.ca; [2]Department of Chemistry, University of British Columbia, Canada; [3]Department of Biochemistry, University of British Columbia, Canada; [4]Integrated Nanotherapeutics, Vancouver, Canada

Diabetes is a devastating disease with an enormous economic burden. Type 2 diabetes (T2D) represents 90% of all diabetes cases and is associated with obesity, insulin resistance, and progressive dysfunction and loss of insulin-producing beta cells in the pancreas. T2D is an inflammatory state, in which inflammation persists through secretion of pro-inflammatory cytokines by immune cells, particularly macrophages. In T2D, proinflammatory macrophages accumulate in adipose tissue and in pancreatic islets, leading to local production of IL-1β by a mechanism involving activation of the NLRP3 inflammasome (see Figure). As inflammation in adipose and islets is a driver of T2D pathogenesis, anti-inflammatory drugs should be a plausible treatment option, but clinical use of anti-inflammatory drugs in T2D is hampered by lack of cell specificity and off-target side-effects. We aim to create a nanomedicine therapeutic for T2D that will target the NLRP3 inflammasome specifically in macrophages, inhibiting local production of IL-1β and thus enhancing insulin sensitivity and beta-cell function.

Since phagocytic cells (including macrophages) take up lipid nanoparticles (LNP) preferentially, we are developing pro-drug inhibitors of the NLRP3 inflammasome encapsulated in LNP. Our data indicate that these LNP are taken up preferentially into tissue-resident macrophages (including macrophages in pancreatic islets and in adipose tissue). Ex vivo studies in bone-marrow derived macrophages indicate that these LNP, with our current lead pro-drug, inhibit release of IL-1β stimulated by treatment with LPS plus ATP. We aim to test these LNP in mouse models of T2D and obesity with both islet and adipose tissue inflammation.

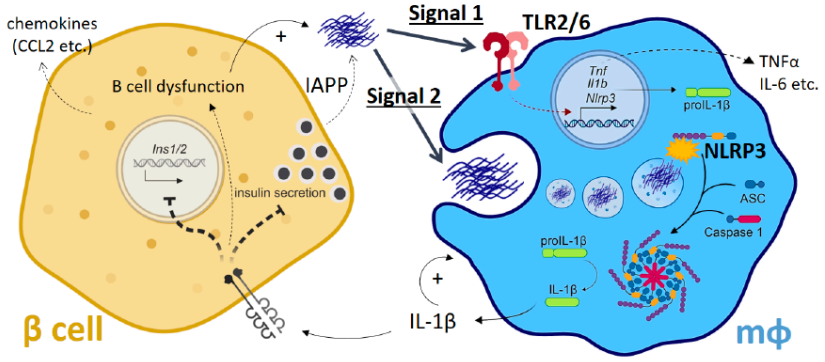

Figure 1: *Pathway for NLRP3 activation and IL-1β release from islet macrophages in response to amyloid deposits in T2D, a major stimulus for NLRP3-mediated IL-1β production*. Our nanomedicine therapeutic aims to target the NLRP3 inflammasome in tissue-resident macrophages, including those in islets and in adipose tissue.

Invited Talk # 16

Design of Lipid Nanoparticle Delivery Systems That Enable Gene Therapies

Pieter R. Cullis

Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada;

Sophisticated delivery systems are required in order to enable RNA and DNA-based macromolecules to be used as therapeutics. These delivery systems must protect encapsulated nucleic acid polymers from degradation in the circulation, promote accumulation in target tissue and facilitate intracellular delivery into target cells following systemic administration. Lipid nanoparticles (LNP) are currently the leading systems for satisfying these demands. In particular, LNP siRNA systems containing optimized ionizable cationic lipids have now been developed that are highly potent and relatively non-toxic agents for silencing target genes in hepatocytes following i.v. injection. This has led to an LNP siRNA product that has recently been approved by the FDA and EMA to treat transthyretin-induced amyloidosis, a fatal hereditary disease that usually leads to death within five years of diagnosis. This is the first drug based on RNA interference that has received regulatory approval. Importantly, the LNP delivery systems developed for siRNA are also effective for delivery of mRNA, plasmids and constructs such as CRISPR Cas 9. Such systems are showing promise for gene replacement therapies, vaccine applications and gene editing procedures. It is expected that these advances will soon include extrahepatic tissues and lead to widespread applications of gene therapies to treat many human diseases.

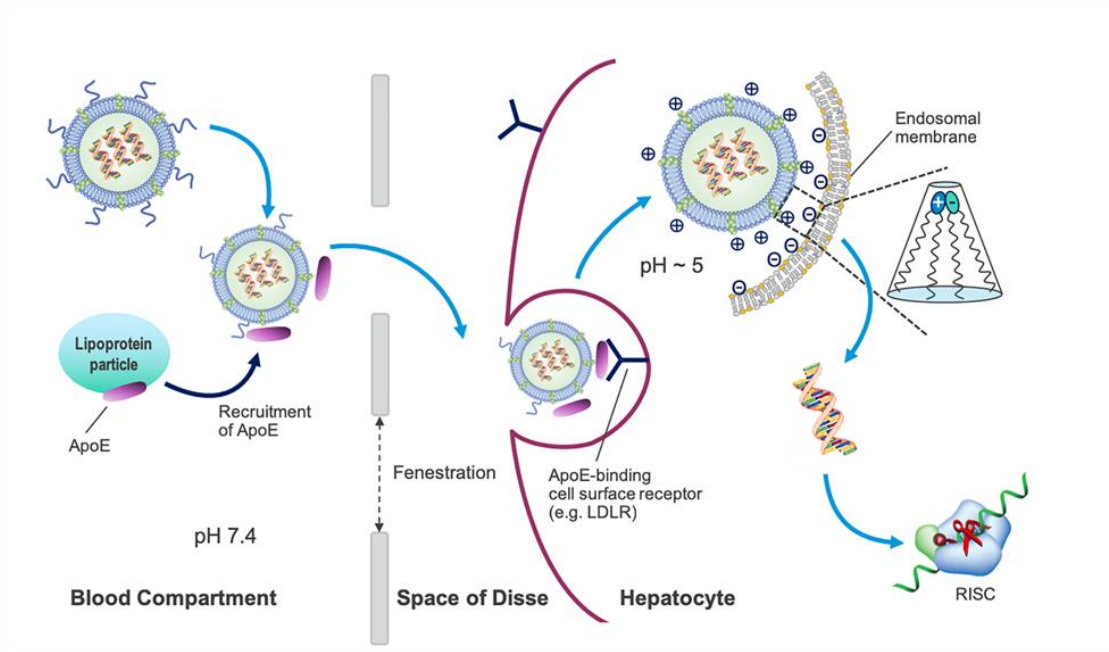

Figure 1. Integrated model of LNP-mediated *in vivo* delivery of siRNA to hepatocytes; key steps include (i) dissociation of PEG-lipids from the particle surface, (ii) recruitment of endogenous ApoE to the LNP surface, (iii) trafficking of LNPs through fenestrated endothelium and binding to LDLR and other ApoE-binding receptors on hepatocytes, (iv) internalization of LNPs via endocytosis and (v), as pH decreases in the endosome, interaction of the protonated ionizable lipid with negatively charged endogenous lipids, which results cone-shaped ion pair formation, destabilization of the endosomal membrane and release of siRNA into cytoplasm where it can engage with the RNAi machinery.

Talk # 17

Single-cell analysis using Convex Lens-Induced Confinement (CLiC) imaging

Ndeye Khady Thiombane[1], Nicolas Coutin[1], Daniel Berard[2] , Radin Tahvildari[2] , Sabrina Leslie[2], Corey Nislow[1]

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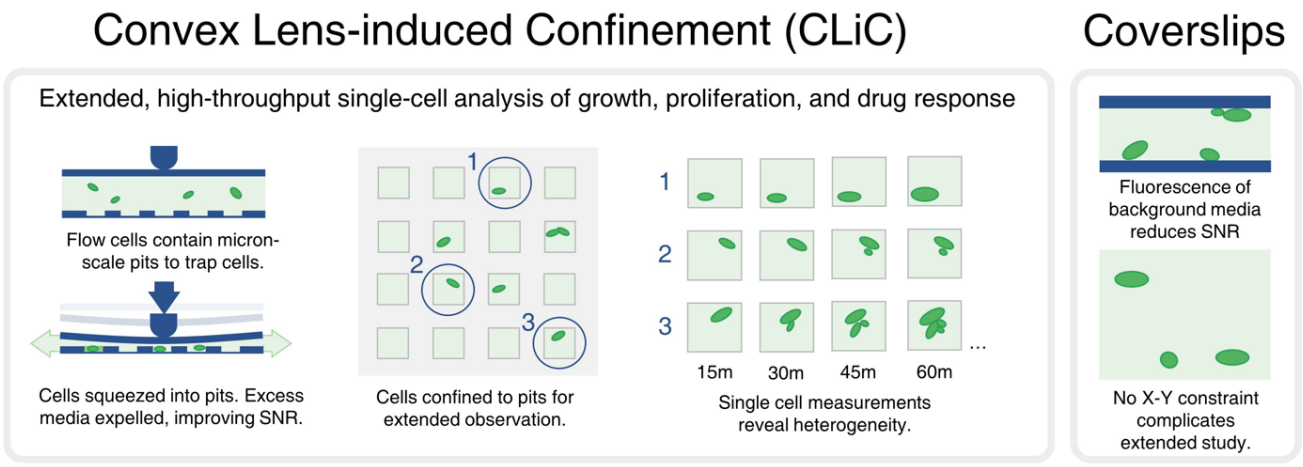
New technologies and methods in molecular and cell biology have been essential for powering the rapid advances in the life sciences over the past several decades; including dramatic improvements in cellular imaging, genomics and phenotypic analysis, to name just a few. For practical reasons, most of these methods operate at the level of cell or sample populations. As such, they provide statistical averages of aggregated data, and must rely on inference to represent the behavior of individuals from the sampled population. It has become increasingly clear that such population measurements do not capture the diversity within any population. Indeed, the explosion of 'single-cell' technologies in genomics, transcriptomics and imaging have provided unique insights that would have been missed by aggregate approaches. Furthermore, single-cell heterogeneity is a known and confounding variable for understanding the drug response which greatly complicates drug discovery and development. Here we demonstrate a new single-cell approach for drug development based on CLiC (Convex Lens-Induced Confinement) microscopy, originally developed for the study of single particles and biomolecules. We validated this platform on yeast cells confined in pits, demonstrating subcellular localization with an enhanced signal-to-noise ratio as compared to a traditional microscope slide. Nuclear localization revealed a higher sensitivity for DAPI detection using the CLiC flow cell. Yeast cells confined in a CLiC flow cell were viable and their doubling time was consistent with propagation in flasks. Additionally, we used the live cell CLiC assay to study the response of cells to drug perturbation. We assessed the effects of methotrexate on cell morphology and mitochondria integrity. No mitochondrial structural damage was detected with mitotracker staining, but we did observe a 5-fold increase in the size of treated cells. Taken together, the data collected using the CLiC system validates this platform for yeast single-cell studies, and demonstrates how it can be used to observe the heterogeneous response of populations of cells to drugs and other perturbations at a single-cell level and with high quality imaging conditions. Furthermore, by confining cells in well-defined, addressable locations, it enables the evolution of individual cells registered within a population to be followed for long time periods.



Talk # 18

Development and Preclinical Evaluation of a Targeted Theranostic Nanomedicine in a Mouse Model of Rheumatoid Arthritis

Zeynab Nosrati, Tullio V. Esposito, Marta Bergamo, Cristina Rodriguez-Rodriguez, Katayoun Saatchi* and Urs O. Häfeli*

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Aim/Introduction: Rheumatoid arthritis (RA) patients are frequently given anti-arthritic drugs including nonsteroidal anti-inflammatory drugs, glucocorticoids, disease-modifying anti-rheumatic drugs and biologics, or combinations thereof. Many patients fail to respond satisfactorily to these treatments or experience side effects. The main reason for non-ideal treatment is that insufficient drug doses reach the joints, therefore higher and more frequent doses needed.

To improve the drug pharmacological profile and direct the anti-inflammatory activity to the site of disease, *i.e.*, the inflamed joints, we synthesized a pro-drug that increased circulation time resulting in sustained release when it reaches the microenvironment –of the inflamed joints.

Materials and Methods: Our methods encompass the chemical syntheses of the polymeric prodrugs and the investigation of their stability and release kinetics. The pharmacokinetics of the prodrug was established after radiolabeling with In-111, and preclinical SPECT/CT imaging in an RA mouse model. The efficacy of the prodrugs is also established in the same RA model and compared to the free drug given in the same form/ timing as it is currently administered to patients. *In vitro* stability measurements of the prodrugs in human synovial fluid from rheumatoid arthritis patients is ongoing to better understand the impact of the local environment of an inflamed joint and how that influences drug release.

Results: Results show that delivering methotrexate (MTX) bound to a carrier polymer produces a significant increase in drug uptake in the inflamed joints. When MTX was delivered as a prodrug, up to 4 times lower dose given every two weeks was just as effective as two standard dosages per week of free MTX. In addition, attaching folic acid (a targeting ligand that selectively binds to folate receptors) to the polymeric carrier helped to keep the active drug longer in the targeted lesion.

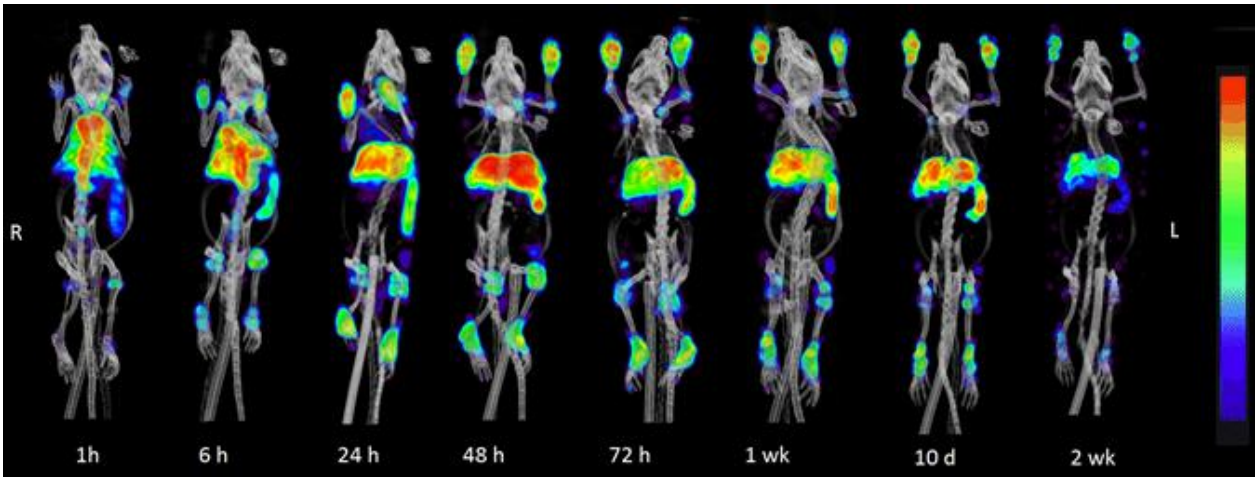


Figure 1: Biodistribution of ^{111}In-HPG-FA conjugated to MTX in an arthritic mouse model.

Conclusion: In this study, using SPECT/CT we show our prodrug approach delivers higher concentrations of anti-arthritic drugs to inflamed joints than has previously been possible, despite lower and less frequent drug doses. Current studies include to utilize and test our novel pro-drug delivery system with other effective RA drugs to further improve their pharmacological profile in patients with RA.

Nanomedicine in Skin Research – Where are we at?

Sarah Hedtrich[1]

[1] Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC, Canada

The human skin is crucial for our survival. It prevents the human body from dehydration and environmental factors such as irradiation and microorganism. This, however, also means that very few, mainly small and moderately lipophilic molecules are able to surmount the skin barrier which greatly limits the treatment options of various local and systemic diseases. Hence, over the past years, various types of nanoparticles have been developed aiming to overcome this bottleneck. Nanoparticles have demonstrated a great potential for topical applications facilitating the drug delivery very efficiently past the stratum corneum. Hence, in this talk, recent advancements in the topical delivery of small and biomacromolecules using polymeric nanoparticles will be highlighted and current limitations will be discussed.

Talk # 20

Self-Assembling Polymer-Drug Nanoparticles: A Platform Technology for Nanomedicine Optimization

Anne Nguyen[1], Shyh-Dar Li[1]

[1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada

Over 40% of new drugs need formulation help to improve low solubility, poor permeability, or off-targeting effects. These limitations hinder clinical application of powerful drugs for the treatment of a wide range of diseases, including cancer and autoimmune diseases. Nanoparticle-based drug delivery can be used to improve the solubility of poorly water-soluble drugs as well as prolong the half-life of drug circulation, leading to improved pharmacokinetics. Chemical modifications can be made to the drug to include solubilizing and/or targeting entities in such a way to promote their self-assembly into nanoparticles. These prodrugs are ultimately their own delivery vehicles, eliminating the need to incorporate other components to make up the nanoparticle. This offers several advantages, such as simplifying formulation optimization processes and limiting the interdependence of the different components that would traditionally be developed individually.

A robust synthetic platform was developed to maximize the efficiency in designing such prodrugs. Three hydrophobic drugs - Gambogic Acid (GA), Podophyllotoxin (PPT), and Paclitaxel (PTX), were modified using this synthetic platform to produce nine different polymer-drug conjugates that were formulated into uniform nanoparticles (NPs). Particle formation, stability, and drug release data were collected from all NPs. This simple synthetic platform can be used in the design and optimization of prodrug-based nanoparticles.

The efficacy of GA-NPs was tested in an *in vivo* rheumatoid arthritic model. Results suggest that GA-NPs were more efficient at suppressing inflammation in the paws of arthritic mice compared to free GA. Future work will focus on studying the effect of modulating physicochemical properties of polymer-drug conjugates on drug release, particle formation, and efficacy in different *in vivo* models.

[1] M Ku (2012) Pharm. Dev. Technol. **17**, 285-302.

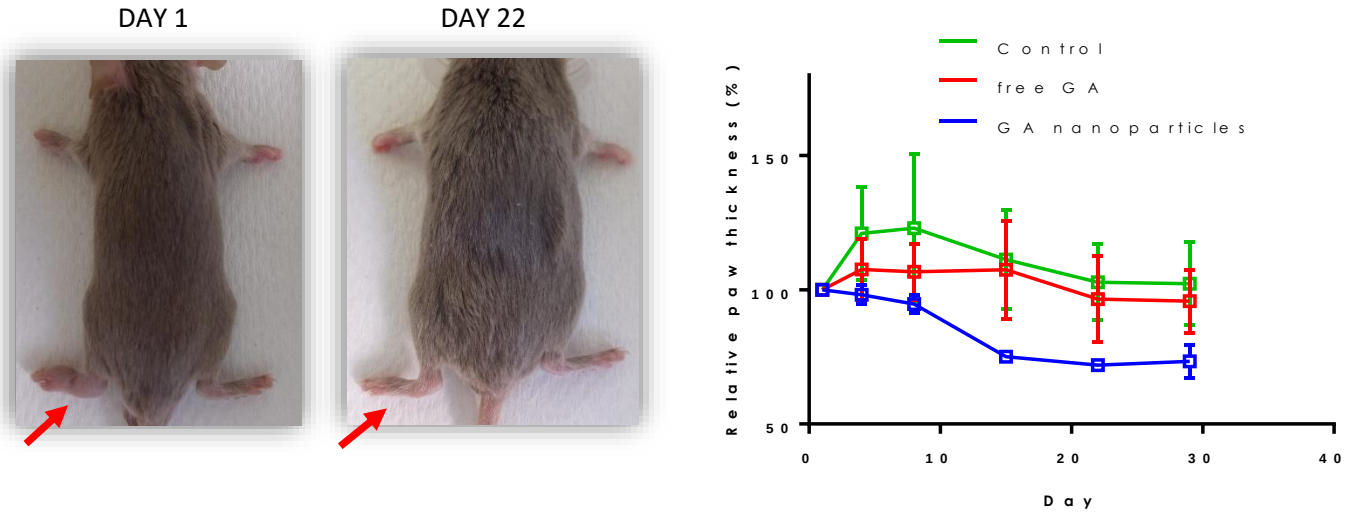


Figure 1: GA-nanoparticles are more effective at suppressing inflammation than free drug.

VANCOUVER

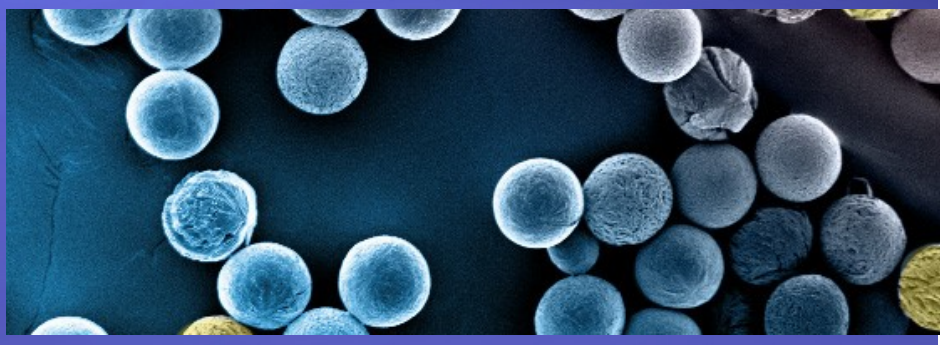
NANOMEDICINE DAY 2019

Thursday, September 12

The Nest, UBC

Poster

Presentations



Poster Session for Vancouver Nanomedicine Day 2019 - September 12, 2019

Poster #	Presenting Author	Institution/Company	Abstract Title
1	Anthony Boey	adMare Bioinnovations	Nanoparticle Intracellular Drug Delivery of Vancomycin into Liver Kupffer Cells Targeting MRSA Bacteria
2	Angelo Lanzilotto	AMGEN - UBC, Pharmaceutical Sciences	Linking IgG Heavy and Light Chains to Preserve Native Sequence Pairing
3	Devon Heroux	UBC, BC Cancer Research Centre	A Liposomal Formulation of Copper Diethyldithiocarbamate for Treatment of Triple-Negative Breast Cancer
4	Helene Stütz	UBC, Pharmaceutical Sciences	Macroaggregated Albumin Microspheres Containing Amphotericin B
5	Ellen Wasan	University of Saskatchewan, Pharmacy	A Lipidic Delivery System of a Triple Vaccine Adjuvant Enhances Mucosal Immunity Following Nasal Administration in
6	Daniel Pletzer	UBC, Microbiology and Immunology	Synthetic Peptides as Adjuvant Therapy for Acute and Chronic Pseudomonas Aeruginosa Skin Infections
7	Tiffany Carlaw	UBC, Medical Genetics	Development of a eGFP Reporter Assay Model to Evaluate the Therapeutic Efficacy of CRISPR/Cas-9 Base Editing
8	Yuhang Huang	University of Victoria, Chemistry	Microfluidic Control of Structure and Drug Delivery Properties of Biological Stimuli-Responsive Polymer Nanoparticles
9	Afsaneh Lavasanifar	University of Alberta, Pharmacy & Pharm Sci	Nanomedicine for Tumor Targeted Delivery of Novel Inhibitors of DNA Repair to EGFR Expressing Orthotopical Colorectal Cancer Xenografts in Mice
10	Sams Sadat	University of Alberta, Pharmacy & Pharm Sci	Nano-Delivery of PNKP Inhibitor Exhibits Synthetic Lethality in PTEN-Deficient Colorectal Cancer Xenograft Mice
11	Karan Khanna	UBC, Pharmaceutical Sciences	Liquid Biopsy for Risk-Stratification in Prostate Cancer
12	Samuel Berryman	UBC, Mechanical Engineering	Image-Based Cell Phenotyping Using Deep-Learning
13	Gilbert Walker	University of Toronto	Nanoparticles for Surface Enhanced Raman Scattering Detection of Cell Surface Proteins
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15	Samuel Clarke	Precision Nanosystems Inc.	Nanoparticle Mediated Non-Viral Gene Delivery to Enable Development of Next Gen T Cell Therapies
16	Jennifer Brown	UBC, Pharmaceutical Sciences	The Application of Differential Scanning Fluorimetry in Exploring Bisubstrate Binding to Protein Arginine N-
17	Jerry Leung	UBC, Biochemistry and Molecular Biology	Fusion-Dependent Formation of Lipid Nanoparticles Containing Macromolecular Payloads
18	Kelsi Lix	UBC, Chemistry	Dextran-Functionalization of Semiconducting Polymer Dots and Conjugation with Tetrameric Antibody Complexes for Bioanalysis and Imaging
19	Alexander Dhaliwal	University of Toronto, Medical Biophysics	Elucidating the Mechanism of the Acoustic Conversion of Microbubbles to Nanostructures for EPR-Independent Delivery to Tumors
20	Blair Morgan	McMaster University, Chemical Engineering	Acoustic Levitation as a Screening Method for the Development of Dry Powder Vaccines
21	Jacqueline Lai	UBC	Microparticulate-Encapsulated Antigen with Split Topical CpG Oligodeoxynucleotide Adjuvant as a Single-Injection Immunization Strategy
22	Lily Takeuchi	UBC	Development of Targeted Macromolecular Iron Chelators for Treatment of Cardiac Iron Overload
23	Anitha Thomas	Precision Nanosystems Inc.	Low Volume Production of Nanoparticles That are Effective Transfection Systems in iPSC-Derived Cells, Immune Cells and Other Primary Cell Cultures
24	Lloyd Jeffs	Precision Nanosystems Inc.	Scalable Manufacture of mRNA Lipid Nanoparticles using a Novel Microfluidic Mixing Architecture
25	Mandy Chan	STEMCELL Technologies, R&D	Immunomagnetic Purification of Extracellular Vesicles from Bofluids in Less Than 30 Minutes

Poster #	Presenting Author	Institution/Company	Abstract Title
26	Qihao Liu	UBC, ECE	Dual-Wavelength Fiber Laser Multiphoton Endoscopy for Oral Cancer Detection
27	Terri Petkau	UBC, Medicine	Development and Optimization of LNP-based Gene Therapy Approaches in the Brain
28	Alice Man	UBC, BC Cancer Research Centre	Coumarin-3-Carboxylic Acid: A Detector for Hydroxyl Radicals Generated by Orthovoltage and Proton Radiation
29	Kent Tsung Jeng Chen	UBC, BC Cancer Research Centre	Liposomal Flavopiridol – a Novel Formulation for the Treatment of Acute Myeloid Leukemia
30	Masoud Norouzi	University of Toronto, Pharmacy	Portable, On-Demand Biofunctionalization for Nanomedicines
31	Morteza Kafshgari	Polytechnique Montreal, Engineering Physics	Targeted siRNA Delivery with Antibody Conjugated Gold Nanostars-Assisted Ultrafast Femtosecond Laser Optoporation
32	Griffin Pauli	UBC, Pharmaceutical Sciences	Liposomal Delivery of an Immunomodulating Agent to the Tumour Microenvironment: PK/PD Studies
33	Siyue Yu	UBC, Pharmaceutical Sciences	Therapeutic Genome Editing: Development of a Reporter System to Optimize and Evaluate in vivo CRISPR/CAS9 Base
34	James Saville	UBC, Biochemistry and Molecular Biology	Peptidisc Applications for the Discovery and Characterization of Antibodies Against Membrane Proteins
35	Morgan Alford	UBC, Microbiology and Immunology	Influence of Nitrogen Source and Metabolism on Virulence of Pseudomonas Aeruginosa
36	Reka Geczy	UBC, Pharmaceutical Sciences	Microfluidic Approaches for the Production of Monodisperse, Superparamagnetic Microspheres in the Low Micrometer Size
37	Melanie Dostert	UBC, Microbiology and Immunology	Genes Regulating Biofilm Formation as Novel Biofilm-specific Drug Targets
38	Presley MacMillan	University of Toronto, Chemistry	Three-Dimensional Microscopy of Administered Substances in Cleared Tissues
39	Alessia Pallaoro	UBC, Mechanical Engineering	High Content Imaging of Endocytosed Nanoparticles and F-Actin for a New Perspective in Cytoskeletal Mechanics
40	Adria Encinas Casas	UBC, Pharmaceutical Sciences	Development of Novel Cell Proliferation Reagent for Mass Cytometry
41	Felix L. Santana	UBC, Microbiology and Immunology	Functional Characterization of Four Novel Crocodylian Cathelicidin Peptides
42	Michael Dunne	University of Toronto, Pharmacy	Hyperthermia and Radiation Therapy as Modulators of the Accumulation and Efficacy of Nanomedicine-Encapsulated Chemotherapies in Breast Cancer
43	Corrie Belanger	UBC, Microbiology and Immunology	How physiologically relevant conditions affect mechanisms underlying susceptibility of Pseudomonas aeruginosa to antimicrobial agents
44	Hagar Labouta	University of Manitoba, Pharmacy	Arriving to Modeling Frameworks For Assessing Potential Nanoparticle Cytotoxicity Risks
45	Rana Faryad Ali	Simon Fraser University	Size-Controlled Synthesis of Second Harmonic Active Lithium Niobate Nanocrystals through Solution-Phase Methods
46	Henry Kang	Simon Fraser University	Tunable Functionalization of Silica Coated Iron Oxide Nanoparticles Achieved through a Silanol–Alcohol
47	Hoda Bagheri	Simon Fraser University	A Mechanically Driven Magnetic Particle Imaging (MPI) & Phase-Weighting Implementation

Poster # 1

Nanoparticle intracellular drug delivery of vancomycin into liver Kupffer cells targeting MRSA bacteria

Anthony Boey[1], Jason Crawford[1], Liliam Oliveira[1], Paul Kubes[2], Bas Sureward[2]

[1]adMare Bioinnovations, Vancouver, British Columbia, Canada
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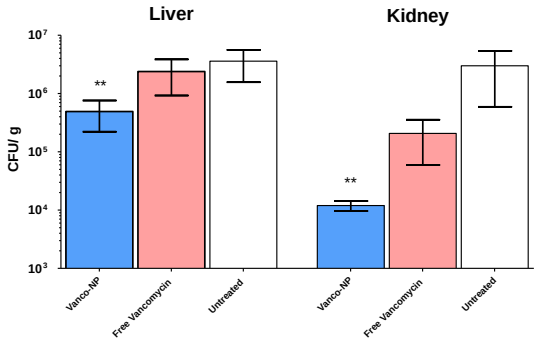
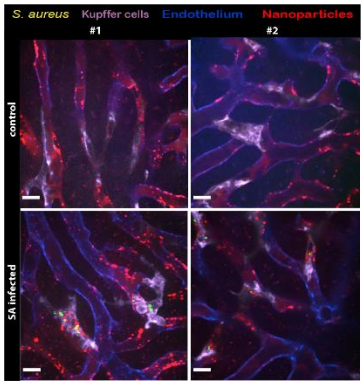
Staphylococcus aureus is a clinically important pathogen that contributes to over 10,000 annual deaths in North America and is notorious for its ability to become resistant to antibiotics. Of great concern is the increasing number of drug resistant strains, such as methicillin-resistant *S. aureus* (MRSA), making vancomycin one of the few remaining useful antibiotics. Vancomycin therapy requires intravenous administration for 2–6 weeks with relapses of vancomycin susceptible strains is not uncommon. Vancomycin treatment is suboptimal due to the existence of an intracellular MRSA niche where vancomycin by itself cannot penetrate.

The aim of this collaboration work was to develop a formulation that can potentially target the intracellular MRSA present in the liver Kupffer cells. Although other nanoparticle approaches has been used for the cellular targeting of vancomycin (e.g. liposomal vancomycin), we developed a simple nanoparticle complex of vancomycin that has potentially better circulation lifetime and lower toxicity as compared with vancomycin for injection.

Intravenous administration of the vancomycin nanoparticles (VNPs) and free vancomycin in a MRSA infection mouse model (Figure 1), showed that VNPs were able to be taken up by MRSA infected liver Kupffer cells, while free vancomycin was not. VNPs significantly reduced both systemic and intracellular liver infections, in comparison to free Vancomycin which had no effect on intracellular infections. Additionally, when the same VNPs were intravenously administered into healthy non-infected mice, no intracellular uptake of the vancomycin nanoparticles was observed.



(A)



(B)

Figure 1. (A) Comparison of knockdown of MRSA infection in liver and kidney (C57Bl/6 mice), between VNP, free vancomycin, and untreated animals. (B) Microscopy images of non-infected (Control, top panels) and infected with MRSA (S. aureus infected, bottom panels). Mouse liver at 3 hours post-injection of VNP (#1, left panel) and vancomycin-containing DSPE-PEG and DSPE-PEG-Mal NP (#2, right panel). Yellow color: S. aureus; Pink color: Kupffer cells; Blue color: endothelium; Red color: VNPs.

Poster # 2

Linking IgG Heavy and Light Chains to Preserve Native Sequence Pairing

A. Lanzilotto[2,3], S. Kesavan[1,3], W. Matochko[3], P.C.H. Li[1], C. Nislow[2], K. Oxenoid[3]

[1]Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Dr, Burnaby BC,
[2]Faculty of Pharmaceutical Sciences, University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC
[3]Therapeutic Discovery, Amgen British Columbia Inc , 7990 Enterprise St, Burnaby, BC

Current antibody production involves immortalization of B cells by fusion with myeloma cells to make hybridomas This is a very inefficient process 99.9% of B cells are lost. Bypassing hybridoma generation has many advantages, especially for rare clones. One of the key steps in the discovery of novel antibodies is the recovery of the heavy chain (HC) and light chain (λC, κC) sequences. Here we present the preliminary data towards an alternative method of recovering antibody sequences. Traditionally, HC and λC/κC are expressed from two separate plasmids. This method aims to eventually recover and subsequently pair native monoclonal antibody (HC and λC/κC) into one sequence directly from the B cell, bypassing hybridomas. The sequence would then be expressed in one vector with a bidirectional promoter within the overlap extension region to produce complete antibodies (Figure 1).

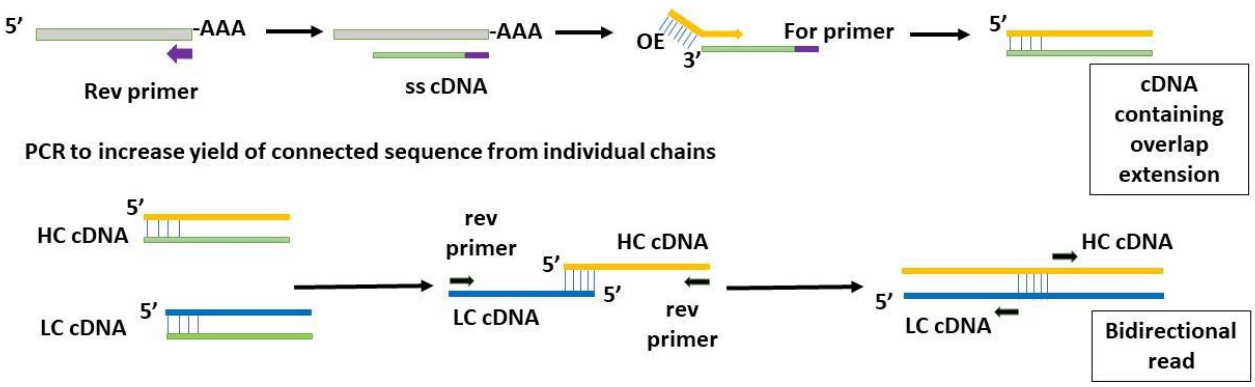


Figure 1 Mechanism of heavy and light chain pairing (HC and λC/κC are expressed on same expression vector and read outwards on complementary strands.

Poster # 3

A liposomal formulation of copper diethyldithiocarbamate for treatment of triple-negative breast cancer

Heroux D.*[1,2], Leung A.[2,3], Wehbe M.[2,5], Bally M.[1,2,3,4,5]

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[2]Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, Canada
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[5]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada

Disulfiram is an FDA-approved drug for the treatment of alcoholism, and functions by producing an acute sensitivity to ethanol. Since the 1960's, it has also been shown to have an anti-cancer effect in pre-clinical models, as well as in small clinical trials[1-3]. After oral administration, disulfiram is reduced to diethyldithiocarbamate (DDC), which subsequently requires copper for activity. This complex has low solubility, and the objective of this project is to develop a formulation that can be filter sterilized and examined further for pharmacokinetics and anti-tumour efficacy in a mouse model of triple-negative breast cancer.

A new formulation was developed using DSPC/Chol liposomes and pre-formed $Cu(DDC)_2$. This method resulted in a five-fold higher retention of the drug after filtration than a previous formulation, and fractionation also showed a greater association between the liposome and drug, with a closer elution profile of lipid and $Cu(DDC)_2$. Various forms of copper were examined for optimal retention, and the influence of the order of addition of DDC and copper was also investigated. Although pharmacokinetic studies demonstrated rapid drug release from the liposome, anti-tumour efficacy studies produced a 40% decrease in tumour growth in a mouse model of triple-negative breast cancer, relative to controls. Additional studies are ongoing to optimize its therapeutic potential, by controlling release kinetics, as well as enhancing its activity through combination studies with other compounds.

[1] Schirmer HK, Scott WW. *Trans Am Assoc Genitourin Surg*. **1966**;58:63-6; [2] Wattenberg LW. *J Natl Cancer Inst.* **1975**; 54(4):1005-6 [3] Nechushtan H, et al. *Oncologist*. **2015**20(4):366-7

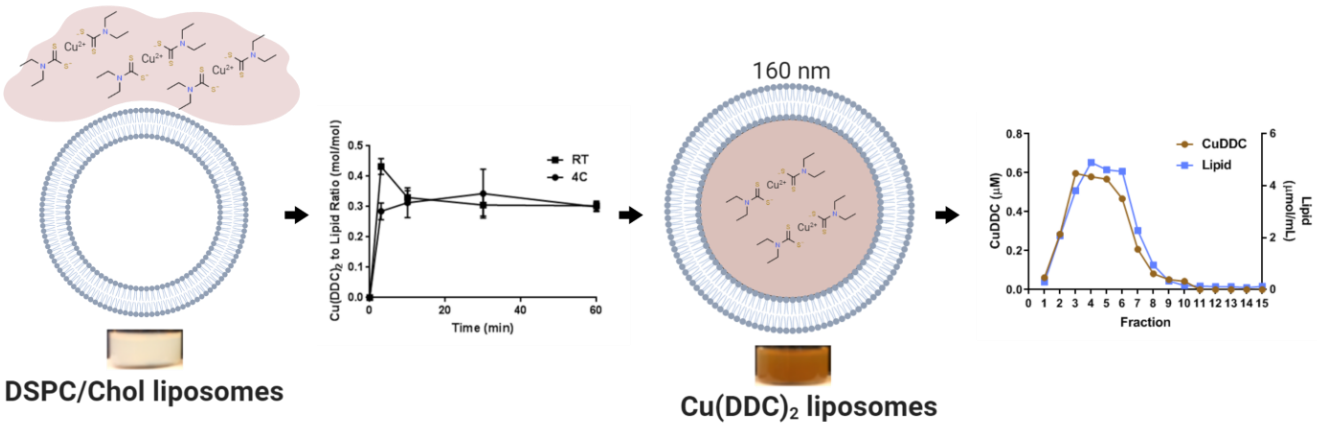

Figure 1: A new method of preparing $Cu(DDC)_2$ liposomes.

Macroaggregated albumin microspheres containing Amphotericin B

Helene Stütz [1,2] , Tullio V. Esposito [1] , Réka Géczy [1,3] , Kathy Saatchi [1] , Urs Häfeli [1,3]

[1] Faculty of Pharmaceutical Science, University of British Columbia, Vancouver, Canada
[2] Department of Life Science, University of Applied Science, Krems, Austria
[3] Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

Pulmonary aspergillosis, is caused by infection of lung tissue by *Aspergillus sp*. in immunosuppressed patients, which is currently treated with azole therapy. A critical bottleneck in the therapy with azoles, is the development of resistances by *Aspergillus sp.* against the applied azole. [1] Lipid formulations of Amphotericin B applied as aerosol are currently used as prophylaxis treatment of Aspergillosis in lung transplant recipients, HCST receiving patients and leukemia patients. [1, 2] Amphotericin B binds to ergosterol in the fungal cell wall and forms a pore, which leads to rapid leakage of monovalent ions and cell death. [3]

The aim of my project is to produce microspheres with an on-chip method. The used thiol-ene chip and the underlying microfluidics system will be beneficial to produce microspheres with only small distribution in size. In this microfluidic system the continuous phase consists of mineral oil with an surfactant, to avoid coalescence of the produced microspheres. The dispersed phase however, consists of Amphotericin B, iron chloride and serum albumin. A temperature of 70°C is applied to the chip, during production of microspheres, to denature the protein and form stable microspheres.

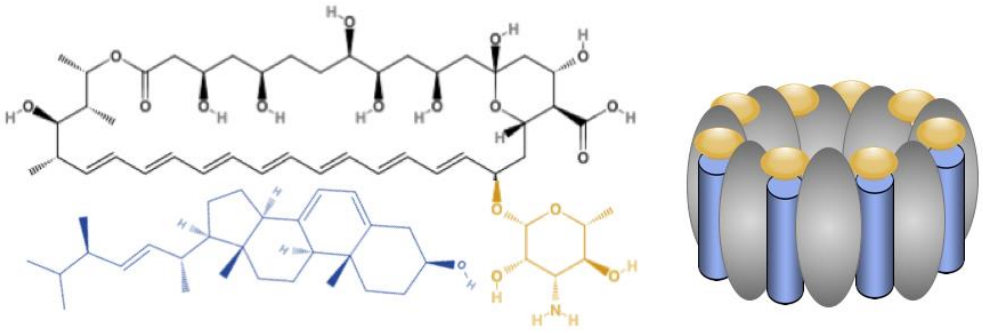

Figure 1: *Formation of an ion channel by Amphotericin B: the mycosamine group (yellow) binds to ergosterol (blue) of the fungal cell membrane*

1. Patterson, T.F., et al., *Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America.* Clin Infect Dis, 2016. **63**(4): p. e1-e60.
2. Hayes, G.E. and L. Novak-Frazer, *Chronic Pulmonary Aspergillosis-Where Are We? and Where Are We Going?* J Fungi (Basel), 2016. **2**(2).
3. Stone, N.R., et al., *Liposomal Amphotericin B (AmBisome((R))): A Review of the Pharmacokinetics, Pharmacodynamics, Clinical Experience and Future Directions.* Drugs, 2016. **76**(4): p. 485-500.

Poster # 5

A lipidic delivery system of a triple vaccine adjuvant enhances mucosal immunity following nasal administration in **mice**

Ellen K. Wasan[a], Jaweria Syeda[a], Stacy Strom[b] , Jacqueline Cawthray [a], Robert E .Hancock[c], Kishor M. Wasan[a], Volker Gerdts[b]

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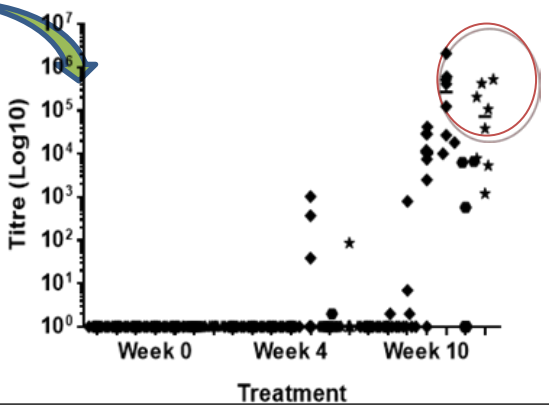
[b]Vaccine and Infectious Disease Organization-International Vaccine Centre, VIDO-InterVac, University of Saskatchewan, Saskatoon, SK S7N 5E3, Canada
[c]Dept. of Microbiology and Immunology, Faculty of Science, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

[1]We previously developed a highly efficacious triple combination adjuvant comprised of innate defense regulator (IDR)-1002 peptide, poly(I:C) RNA and polyphosphazene (TriAdj). Here, we aimed to design and test the *in vivo* efficacy of a mucoadhesive nasal formulation of this adjuvant. The triple combination adjuvant TriAdj self-assembles in solution forming an anionic nanocomplex (TriAdj). Cationic liposomes comprised of didodecyl dimethylammonium bromide (DDAB), dioleoyl phosphatidylethanolamine (DOPE) (50:50 or 75:25 mol:mol) and DDAB, L-α-phosphatidylcholine (egg PC) and DOPE (40:50:10 mol:mol:mol) were prepared by the thin-film extrusion method. The lipids and pre-assembled TriAdj were combined by simple mixing, forming an electrostatically condensed cationic nanoparticle complex, L-TriAdj. Physical characterization included dynamic light scattering, zeta potential, mucin interactions and transmission electron microscopy. Exposure of RAW267.4 mouse macrophage cells *in vitro* to TriAdj alone vs. L-TriAdj indicated that DDAB/DOPE (50:50) and DDAB/EPC/cholesterol (40:50:10) complexation reduced TriAdj cellular toxicity according to an MTT assay. L-TriAdj was also prepared with various component ratios to optimize size, stability and to achieve the desired positive charge. Transmission electron microscopy showed rearrangement of lipid structures on binding of lipids to TriAdj and to mucin. L-TriAdj particles were ~200 nm over 24 h and showed greater mucin binding when containing DDAB/DOPE compared to DDAB/EPC/DOPE. To demonstrate the *in vivo* efficacy of L-TriAdj as a vaccine adjuvant, the DDAB/DOPE + TriAdj complex was administered intranasally to mice with ovalbumin as the model vaccine antigen. The immunogenic response was assessed by quantifying serum levels of IgG1, IgG2a, IgA over time post vaccination and by measuring ex vivo T cell (splenocyte) response to antigen challenge after vaccination by release of IL-5 and IFN-γ. Mice administered adjuvant with the vaccine showed a significantly greater immune response with L-TriAdj compared to TriAdj alone, with a dose-response proportionate to the triple adjuvant content, and an overall balanced Th1/Th2 immune response representing both systemic and mucosal immunity. Mice did not exhibit signs of toxicity.

Nasal administration of model vaccine with lipidic formulation of triple adjuvant (L-TriAdj)



Lipidic adjuvant vaccine shows superior IgG1, IgG2a and IgA response compared to adjuvant alone

1 Wasan, E. K. *et al.* A lipidic delivery system of a triple vaccine adjuvant enhances mucosal immunity following nasal administration in mice. *Vaccine* **37**, 1503-1515, doi:https://doi.org/10.1016/j.vaccine.2019.01.058 (2019).

Synthetic peptides as adjuvant therapy for acute and chronic *Pseudomonas aeruginosa* skin infections

Pletzer Daniel[1,*], Choi Ka-Yee Grace[1], Esposito Tullio V[2], Coleman Shannon R[1], Blackadar Colin[2], Rodriquez Cristina[2], Lee Amy HY[1], Saatchi Katayoun[2], Hafeli Urs O[2], and Hancock Robert EW[1]

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Abstract:
There has been enormous publicity about the inexorable rise of antibiotic resistance and the lack of new therapies. However, less attention has been placed on adaptively multidrug-resistant high-density bacterial infections for which antibiotics are highly used but no effective therapies currently exist. Here we show how synthetic peptides can be used as an adjuvant therapy to target bacterial infections in a cutaneous mouse model of acute and chronic infection. In particular, the commonly used *Pseudomonas aeruginosa* PA14 strain was highly aggressive, responsible for rapid lethality of about 40% of mice over three days, when delivered in high-densities under the skin. We hypothesized that bacterial motility, a mechanism by which organisms move and colonize, was a key characteristic during acute infection. Previously, we identified that the anti-biofilm peptide 1018 suppressed *P. aeruginosa* swarming motility *in vitro*, and therefore we investigated the potential of the peptide to reduce motility *in vivo*. We found that peptide treatment reduced abscess formation and bacterial dissemination into various body organs. We adapted this model to enable chronic infection with *P. aeruginosa* strain LESB58 for three-weeks, by using hydroxyapatite, a natural mineral found in bones and teeth, as a growth surface within the host; this led to the formation of an internal abscess capsule. Using *in vivo* live imaging technology, the presence of neutrophils and metalloproteases were confirmed at the infection site. Treatment of the tissue lump with daily intra-abscess or intra-venous administration of the immunomodulatory peptide 1002 reduced the bacterial load and modulated the host immune system. Moreover, pharmacokinetics of peptide 1002 revealed that the peptide remained as within the host tissue for several hours to days depending on the route of administration. These peptides have therefore the potential to broaden our limited antibiotic arsenal for extremely difficult to treat infections caused by resistant pathogenic bacteria.

Poster # 7

DEVELOPMENT OF A eGFP REPORTER ASSAY MODEL TO EVALUATE THE THERAPEUTIC EFFICACY OF CRISPR/CAS-9 BASE EDITING

Tiffany Carlaw[1], Siyue Yu[2], Linhua Zhang[2], Colin Ross[2]

[1]Department of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, BC

[2]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC

BACKGROUND

Each copy of our genome consists of three billion nucleotides of DNA that encodes over 25,000 genes that modulate all of the processes within living cells. A single change in the DNA sequence can lead to a devastating genetic disease or cancer. The ability to safely and efficiently repair a mutation in our DNA would have a profound impact on our ability to prevent genetic diseases, cancer, and even susceptibility to certain infectious diseases. Currently >5% of human genetic diseases have approved treatments and these are often of limited therapeutic benefit to patients, highlighting the need for improved therapeutic efficacy. Gene therapy is an approach to treat genetic diseases that directly targeting the cause of the disease: the mutated gene. Recent advances in gene therapy, in particular the development of CRISPR/Cas-9 base editors, provide a novel approach to treat numerous genetic diseases. We sought to design a reporter model system to evaluate and optimize the *in vitro* and *in vivo* therapeutic potential of CRISPR/Cas-9 base editing.

METHODS

In order to evaluate the safety and effectiveness of therapeutic CRISPR/Cas-9 base editing, we designed a series of mutations in the eGFP gene, tested each for their ability to knockdown fluorescence signal, and demonstrated *in vitro* proof-of-principle with efficient repair of mutations in HEK293 cells via base editing.

RESULTS

We successfully designed two mutations in eGFP that abrogate fluorescence signal. Using CRISPR/Cas-9 base editing we were able to restore fluorescence signal for both mutants. We then stabilized our eGFP mutant constructs into the genome of HEK cells using the FLP-In Trex system.

CONCLUSIONS

Using our newly created stabilized cell lines we will be able to rigorously optimize each component of base editing, as well as access the safety of therapeutic base editing. These mutant models are currently being replicated in mice.

Poster # 8

Microfluidic Control of Structure and Drug Delivery Properties of Biological Stimuli-Responsive Polymer Nanoparticles

Yuhang Huang[1], Arman M. Jazini[2], Elliot P. Howell[1], Jung Kwon Oh[2], Matthew G. Moffitt[1,*]

[1]Department of Chemistry, University of Victoria, Victoria, British Columbia, Canada
[2]Department of Chemistry and Biochemistry, Concordia University, Montreal, Quebec, Canada

Polymer nanoparticles (PNPs) for drug delivery have received significant attention due to their morphological variability, robustness and ease of functionalization. PNPs based on amphiphilic block copolymers exhibiting stimuli-responsive degradation can maximize the therapeutic efficiency through targeted and controlled release of encapsulated agents under specific biological stimuli. Conventional synthetic methods for PNP manufacturing rely exclusively on intermolecular bottom-up forces requiring variations in the chemistry of formulation. On the other hand, our group has shown that tunable shear forces in two-phase, gas-liquid microfluidic reactors introduce top-down control of PNP structure and drug delivery properties *via* shear processing without changes in the formulation chemistry. Herein we demonstrate the effects of microfluidic manufacturing and flow-variable shear processing on stimuli-responsive degradation PNPs, focusing on aspects of shear processing control over PNP morphologies, drug loading efficiencies, stimuli-responsive degradation, and stimuli-triggered release kinetics.

[1] N Chan, B Khorsand, S Aleksanian and J K Oh (2013) *Chem. Commun.* **49**, 7534-7536

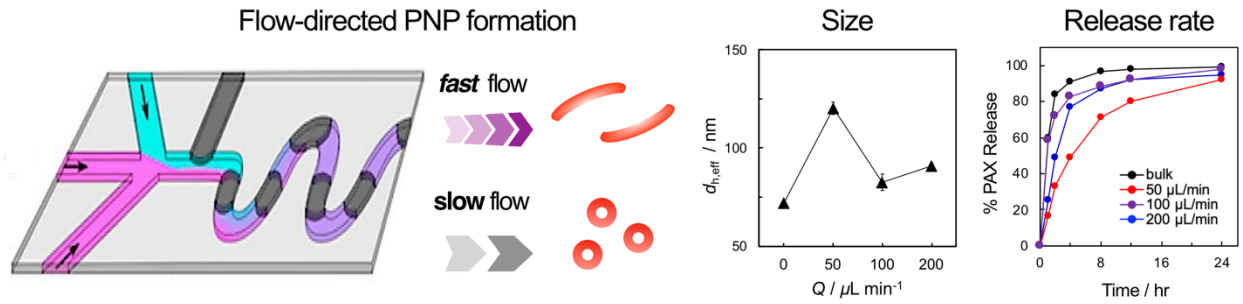

Figure 1. The shear processing control of PNP morphologies, sizes, and stimuli-triggered release rates.

Poster # 9

Nanomedicine for tumor targeted delivery of novel inhibitors of DNA repair to EGFR expressing orthotopical colorectal cancer xenografts in mice

Igor M. Paiva[1], Sams Sadat[1], Mohammad R. Vakili[1], Marco Paladino[3], Dennis G. Hall[3], Michael Weinfeld[2], Afsaneh Lavasanifar[1].

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Colorectal cancer (CRC) affects nearly 1.4 million people worldwide and over 60% of CRC patients will eventually relapse or develop *de novo* metastatic disease. Our research group has previously reported on the development of an inhibitor of a DNA repair enzyme, i.e., polynucleotide kinase/phosphatase (PNKP), known as A83B4C63, and its polymer based nanoparticle formulations as novel drugs against CRC. We have also shown that the pharmacological inhibitors and genetic silencers of PNKP to be synthetic lethal partners of a tumor suppressor protein, phosphatase and tensin homologue (PTEN), which is frequently disrupted in CRC. The aim of current study was to assess the activity of a second-generation nanoparticle formulation of A83B4C63, i.e., nanoparticles modified with epidermal growth factor (EGFR) targeting GE11 peptide on their surface (GE11-NP's), in CRC models. We, hypothesized that GE11 modified nanocarriers would provide a superior delivery of A83B4C63 to CRC, leading to improved anticancer outcome in PTEN-negative CRC models both *in vitro* and in orthotopic CRC models *in vivo*. Our data corroborated that GE11 modification on the nano-delivery system to positively impact the uptake of nanoparticles by EGFR-expressing CRC cells, without any negative impact on their physicochemical properties. GE11-NP's loaded with A83B4C63 were also found to be more effective in inhibiting the growth of PTEN negative CRC tumors, when compared to the unmodified A83B4C63 formulations, leading to elongated mice survival, *in vivo*.

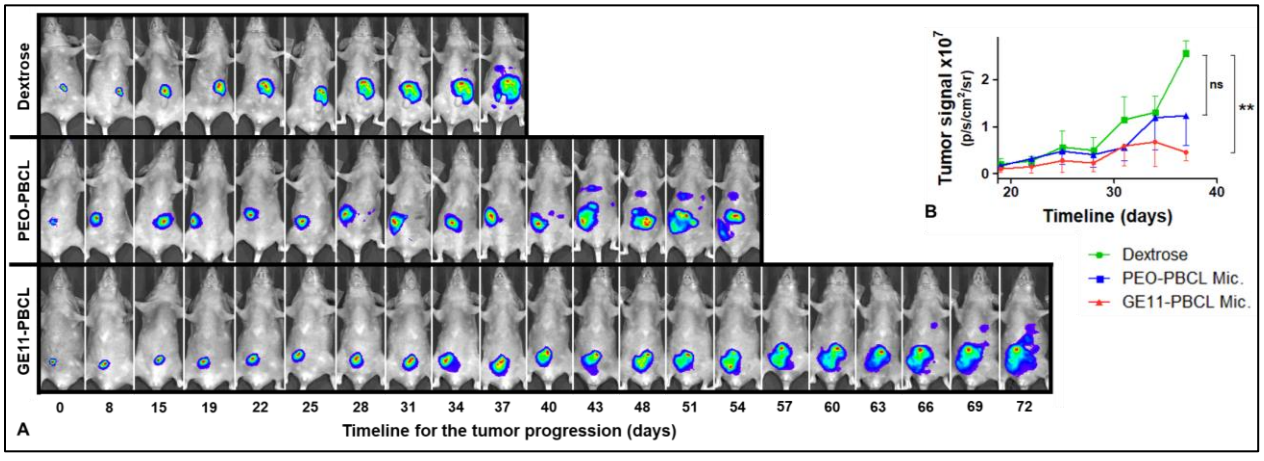

Figure 1. Mice treatment with nano-formulation of A83B4C63 compound (6 i.v. injections of 25 mg/Kg drug). (A) Tumor growth development monitoring through luminescence signal from HCT116-Luc+ PTEN-/- cells orthotopically implanted in NIH-III mice. (B) Highlighted timepoints in the treatment course, indicating enhancement in efficacy by using GE11 peptide micellar surface modification. Values are the average of least three mice per group ± SEM. Unpaired t-test, ^{ns} $\rho > 0.05$, ** $\rho \leq 0.01$.

Poster # 10

Nano-delivery of PNKP inhibitor exhibits synthetic lethality in PTEN-deficient colorectal cancer xenograft mice.

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Purpose: Phosphatase and TENsin homolog deleted on chromosome 10 (PTEN), encoded by the PTEN gene, is a tumor-suppressor protein that is lost in up to 75% of aggressive colorectal cancers (CRC). Our research group has identified that the co-depletion of PTEN and the DNA repair protein, polynucleotide kinase phosphatase (PNKP), leads to synthetic lethality in several cancer cell lines including CRC cells. This finding inspired the development of novel PNKP inhibitors and their nanoparticle (NP) formulations as potential new drugs for treating PTEN-negative CRC. The objective of this study was to investigate the specificity and anticancer activity of our lead PNKP inhibitor (A83B4C63) as free or NP formulation in wild-type $PTEN^{+/+}$ and $PTEN^{-/-}$ CRC xenograft in mice following intravenous administration.

Methods: A cellular thermal shift assay (CETSA) was developed to assess both *in vitro* and *ex vivo* binding capacity of A83B4C63 to intracellular PNKP in wild type ($HCT116/PTEN^{+/+}$) and PTEN-negative ($HCT116/PTEN^{-/-}$) CRC cells and their xenografts. A83B4C63 was either encapsulated in methoxy poly(ethylene oxide)-*b*-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-*b*-PBCL) NPs or solubilized with the aid of Cremophor EL: Ethanol (CE). Maximum tolerable dose (MTD) of free versus NPs of A83B4C63 was determined in healthy CD-1 mice following 3 intravenous (IV) injections at a dose range of 2.5-50 mg/kg (n=4). Histopathological evaluation for different organs was performed to identify the toxicity of A83B4C63 as free drug and its NP formulation following 3 IV injections at a dose of 50 mg/kg (n=4). The anticancer activity of both formulations was determined in $HCT116/PTEN^{-/-}$ and $HCT116/PTEN^{+/+}$ xenograft in NIH-III nude mice (n=5) after 3 IV injections of A83B4C63 at 2 different doses of 10 and 25 mg/kg, every other day. Tissue and plasma concentrations of A83B4C63 for both formulations were also determined in the tumor bearing animals (n=3) 24 h after the last IV injection for the 25 mg/kg dose.

Results: CETSA confirmed the binding of A83B4C63 to intracellular PNKP at 10 μM concentration (*in vitro*) and at 1 mg/kg dose (*ex vivo*) of 3 intratumoral injections given 2 days apart. The MTD study revealed no significant change in weight gain or biochemical indicators of toxicity in animals receiving A83B4C63 compared to saline control. The nanocarriers of A83B4C63 reduced the rate of $HCT116/PTEN^{-/-}$ xenograft growth more efficiently than free drug. This was in contrast to wild-type $HCT116/PTEN^{+/+}$ xenografts, which showed similar growth rates following administration of A83B4C63 in either formulation, formulation excipients (without drug) or 5% dextrose. Moreover, no toxicity was observed by histology in different organs of the A83B4C63-treated animals. A significantly higher concentration of A83B4C63 was measured in blood plasma and tumor when delivered by NPs compared to CE formulation in the $HCT116/PTEN^{-/-}$ xenografts. A similar trend was observed in animals with $HCT116/PTEN^{+/+}$ xenografts. Analysis of other tissues is currently underway.

Conclusion: Delivery of A83B4C63 by PEO-*b*-PBCL NPs demonstrates a promising new monotherapeutic option in PTEN-deficient CRC.

Poster # 11

Liquid Biopsy for Risk-Stratification in Prostate Cancer

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Prostate cancer (PCa) is the most commonly diagnosed cancer in men. Current screening methods using prostate-specific antigen (PSA) testing has led to more patients presenting with earlier and more indolent, low-risk, forms of PCa. This has resulted in overtreatment of indolent disease through unnecessary surgical procedures. Thus, identifying individuals with elevated PSA levels who have aggressive versus indolent disease could greatly reduce the number of unnecessary surgical procedures.

This project looks to identify men with aggressive PCa using an extracellular vesicle (EV)-based blood biopsy. Previous studies have demonstrated that elevated levels of PCa EVs are associated with aggressive disease. We have built on these studies to develop a robust test capable of risk-stratifying men with elevated PSA levels. Using nano-flow cytometry in combination with biomarkers for prostate-derived EVs (PSMA and STEAP1) and our aggressive cancer biomarker (polysialic acid, polySia) we can enumerate EV levels in patient plasma.

As polySia has not been previously reported in PCa, we analyzed polySia expression levels using a tumor tissue microarray and found elevated levels were associated with poor patient outcomes. Next, we analysed plasma from low-risk and high-risk PCa patients for EVs expressing polySia-PSMA-STEAP1. Triple Positive (polySia-PSMA-STEAP1) EV levels were significantly elevated in high-risk patients compared to low-risk patients. In combination with PSA, we found that a Triple Positive EV-based test could improve PCa diagnostics (AUC=0.82). Our results highlight the potential utility of a non-invasive EV-based blood test, in combination with PSA, for guiding patient management and reducing overtreatment in PCa.

Poster # 12

Image-based Cell Phenotyping Using Deep-Learning

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The ability to phenotype cells is fundamentally important in biological research and medicine. Current methods of phenotyping cells rely primarily on flow cytometry to detect specific fluorescent markers. There are many situations where this this approach is undesirable, such as problems with availability, specificity, cross-reactivity and cost of phenotyping markers. Furthermore, the number of markers required can increase the complexity, may exceed the detection limit, and even activate or decrease the viability of some cells. Finally, cells that are non-spherical or are too few in number are sometimes not compatible with flow cytometry. For these reasons, alternate methods for phenotyping are sought after, with the focus on live-cell imaging. Here, we investigate the potential to develop a deep-learning model to phenotype cells directly from brightfield and non-specific fluorescence microscopy images.

Our Convolutional Neural Network (CNN) was trained on seven standard cancer cell lines (MCF7, MDA-MB-231, LnCAP, PC3, dHL60, Jurket and Raji). Using five-fold cross-validation, we show that the CNN was able recognize each cell line with a 97.5% average accuracy. Our results demonstrate the ability to use deep-learning to phenotype cells directly from microscopy images without specific markers. This capability will be valuable for situations where phenotyping markers are unavailable or the cell sample cannot be stained, such as prior to therapeutic use. We envision this approach to be a general method for identifying cell types directly from image data in order to identify the emergence of phenotypic shifts or new cell types.

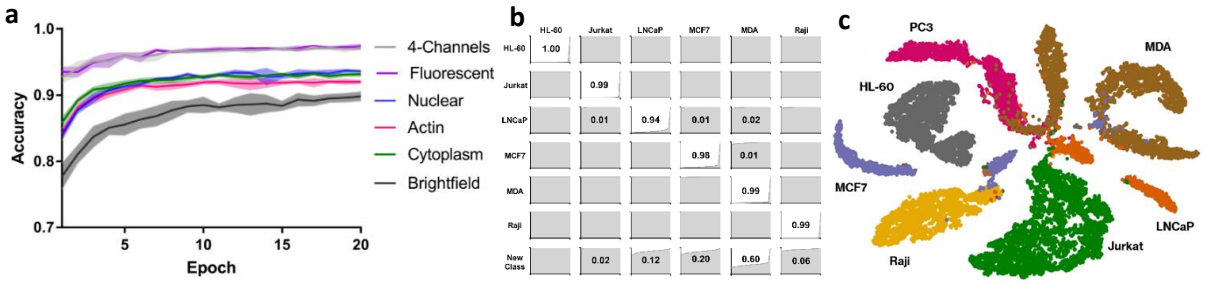

Figure 1| a, 5-fold exhaustive cross validation on single-channel and multi-channel images. **b,** Confusion matrix of 4-channel model on the 20,000 validation images with cumulative distributions. **c,** t-SNE plot of the 64-feature output, from the layer preceding the final output layer of the 4-channel model, using the 20,000 validation images.

Poster # 13

Nanoparticles for Surface Enhanced Raman Scattering Detection of Cell Surface Proteins

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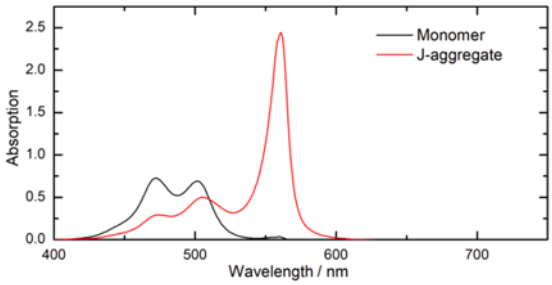
The multiplexed detection of cell surface proteins plays an integral role in the differentiation of cell types and diagnosis of disease. Currently, the use of flow cytometry to evaluate fluorescently-labelled surface proteins is the gold standard technique for the diagnosis of diseases such as chronic lymphocytic leukemia, the most prevalent blood cancer in the western world. However, the number of proteins that can be detected at once is limited by the broad emission signals and there is a current push to create Raman-based labels whose narrow spectral signatures allow for greater multiplexing. Molecular Raman scattering is typically weak however, and methods to enhance the scattering and decrease unwanted fluorescence background are desired. Detection methods focusing on the coupling of the localized surface plasmon resonances (LSPRs) of plasmonic surfaces with Raman active dyes as a way to generate probes have been shown to be the most promising methods to address the brightness limitation. To be practical alternatives to fluorescent markers, these probes must: 1. Produce strong & detectable SERS signals, 2. Produce signals that are consistent over time & between batches and a quantitative technique; 3. Demonstrate colloidal stability in physiological media, and 4. Specifically target proteins with minimal non-specific binding. We will describe 3 particle types: PEGylated gold core NP, liposome encapsulated gold nanoparticles, and j-aggregate metal core np.

The j-aggregate particles are especially bright. They exhibit an excitonic resonance of the J-aggregate is red-shifted and higher intensity from that of the monomer; this is key in setting up a strong electric field enhancement in the j-aggregate layer. The collective absorption of J-aggregates can tightly couple with LSPRs of NPs to results in greater SERS. In producing these, acationic linker facilitates formation of J-aggregates on the NP surface with greater control & reproducibility. Silica encapsulation provides colloidal stability and brings dyes closer to surface (within a few nm) to provide fluorescence quenching.



Liposomes composed of zwitterionic lipids are valuable both to limit biofouling and to serve as a modular matrix to incorporate a variety of functional molecules, and hence are reported here as vehicles for SERS-active materials. Dark field microscopy and SERS represent new combined functionalities for targeted liposomal probes. Two methods of antibody conjugation to SERS liposomes are reported: (i) direct conjugation to functional groups on the SERS liposome surface, or (ii) post-insertion of lipid-functionalized antibody fragments (Fabs) into preformed SERS liposomes. In vitro experiments targeting both the lymphoma cell line LY10 and primary human chronic lymphocytic leukemia (CLL) cells demonstrate the usefulness of these probes as optical contrast agents in both dark field and Raman microscopy.

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Poster # 14

^{177}Lu-Chelated Nanotexaphyrins for PDT/SPECT Theranostics

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Texaphyrins are expanded porphyrins capable of stable chelation of lanthanide metals that have been clinically investigated for precision imaging and ablative therapies (e.g. radiosensitization and photodynamic therapy (PDT)) [1]. We recently developed nanotexaphyrin with texaphyrin-lipid conjugates that self-assemble into liposome-like nanoparticles. These nanoparticles exhibit good biocompatibility and stably chelate large metal ions with high loading capacity [2]. The ^{177}Lu-texaphyrin complex is both a singe-photon emission computed tomography (SPECT) contrast agent and a potent photosensitizer [1], presenting us with a unique opportunity for image-guided PDT in the treatment of oligometastatic diseases.

To optimize Lu-nanotexaphyrin for sensitive SPECT imaging and potent PDT, a hybrid ^{177}Lu/^{175}Lu-nanotexaphyrin will be synthesized. Texaphyrin-lipids will primarily be chelated with the "cold" (stable) ^{175}Lu for PDT efficacy, while a small percentage will be chelated with "hot" (radioactive) ^{177}Lu as a radiotracer. This mixed-and-matched chelation strategy has been successfully developed in our lab, which allows for quantitative post–insertion of "hot" ^{111}In into pre-formed nanotexaphyrins containing 90% Mn-texaphyrin and 10% metal-free texaphyrin, resulting in a structurally stable, mixed ^{111}In-Mn-nanotexaphyrin for dual-modal SPECT/MRI imaging. Having already developed stable, cold Lu-nanotexaphyrin, we will now apply this post-insertion method to modulate the ratio of "hot" and "cold" Lu to achieve an optimal balance between imaging sensitivity and radiotoxicity following the strategy in Figure 1. We hope to harness the safety profile of metallo-nanotexaphyrins to enhance therapeutic efficacy by SPECT-guided PDT in order to truly meet clinical needs for precision imaging and therapy of oligometastatic diseases.

[1] J. M. Keca and G. Zheng (2019) Coordination Chem. Rev. **379**, 133-146; [2] J. M. Keca et al. (2016) Angewandte Chemie **55**, 6187-91.

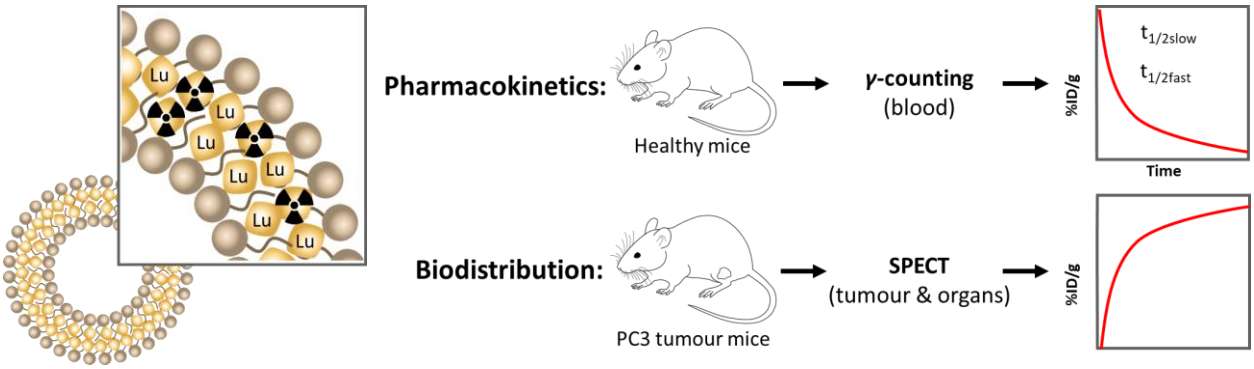

Figure 1: Hot/cold Lu-nanotexaphyrin will be assessed in rodents.

Nanoparticle Mediated Non-Viral Gene Delivery to Enable Development of Next Gen T Cell Therapies

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The next generation of CAR T and T cell therapies hold tremendous promise to revolutionize cancer treatment. Compared to Kymriah® and Yescarta®, these next generation therapies will have an improved safety profile, they will be used in earlier lines of treatment, they will be applied for a wider range of liquid and solid cancers, and they will be more cost-effective and supplied off-the-shelf. However, progress in this field is hampered by the safety, manufacturing and performance limitations of viral vectors and electroporation for engineering gene expression in cells. In this work, we will describe a novel hybrid lipid nanoparticle (LNP) technology for gene delivery. These LNP encapsulate nucleic acids and deliver them to the cytoplasm of cells using natural endocytic pathways. They are chemically-defined, rapidly manufactured at any scale using microfluidic mixing, and easily administered to cells in a single step. We will present a case study demonstrating LNP-mediated delivery of messenger RNA to human T cells *ex vivo,* achieving target gene expression in greater than 80% of cells with no measurable effect on cell viability or proliferation. Overall, these results support the utility of this new hybrid LNP technology to aid in the development of next generation cell-based therapies.

Poster # 16

The Application of Differential Scanning Fluorimetry in Exploring Bisubstrate Binding to Protein Arginine *N*-Methyltransferase 1

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Protein arginine *N*-methyltransferases (PRMTs) catalyze methylation of substrates through use of a methyl-donor cofactor. The PRMT mechanism has been largely explored through a variety of kinetic experiments, but analysis of these results can be difficult, subjective, and sometimes inconclusive. To ensure a robust understanding of the PRMT bisubstrate mechanism, a complement of structural and biophysical studies in addition to kinetic experiments have proven vital. We hypothesize that thermal shift assays may be used in a novel application to explore bisubstrate binding to PRMT1.

We used differential scanning fluorimetry (DSF) to measure protein melting temperature changes in response to ligand binding. We investigated the stability of human PRMT1 in the presence of different binding factors, including: the methyl-donor cofactor *S*-adenosyl-L-methionine (SAM) and its analogues; peptide substrates representing two *in vitro* target proteins of PRMT1; and a potent PRMT1 inhibitor, MS023, which binds in the arginine binding pocket. We predicted that ligands which result in a stabilizing thermal shift are those that bind to the enzyme first in succession whereas subsequently binding ligands do not stabilize the enzyme. Interestingly, not all substrates interact with PRMT1 in a stabilizing manner. We found that the methylation cofactor and its analogues stabilize the enzyme, while peptide substrates destabilize the enzyme. However, an additional stabilizing effect is seen when titrating an active-site ligand in the presence of a saturating concentration of cofactor. We similarly found that MS023 alone does not interact with the enzyme but when incubated with cofactor, stabilizes PRMT1 (Figure 1). We resolve that DSF can be used to ascertain the order of substrate binding. This evidence supports other literature findings that describe how cofactor binding precedes target substrate binding to allow for methyltransfer to occur. Therefore, DSF is a suitable biophysical method to explore PRMT bisubstrate sequential binding and supplements other structural and kinetic experiments.

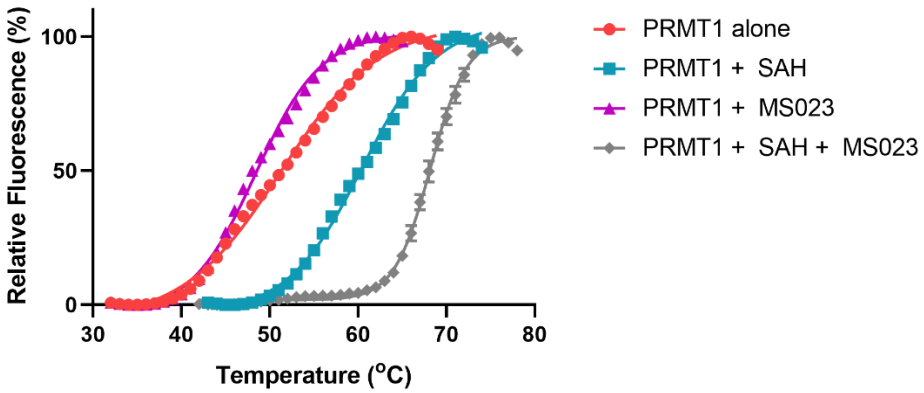

Figure 1. Ligands which tightly bind to and stabilize the enzyme result in a positive thermal shift of the enzyme melt curve. The active site ligand MS023 only interacts with and stabilizes PRMT1 in the presence of saturating cofactor SAH, which provides evidence for a sequential ordered mechanism where cofactor binding to enzyme precedes binding of the active site ligand.

Poster # 17

Fusion-dependent Formation of Lipid Nanoparticles Containing Macromolecular Payloads

Jerry Leung[1], Jayesh A. Kulkarni[1,2,*], Dominik Witzigmann[1], Roy van der Meel[1,3,4], Josh Zaifman[1,5], Maria M. Darjuan[1,5], Hiu Man Grisch-Chan[6], Beat Thöny[6], Yuen Yi C. Tam[1,5], Pieter R. Cullis[1,*]

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The success of Onpattro™ (patisiran) clearly demonstrates the utility of lipid nanoparticle (LNP) systems for enabling gene therapies. These systems are composed of ionizable cationic lipids, phospholipid, cholesterol, and polyethylene glycol (PEG)-lipids, and are produced through rapid-mixing of an ethanolic-lipid solution with an acidic aqueous solution followed by dialysis into neutralizing buffer. A detailed understanding of the mechanism of LNP formation is crucial to improving LNP design. Here we use cryogenic transmission electron microscopy and fluorescence techniques to further demonstrate that LNP are formed through the fusion of precursor, pH-sensitive liposomes into large electron-dense core structures as the pH is neutralized. Next, we show that the fusion process is limited by the accumulation of PEG-lipid on the emerging particle. Finally, we show that the fusion-dependent mechanism of formation also applies to LNP containing macromolecular payloads including mRNA, DNA vectors, and gold nanoparticles.

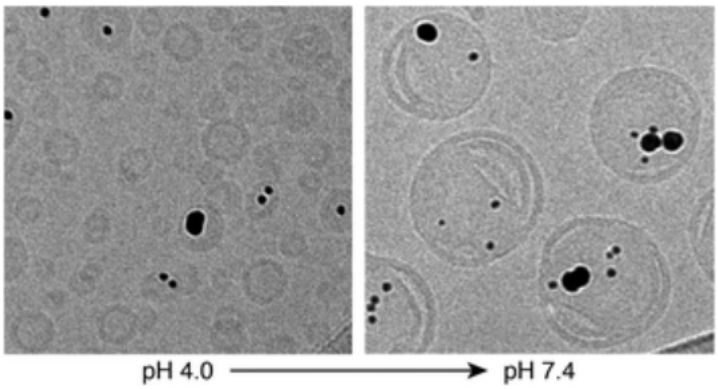

Figure 1. LNPs are formed via the fusion of pH-sensitive liposomes upon pH neutralization, as illustrated by cryogenic transmission electron microscopy images showing that liposomes contain singly-sized gold nanoparticles at pH 4 while LNPs contain gold nanoparticles of varying sizes instead when the pH is neutralized.

Dextran-Functionalization of Semiconducting Polymer Dots and Conjugation with Tetrameric Antibody Complexes for Bioanalysis and Imaging

Kelsi Lix, Michael V. Tran, Melissa Massey, Kelly Rees, Ethan R. Sauvé, Zachary M. Hudson, W. Russ Algar*

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Semiconducting polymer dots (Pdots) are emerging as a powerful fluorescent probe in bioanalysis and imaging due to their extremely high brightness and biocompatibility. However, Pdots suffer from several key limitations including poor stability, non-specific adsorption, and relatively few reported surface chemistries and bioconjugation strategies. We have developed dextran-functionalized Pdots (Dex-Pdots, Figure 1) to address these limitations. Dex-Pdots show improved stability over a range of pH and at high ionic strength. Pdot immunoconjugates were prepared via bifunctional tetrameric antibody complexes (TAC) and were used in a proof-of-concept fluorescence-linked immunosorbent assay (FLISA) for human erythropoietin (EPO) and for the immunolabelling of human epidermal growth factor receptor 2 (HER2)-positive SK-BR3 breast cancer cells. Specific labelling was achieved with minimal non-specific binding compared to Pdots without dextran coatings. Our results show that dextran functionalization is a promising strategy to improve Pdot performance in biological applications.

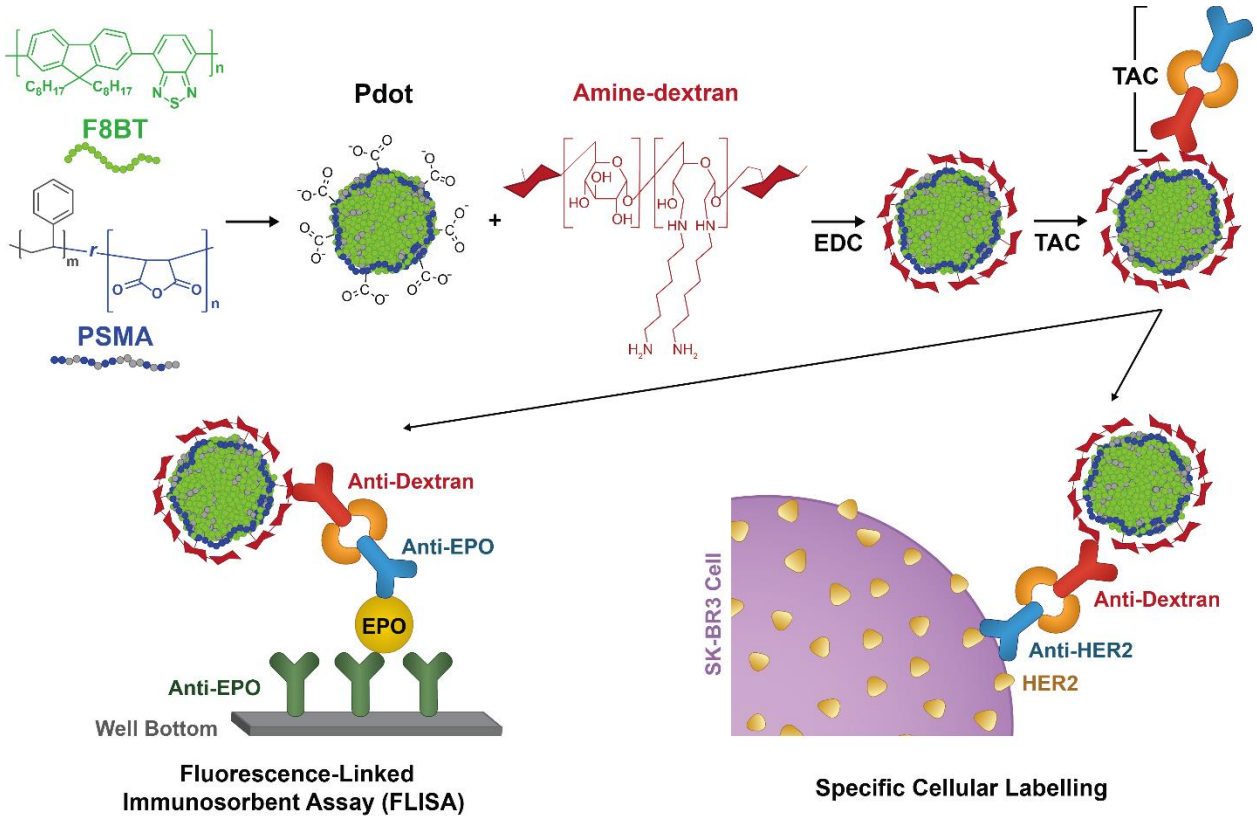

Figure 1. Pdot synthesis, functionalization with dextran, preparation of TAC conjugates and use in specific cell labelling and immunoassay (FLISA) applications.

Poster # 19

Title: **Elucidating the mechanism of the acoustic conversion of microbubbles to nanostructures for EPR-independent delivery to tumors**

Authors: A. Dhaliwal^1,2 (pres.), M. Rajora^1,3, C. Pellow^1,2, Goertz, D.^4, G. Zheng*^1,2,3

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INTRODUCTION – The field of nanomedicine has promised to revolutionize cancer therapy by utilizing multidisciplinary innovations to enhance diagnosis and treatment. However, it has seen limited clinical translation despite preclinical success, a problem largely attributed to the widespread reliance on the enhanced permeability and retention (EPR) effect for tumor accumulation[1–3]. As growing evidence suggests that the passive EPR effect has a heterogeneous clinical profile[4,5], the field has been driven to consider more active strategies to improve delivery. One such platform that may bypass the EPR effect is the *in situ* ultrasound-stimulated conversion of microbubbles to nanostructures – a micro-to-nano conversion – which was first explored using porphyrin-containing microbubbles for the localized delivery of photosensitive nanostructures to tumors[6]. However, in order to facilitate an intentional design process for the development of this technique as a versatile means of stimulated nanoparticle delivery, a thorough characterization and optimization of the micro-to-nano conversion is required.

OBJECTIVE – To elucidate the mechanism and pertinent parameters that enable the microbubble-to-nanostructure conversion under clinically relevant ultrasonic conditions and verify their effect on tumor delivery.

METHODOLOGY & FINDINGS – A library of microbubbles was formulated to assess the impact of lipid chain length, composition, and agent concentration on the micro-to-nano conversion efficiency. These microbubbles were characterized morphologically and optically using particle sizing, microscopy, and fluorescence. Irradiation under a variety of ultrasound pressure and pulse length regimens was conducted in a channel phantom to optimize conversion criteria, and the irradiated products were characterized via acoustic detection and particle sizing. Insight into the mechanism of the conversion was gained via FRET using microbubbles functionalized with fluorescent lipid probes.

IMPACT & INNOVATIONS – The *in situ* microbubble-to-nanostructure acoustic conversion represents a novel strategy for the stimulated delivery of nanoparticles to tumors independently of the EPR effect. Through a deeper understanding of the material properties and acoustic conditions which influence this phenomenon, its use as a generalized platform of EPR-independent delivery of nanoparticles can be utilized in a predictive and consistent manner. By addressing this obstacle in clinical translation, the micro-to-nano approach is capable of facilitating and enhancing agent delivery for a more diverse clinical population, broadening the impact of nanomedicine for cancer therapy and diagnostics.

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Poster # 20

Acoustic levitation as a screening method for the development of dry powder vaccines

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Spray drying has emerged as a promising technique to create thermally stable vaccine powders containing viral vectors. Thermally stable vaccine powders reduce the cost and potential damage that are inherent to the traditional cold chain required to maintain vaccine stability during transportation and storage. Powdered vaccines can be delivered either via inhalation or oral administration to avoid the use of needles, or can be more traditionally delivered by reconstituting and injecting the dissolved powder. Identifying and optimizing the excipient carriers used for stabilization with the spray drying method is a time intensive task and can use large amounts of costly viral material, creating a need for an efficient screening method. In this work, an acoustic levitator modified with a heating system was used to model the spray drying process in order to understand the effects of the environment on spray dried materials and determine its effectiveness as a screening method. Excipient blends of mannitol/dextran, xylitol/dextran, and lactose/trehalose were used to stabilize a recombinant human type 5 adenovirus (AdHu5) as a model viral vector. The levitated particles were characterized by scanning electron microscopy (SEM), differential scanning calorimetry, thermal gravimetric analysis, and X-ray diffraction and compared to spray dried powders. Stability of the AdHu5 was investigated *in vitro* by infecting A549 lung epithelial cells with a green fluorescent protein expressing variant of AdHu5. Similar particle texture was observed for levitated and spray dried materials, although the particle size differed by several orders of magnitude. The levitated particles were found to have better activity retention of the AdHu5 in two of the three formulas tested. The differences in activity may be attributed to the fact that levitated particles did not undergo the application of shear stress that is present during spray drying. When heat was added to the levitation chamber, viral activity increased up to 40°C, but at higher temperatures it began to decrease. Although the magnitude of activity losses for levitated particles at 40°C and spray dried powders was different, overall trends in viral activity between the excipient formulas were the same. This research will show that acoustic levitation is a promising screening method for spray drying thermally stable dry powder vaccines.

Poster # 21

Microparticulate-Encapsulated Antigen with Split Topical CpG Oligodeoxynucleotide Adjuvant as a Single-Injection Immunization Strategy

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Antigen-presenting cells (APCs) are responsible for presenting foreign antigens to, and activating T cells to fight off infection. Manipulation of APCs to modulate immune responses to immunization has been a focus in vaccinology. Due to high frequencies of APCs present in the skin, we are interested in the skin as a site for immunization. Keratinocytes and skin-resident plasmacytoid dendritic cells can be activated via binding of their Toll-like receptor 9 to CpG oligodeoxynucleotides (CpG ODN) – synthetic DNA that mimic unmethylated CpG bacterial DNA. We encapsulated ovalbumin (OVA) antigen in polylactide co-glycolide microparticles (PLG-OVA) and show that mice immunized subcutaneously with PLG-OVA generate antigen-specific cytotoxic T cells when CpG ODN is used as an adjuvant topically. Using flow cytometric analysis, 2.3%, 0.7% and 0.5% of antigen-specific cells are detected within the $CD8^+$ cytotoxic T cell population in the peripheral blood, skin draining lymph nodes and the spleen respectively. In addition, 0.8% of $CD8^+$ splenocytes produce interferon-gamma when re-stimulated with the OVA (SIINFEKL) peptide *in vitro*. Our PLG-OVA particles show a triphasic release of the OVA antigen which simulates two doses of antigen injected separately. Fluorescent microscopy shows that both PLG and OVA are present in the skin draining lymph nodes two weeks after immunization. Using the release properties of PLG-OVA and a second topical application of adjuvant, we enhance the generation of antigen-specific cytotoxic T cells in the blood from 0.4 to 0.9% ($P < 0.05$) and OVA-specific total IgG and IgG2c production, effectively generating a single-injection vaccine with split topical adjuvant administration that induces robust cytotoxic T cell and antibody responses. Unlike the adjuvant Alum, subcutaneous injection of PLG-OVA with topical CpG ODN application does not cause the formation of persisting subcutaneous nodules. Generating single-injection vaccines with minimal side effects may enhance compliance with vaccination in the general population.

Development of Targeted Macromolecular Iron Chelators for Treatment of Cardiac Iron Overload

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Purpose: Disorders of hemoglobin, such as sickle cell anemia and thalassemia, are an increasing global health concern requiring chronic transfusion as a life sustaining treatment. Unfortunately, a consequence of chronic transfusion is excess iron burden leading to *transfusion-associated iron overload*. With iron overload, excess iron is available to mediate oxidative damage of cells, and this iron accumulates in vital organs, namely, the liver and the heart. To date, no methods are available to remove iron from specific organs to prevent organ toxicity; consequently, iron overload remains associated with significant morbidity and mortality. In this work, we developed a macromolecular chelator with enhanced specificity toward heart as an effective system to treat cardiac iron overload. Here, we study the cellular and organ targeting ability, biodistribution, and biocompatibility of our novel **c**ardiac-**t**argeted **m**acro**c**helator (CTMC).

Methods: Fluorescently-tagged CTMC and non-targeted controls were incubated in a co-culture model of iPS cardiomyocytes and BJ human fibroblasts. *In vitro* cellular uptake was measured by flow cytometry and laser scanning confocal microscopy methods. Next, *in vivo* organ targeting ability was determined by injecting ^{3}H-labelled CTMC in Balb/c mice. Qualitative distribution of CTMC was assessed through histological examination of heart tissue collected from mice injected with fluorescently-tagged CTMC. Finally, biocompatibility of the system was assessed by MTT cell viability assay.

Discussion/Results: *In vitro* cellular uptake studies demonstrated a 2.5 ± 0.5-fold uptake of CTMC in as limited as 100 nM doses by iPS cardiomyocytes. *In vivo* radiolabeling study of CTMC demonstrated successful organ-targeting ability with 22 ± 4% injected dose/gram of cardiac tissue retained up to 8 hours post-injection, as compared to 7 ± 1% from non-targeted controls. Finally, biocompatibility testing showed that CTMC incubated cells experience 30% higher cell viability compared to cells incubated with a small molecule chelator.

Conclusions: Here, we successfully demonstrate a biocompatible, cardiac-specific targeting approach to chelation therapy. As cardiac failure remains a leading cause of death in iron overload patients the development of chelators targeting heart will be a paradigm shift in the treatment of iron overload.

Low volume production of nanoparticles that are effective transfection systems in iPSC-derived cells, immune cells and other primary cell cultures

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The need for drug delivery and transfection systems is obvious to molecular biologists, disease researchers and scientists in early discovery labs of the pharmaceutical industry. Nanoparticles that serve as these systems must have the potential to be manufactured at sufficiently low volumes to support the genetic manipulation of cells and to conserve costly genetic payloads. Currently, there are no accurate and reproducible production methods to meet the needs of these researchers. The NanoAssemblr™ Spark™ uses a proprietary microfluidics technology for the controlled and reproducible manufacturing of nanoparticles in small volumes (25 – 250 uL). Using a lipid delivery system, that is well described in the literature for the delivery of siRNA, mRNA and plasmid, we have shown the production of lipid nanoparticles (LNPs) that are 60 – 130 nm in size with a polydispersity index (PDI) below 0.2. We have manufactured mRNA-containing LNPs with over 80% encapsulation of payload and shown the effect of various formulation parameters on nanoparticle characteristics. The formulation parameters analyzed included PEG content, N/P ratio, and choice of the cationic/ionizable lipid. mRNA and plasmid LNPs made on the NanoAssemblr™ Spark™ were applied to cells in culture to assess biological activity. Induced pluripotent stem cell (iPSC)-derived neurons were used as neuroscientists are turning to this *in vitro* model to identify potential therapeutic strategies against neurodegenerative diseases. Oligonucleotide-based therapies require the efficient delivery of nucleic acid payloads to these cells, while minimizing cytotoxicity and changes in neuronal physiology. iPSC-derived neurons and iPSC-derived neural progenitor cells (NPCs) were treated with mRNA and plasmid LNPs that were made on the The NanoAssemblr™ Spark™. In this proof-of-concept work, we demonstrate the successful delivery of plasmid LNPs to iPSC-derived NPCs with minimal impact on downstream differentiation into cortical neurons. Over the course of 21 days, these NPCs differentiated into cortical neurons with similar morphological characteristics, such as neurite length, relative to untreated controls. Most critically, this plasmid LNPs treatment had a relatively insignificant impact on neurite length and overall neuron viability. We also show the efficient transfection of mature iPSC-derived cortical neurons using plasmid LNPs. The NanoAssemblr™ Spark™ is ideal for screening novel compositions of nanoparticles that use valuable active pharmaceutical ingredients. This nanoparticle screening platform allows for the rapid advancement of delivery systems that support the development of efficient transfection technologies and the next generation of therapeutics.

Scalable Manufacture of mRNA Lipid Nanoparticles using a Novel Microfluidic Mixing Architecture

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Precision NanoSystems Inc. (PNI) has developed novel microfluidic-based approaches to prepare a wide range of nanoparticles, including lipid-based nanoparticles for delivery of oligonucleotides (e.g., siRNA), larger polynucleotides (e.g., pDNA and mRNA), PLGA particles, liposomes and emulsions. PNI's NanoAssemblr® technology with the established Staggered Herringbone Mixer (SHM) is widely used to produce homogeneous lipid nanoparticles with defined particle sizes, narrow size distributions (PDI < 0.1) and high nucleic acid encapsulation efficiencies (>90%).

PNI has developed a new microfluidic architecture designed for high capacity, homogenous mixing of fluid streams to enable the self-assembly of nanoparticles. This next-generation mixer achieves microfluidic mixing using centrifugal force and allows production rates to be scaled by orders of magnitude with a single mixer, while preserving the precise, time invariant microfluidic mixing properties of the previous generation SHM-based mixer. This new architecture is ideally suited for the reproducible scale-up of mRNA-LNP and other challenging nanomedicine formulations.

Luciferase mRNA-LNP were prepared on the next-generation mixer and SHM under fixed formulation conditions and varied flow-rates. The mRNA-LNP were post-processed using ultra-filtration for buffer exchange and concentration. The particle size and polydispersity index were determined using dynamic light scattering (DLS). Particle morphology was determined using cryo-electron microscopy. The RiboGreen assay was used to determine total mRNA content and encapsulation efficiency. UPLC methods were utilized to determine total lipid concentration and final amine to phosphate (N/P) ratio. Mice were injected intravenously with various Luciferase mRNA-LNP samples and gene expression was determined using luciferase activity (bioluminescence) in an IVIS imaging system.

We show experimentally that Luciferase mRNA-LNP prepared using the SHM at 12 mL/min and the high capacity next-generation mixer at 200 mL/min both gave particles of less than 100 nm, with similar physiochemical properties (i.e., polydispersity, mRNA encapsulation efficiency, particle morphology). These mRNA-LNP also possessed similar biological activity in mice (Luciferase Bioluminescence assay) at doses of 1.0, 0.3 and 0.05 mg/kg, which further demonstrates the parity of the SHM and next-generation mixer architectures.

These experimental results demonstrate the equivalence of the next-generation mixer and SHM allowing for the seamless scale-up of mRNA-LNP nanoparticle drugs. The high throughput next-generation mixer can be used for the GMP manufacture 1 – 100 g batches mRNA-LNP to support clinical development programs.

Immunomagnetic purification of extracellular vesicles from biofluids in less than 30 minutes

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Extracellular vesicles (EVs), including exosomes, are purported to be key regulators of intercellular communication and are produced and secreted by most cell types. EVs contain a variety of cargoes including miRNA, mRNA, and proteins that reflect the status of the originating cell type. Analysis of this cargo can provide insight into intercellular communication under physiological conditions and can also provide valuable diagnostic and prognostic information under pathological conditions, such as cancer and neurological disease. However, EV isolation and purification from complex biofluids remains challenging and time consuming. The current standard isolation method is differential ultracentrifugation using density gradient medium, which takes 16 – 90 hours and is associated with low recovery of EVs. Alternative high recovery methods such as short differential ultracentrifugation and polymer precipitation using polyethylene glycol suffer from low specificity due to co-isolated biological materials, such as proteins. The EasySep™ Human EV Positive Selection Kits provide a fast and easy EV isolation method that utilizes the expression of tetraspanin markers (CD9, CD63, CD81) on the surface of EVs for positive selection without expensive equipment. The 26- to 28-minute protocol can process volumes from 0.5 - 8 mL and EVs can be isolated on the basis of CD9, CD63, CD81 or pan-tetraspanin selection. The protocol involves first incubating target EVs with antibody complexes against individual or pan-tetraspanins, followed by incubation with the magnetic particles. Immunolabeled EVs remain in the hand-held magnet, while unwanted contaminants are poured off. The EasySep™ Human EV isolation kits have been tested using multiple biofluids, including plasma, serum, urine, and various conditioned media. The EasySep™ Human Pan EV isolation kits can provide up to 1.1 - 3.7 times higher EV recovery when compared to short differential centrifugation (n = 4). The EasySep™ protocol achieves similar EV recovery in 30 minutes compared to competitors' kits requiring 1.5-hour isolation. Overall, the EasySep™ human EV Positive Selection kits provide a fast and easy way to isolate high-quality EV and are compatible with common downstream assays, such as Western Blots, and RT-qPCR.

Dual-wavelength Fiber Laser Multiphoton Endoscopy for Oral Cancer Detection

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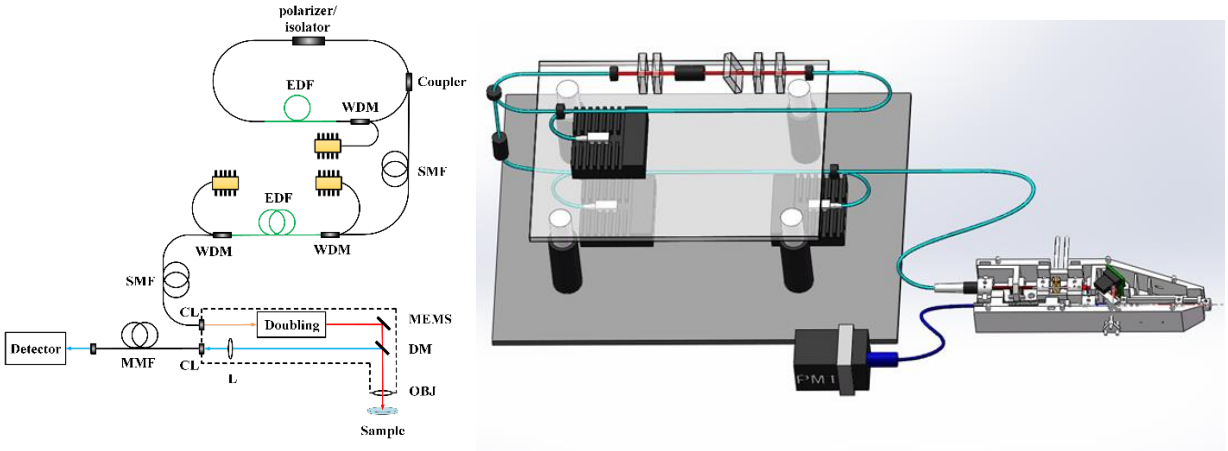
Abstract (250 words)

Currently, a majority (52.7%) of oral cancer diagnosis occurs during stage IV, significantly reducing average patient survival rates within five years compared to that of early detection. Multiphoton microscopy (MPM), which offers high resolution and deep penetration analysis of cellular and extra-cellular features, has shown promising results in distinguishing cancerous tissues in past studies. Unlike traditional biopsy, MPM provides fast, label-free, non-invasive examination of suspicious oral lesions, making it a potential cancer screening tool in the future.

Most MPM systems are built around either two-photons or three-photons excitation. We can work with both by using 1580 and 790 nm dual wavelengths [1]. An Er-doped fiber laser delivers fundamental pulses at 1580 nm and 80 fs for third harmonic generation (THG) [2]. Second harmonic generation (SHG) and two-photon excitation fluorescence (2PEF) is obtained by frequency doubling of 1580 nm with periodically-poled $LiNbO_3$ to 790 nm (80 fs pulse-width). The multimodal MPM allows for comprehensive biological studies as the three signals contain complementary information—SHG from the fibrillar collagen and striated muscle myosin, 2PEF from intrinsic tissue fluorophores, and THG from lipids-tissue interfaces.

However, complexities in multimodal excitation, femtosecond pulse delivery, and scanning mechanism have constrained MPM to large benchtop systems, impractical for clinics. Here, we propose a compact multiphoton endoscope capable of acquiring SHG, 2PEF, and THG images. A MEMS mirror performs lateral scanning while a separate actuator is being integrated to shift the focal plane. The system is compact with all-fiber delivery, provides three complementary 3D images, and is suitable for clinical translation

[1] Huang, L., et al. (2016). Biomed. Opt Express. **7**, 1948-1956; [2] Huang, L., et al. (2018). JBO. **23**, 126503.



Poster # 27

Development and Optimization of LNP-based Gene Therapy Approaches in the Brain

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Brain diseases, particularly age-related diseases such as Alzheimer's disease and Parkinson's disease, are a significant burden to the Canadian health care system. Furthermore, estimates suggest the number of people affected by these diseases is increasing. Treatment of genetic brain diseases, including neurodevelopmental and neurodegenerative disorders, is severely limited by the lack of accessibility of most genetic therapies to the brain due to the presence of the blood-brain barrier. Gene therapy is a viable option for the treatment of brain diseases provided the therapeutic agents can be delivered to neurons, the primary cells of interest in the brain. Current approaches to gene therapy in the brain are centered around the use of antisense oligonucleotides (ASOs) and adeno-associated viral (AAV)-mediated gene delivery. There are numerous limitations to these approaches, leaving abundant space for novel and/or improved gene therapy delivery methods.

Lipid nanoparticle (LNP) based gene therapy approaches hold great promise for the future clinical development of brain disease treatments for two main reasons. First, we and others have shown that neurons, either isolated *ex vivo* or *in vivo*, are highly amenable to transfection by LNPs carrying gene therapy agents. This is in stark contrast to the vast majority of other means of transfection apart from AAV-mediated transduction. Second, LNPs have been proven safe and effective in clinical trials for the treatment of other conditions, with 10 different LNP-based drugs now approved by the FDA. This proposal aims to identify optimal brain-specific LNP formulations for the delivery of two key gene therapy payloads, siRNA and mRNA. These optimized formulations will be further developed for the treatment of multiple genetic brain diseases. To do this, we will establish a pipeline for screening LNP formulations in primary neuronal cultures (*ex vivo*) followed by validation via direct brain injections (*in vivo*) (**Fig.1**). At the completion of this work, we will have identified optimal LNP formulations, doses, and modes of administration for delivery of gene therapy payloads to neurons *in vivo*, setting the stage for future development of target-specific payloads to treat genetic brain diseases.

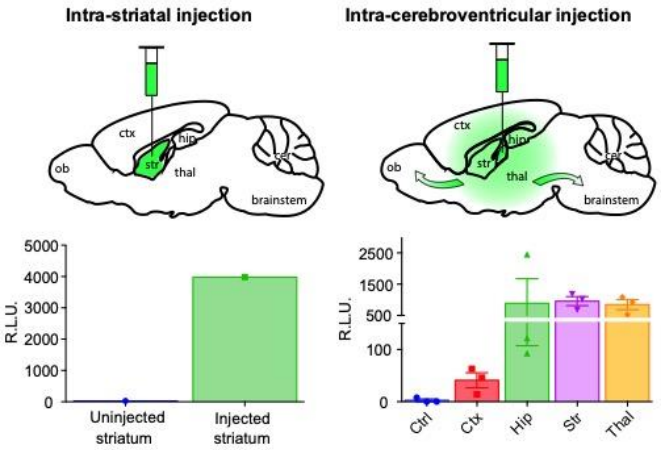

Figure 1. Direct brain injections of LNPs can be given as a targeted bolus to a specific brain region (ie. striatum, left) or introduced into the circulating CSF via intra-ventricular injection (right).

Poster # 28

Coumarin-3-Carboxylic Acid: A Detector for Hydroxyl Radicals Generated by Orthovoltage and Proton Radiation

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Gold nanoparticles (AuNPs) can act as both radioenhancers and radiosensitizers in the presence of X-rays. While radioenhancers are agents which lead to the physical increase in the local energy deposition of X-rays, radiosensitizers interact with the chemical and biological byproducts of radiation. Together, the physical, chemical, and biological interactions of AuNPs with X-rays and their byproducts enhance the formation of DNA-damaging free radicals [1]. While the physical dose enhancement effect of gold nanoparticles has been modelled using Monte Carlo simulations and its radiosensitizing effects shown through cell and mice studies, there are limited studies demonstrating their direct effect in an isolated system containing AuNPs and a detector for reactive oxygen species [2]. The ability to quantify the dose enhancement factor in a simple physical system can elucidate the effect of various irradiation conditions and gold nanoparticle formulation factors such as size, concentration, and surface coatings.

Coumarin-3-carboxylic acid (3-CCA) was studied as a probe for the detection of hydroxyl radicals under kilovoltage and proton irradiation. When exposed to hydroxyl radicals, 3-CCA is oxidized to 7-OH-CCA, a detectable fluorescent species. Coumarin-3-carboxylic acid was chosen over other fluorescent probes due to its high sensitivity and low cost [2]. The sensitive range of the assay was determined, where higher total doses of kilovoltage and proton irradiation produced readings with higher fluorescence intensity. Although the gold nanoparticle fluorescence spectrum was found to interfere with the assay readings, the fluorescence intensity could be recovered by removing AuNPs from the samples by centrifugation. Overall, 3-CCA was shown to be a promising system for the detection of reactive oxygen species.

[1] C Sicard-Roselli (2014) Small **10**, 3338-3346; [2] S Her (2017) Adv. Drug Deliv. Rev. **109**, 84-101

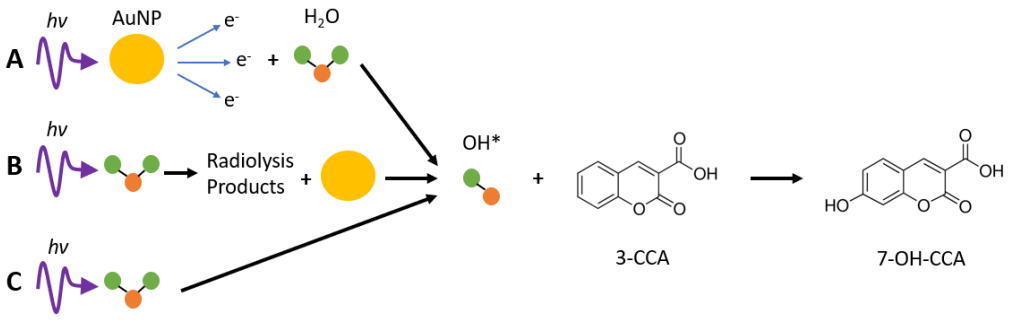

Figure 1: Quantification of radiation dose enhancement by gold nanoparticles (AuNPs) through hydroxyl radical detection. Hydroxyl radicals are formed through direct (A) and indirect (B) interactions with AuNPs or water (C).

Poster # 29

Liposomal flavopiridol – a novel formulation for the treatment of acute myeloid leukemia

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[e]Cuprous Pharmaceuticals ,Vancouver, BC, Canada

For more than 30 years, treatment of acute myeloid leukemia (AML) has remained largely unchanged and continues to rely on chemotherapeutic drug combinations. In light of the recent approval of a combination product (Vyxeos) that showed marked therapeutic improvement against AML, we are considering the development of an alternative combination product for AML. One of the drugs to be used in this combination product is flavopiridol: a semi-synthetic flavonoid that has shown significant activity against AML primarily through the inhibition of cyclin-dependent kinases. However, the development of flavopiridol as an anticancer agent has been hindered by its poor aqueous solubility and its lack of clinical activity due to its affinity to serum proteins. Therefore, in this study, we remedied the clinical limitations by utilizing the copper-binding capabilities of flavopiridol to prepare a safe and injectable formulation with significantly enhanced apparent solubility. The methods rely on adding flavopiridol powder directly to preformed copper-containing liposomes. Flavopiridol in free solution can cross the liposomal lipid bilayer and subsequently form a copper-flavopiridol complex within the liposomes. This flavopiridol formulation is well tolerated in mice following *i.v.* administration (at a dose of 5mg/kg) with no acute or chronic toxicities observed. *In vivo* pharmacokinetics of the resultant liposomal flavopiridol formulation demonstrated a 2-fold improvement in elimination half-life (1 hours versus 0.5 hour) and a 30-fold increase in AUC (67nmol/mL*hr versus 2nmol/mL*hr) compared to free flavopiridol formulation. The resultant liposomal formulation also demonstrated significant therapeutic activity in subcutaneous AML models (MV4-11 and MOLM-13), resulting in regression of tumour sizes and improved overall survival. The successful development of a novel formulation of flavopiridol resolves the therapeutic limitations of flavopiridol and may be more clinically relevant and is suitable for use in the development of a novel combination product that should be particularly active in AML patients.

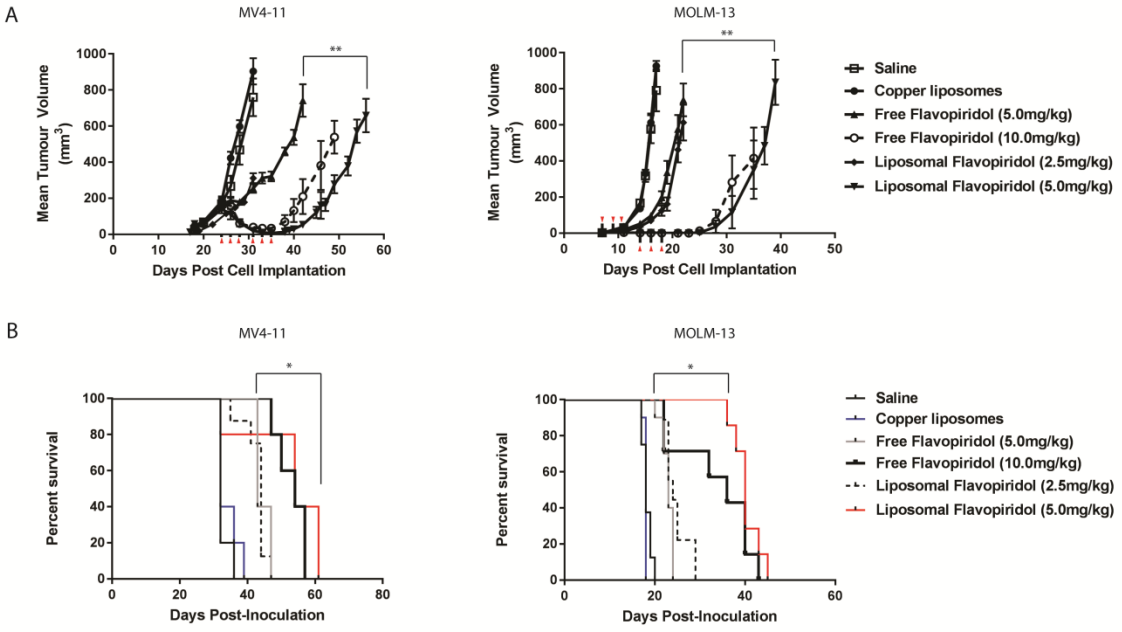

Figure- Study of efficacy of liposomal and free flavopiridol formulations in tumour-bearing NRG immune-compromised mice.

Poster # 30
Portable, On-Demand Biofunctionalization for Nanomedicines

Nanomedicine has revolutionized targeted drug delivery and holds great potential for genome-level countermeasures against genetic diseases. However, the biological components of nanomedicines (e.g. antibodies, RNAs) require highly specialized manufacturing logistics and expertise, limiting access at the point of care and remote areas. We propose to facilitate nanomedicine development and extend its reach by using automated, deployable cell-free biologics manufacturing to functionalize and program lipid nanoparticles (LNPs) with the required targeted delivery and genomic manipulation components. We have previously demonstrated the production of functional biomolecules and therapeutics using our freeze-dried cell-free technology [1]. As proof of concept for nanomedicine applications, we aim to demonstrate the automated biomanufacture of nucleic acids and antibodies to functionalize LNPs for the targeted delivery of CRISPR machinery for *in vivo* reprogramming of T lymphocytes into CAR T cells for the treatment of leukemia. This will involve: (i) the integration of custom-designed hardware and molecular technologies for the automated synthesis of biological components and biofunctionalization of LNPs, (ii) *in vitro* cell-based uptake and efficiency assays followed by mouse pilot studies for LNP distribution and validation of T-cell reprogramming, (iii) cross-network collaboration with a number of NMIN laboratories to demonstrate the developed technology for on-demand biofunctionalization of nanomedicines from these collaborating groups.

[1] Pardee, K. *et al.* Portable, On-Demand Biomolecular Manufacturing. *Cell* **167**, 248-259.e12 (2016).

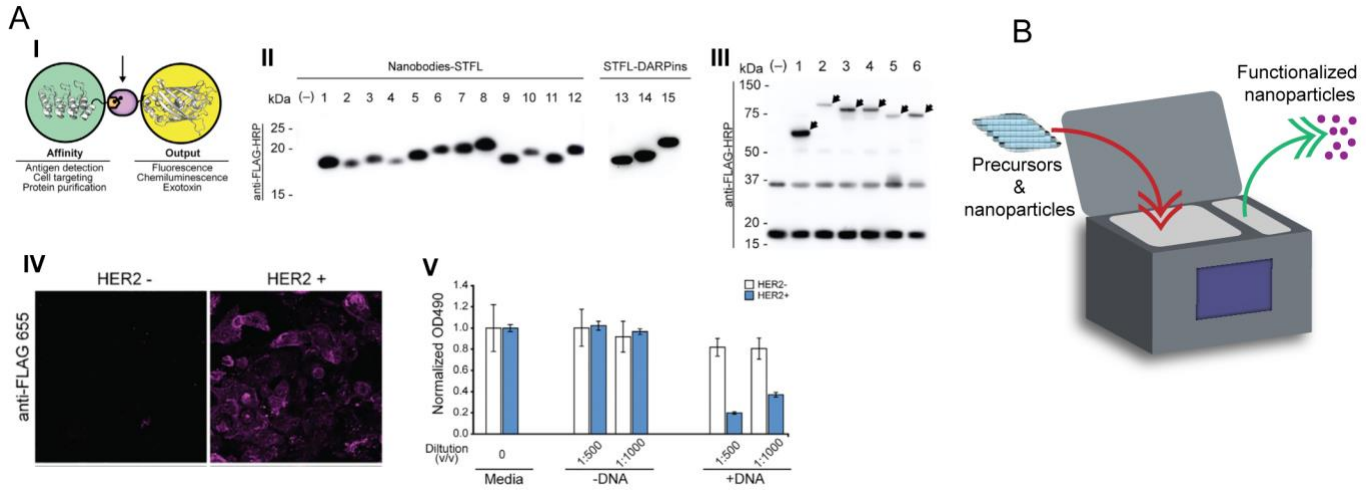

Figure 1: A) **Portable on-demand manufacturing of antibodies.** (**I**) Proof-of-concept spy tag-based affinity conjugation scheme for biomolecules. (**II**) Anti-FLAG Western blot for cell-free expression of 15 single domain antibodies. (**III**) Anti-FLAG Western blot of 1-6 one-pot antibody to protein conjugation reactions (black arrows). (**IV**) Image of anti-HER2 DARPin from cell-free expression for specifically labelling or (**V**) killing HER2+ cells. **B**) Schematic of automated device for cassette-based antibody synthesis and conjugation to LNPs for simple on-demand biofunctionalization.

Poster # 31

Targeted siRNA Delivery with Antibody Conjugated Gold Nanostars-Assisted Ultrafast Femtosecond Laser Optoporation

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Gene therapy as an effective alternative to chemotherapy has been ideally approved in vitro and in vivo. However, the common gene delivery approaches has not been really practical in human clinical trials due to unexpected side effects caused by various viral and non-viral vectors. Optoporation can gently perforate the membrane lipid bilayer of target cells and internalize transgenes into the cytoplasm [1]. Here, optoporation is furthered through the use of an ultrafast-pulsed femtosecond laser at the near-infrared (NIR) region (800 nm). Among a wide range of plasmonic nanostructures, gold nanostars (AuNSs), a branched nanostructure with a core and tips, magnify the local field strength in several folds regardless of the orientation of the incident light compared to gold nanorods [1]. The localized surface plasmon resonance of AuNSs tuned into the NIR region of the electromagnetic spectrum holds remarkable promises for an efficient cellular transfection in deep tissues. The epithelial cell adhesion molecule (EpCAM) antibody conjugated AuNSs (Ab_{EpCAM}-PEG_{5kDa}-AuNSs) improves the cellular binding compared to non-specific PEGylated-AuNSs. An efficient local delivery of cell-impermeable BLOCK-iT™ fluorescent siRNA into NIR laser irradiated Ab_{EpCAM}-PEG_{5kDa}-AuNSs bound ARPE-19 (human retinal pigment epithelial cell line) achieves without affecting the viability. The site-specific optoporation adaptable to a plasmon resonance of different nanostructures may be a promising gene delivery strategy into target cell population in the body.

[1] A Wilson (2018), Nano Lett., **18**, 6981-6988.; [2] CG Khoury (2008), J. Phys. Chem. C, **11**, 18849-18859

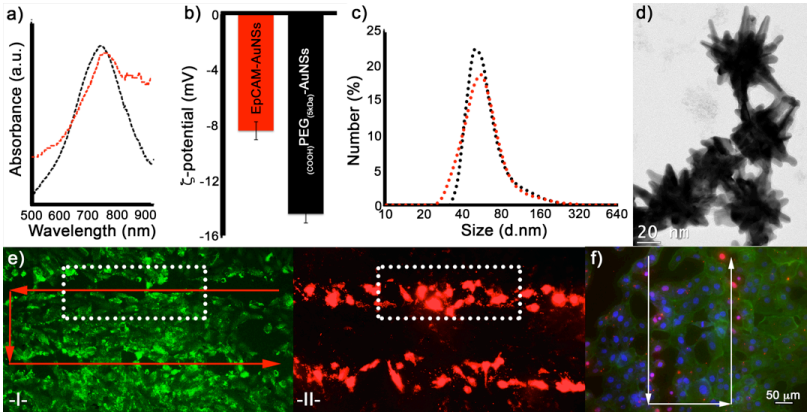

Figure 1. a) Extinction spectrum, b) ζ-potential (mV), c) size measurement of PEGylated-AuNSs (back dashed lines and column) and Ab_{EpCAM}-PEG_{5kDa}-AuNSs (red dashed lines and column), and d) a representative TEM image of Ab_{EpCAM}-PEG_{5kDa}-AuNSs. e) The internalization of propidium iodide (red color) into the NIR treated ARPE-19 cells; (I) the cells stained by means of calcein acetoxymethyl are green, and (II) those treated cells internalized propidium iodide are red. f) The cell-impermeable BLOCK-iT™ fluorescent siRNA (red color) into NIR laser irradiated Ab_{EpCAM}-PEG_{5kDa}-AuNSs bound ARPE-19. The filaments of the cytoskeleton were stained with phalloidin-TRITC (green). The femtosecond NIR laser with a fluence of 120 mJ/cm^2 (Olympus 4x objective and velocity 500 µm/s) was used for the optoporation.

Poster # 32

Liposomal delivery of an immunomodulating agent to the tumour microenvironment

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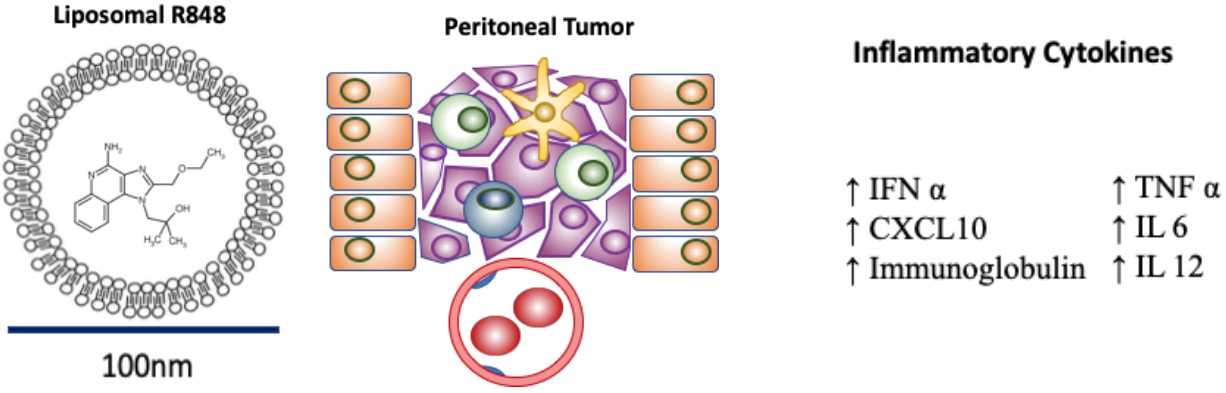
Tumour cells in the peritoneum secrete cytokines to promote an immunosuppressive tumour immune microenvironment (TIME). The TIME is characterized by a decreased infiltration of CD8+ T Cells and an increase in T_{reg} cells, tumour associated macrophages (TAMs), and myeloid derived suppressor cells (MDSCs) [1]. Overall, this cell profile promotes immunosuppression, facilitating tumour growth. The Toll-like receptor 7/8 agonist, R848, can effectively reduce tumour burden by altering cytokine release profiles and repressing immunosuppression. However, this effect is only achieved at doses that elicited toxicity. When R848 is delivered in liposomes, we found that a lower dose could avoid toxicity and remain efficacious. The aim of this study was to optimize a targeted liposomes formulation, measure the pharmacokinetics of R848-liposomes, systemic and local cytokine expression and begin preliminary efficacy and safety studies.

We found that liposomes containing the positive lipid DSTAP (Distearoylpropyl trimonium chloride) were retained in the peritoneal cavity to a significantly higher extent relative to neutral and negative formulations. In accordance with pharmacokinetic studies, DSTAP-liposomes containing R848 also caused significantly increased expression of IFN-α, IL-6 and TNF-α in the peritoneal fluid relative to fre R848, indicating immunostimulatory effects. In preliminary efficacy studies, DSTAP-R848 was not effective a clearing tumours when administered at 1mg/kg. We next investigated the ability of DSTAP-R848 to act as an adjuvant to the antineoplastic compound oxaliplatin. In this study we found that when administered in conjunction with DSTAP-R848 could clear late stage tumours in the peritoneal cavity, and result in long term tumour immunity, possibly due to a synergistic interaction of oxaliplatin induced immunogenic cell death.

R848 encapsulated in DSTAP-liposomes shows encouraging results for promoting cell mediated tumour reduction through cytokine modulation. Future studies are needed to evaluate the change in immune cell composition and elucidate the precise mechanism of action.



Balkwill et al. (2012) Journal of Cell Science **125**: 5591-5596

Poster # 33

THERAPEUTIC GENOME EDITING: DEVELOPMENT OF A REPORTER SYSTEM TO OPTIMIZE AND EVALUATE *IN VIVO* CRISPR/CAS9 BASE EDITING

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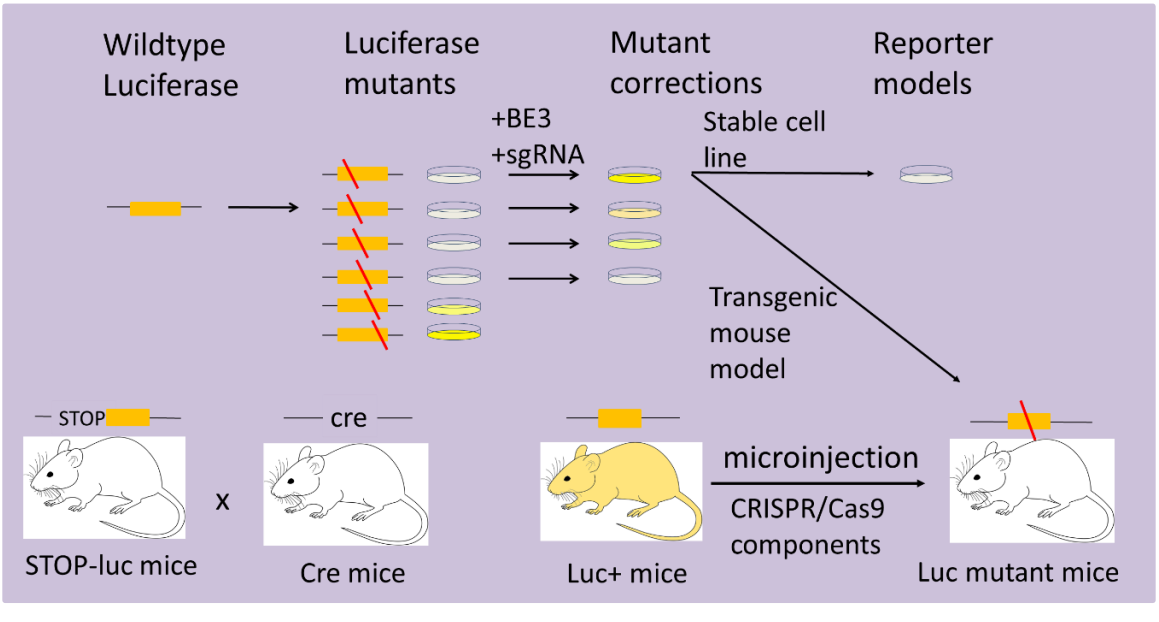
Of the estimated 6,000 human genetic diseases, >95% have no approved therapy. The CRISPR/Cas9 system is a powerful genome editing tool which has the potential to permanently correct pathogenic mutations. New formulations of Cas9 nuclease called base editors have been developed with the ability to target a specific nucleotide in DNA. For example, cytidine base editors (CBE) can convert cytidine to thymine with high specificity and efficiency, for which ~14% of human pathogenic point mutations could potentially be corrected. We sought to develop a reporter system to optimize *in vivo* base editing for the treatment of genetic diseases.

We have identified a luciferase mutant which exhibited 99.96% reduction in activity. This mutant could be efficiently corrected by CBE when transiently transfected, restoring 20.3% of the wildtype activity. Similar correction efficiency was also observed in the mutant stable cell lines. To generate the mutant luciferase mouse model, we have bred mice to homogenously or heterogeneously express luciferase. We used CRISPR/Cas9 to introduce the desired mutations in the luciferase gene within the mouse genome. The mouse model is currently underdevelopment. It will be a valuable tool for comparing new generation of base editors and optimizing delivery systems improve the specificity and efficiency.



Peptidisc Applications for the Discovery and Characterization of Antibodies Against Membrane Proteins

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Membrane proteins comprise one third of the total cellular proteome and constitute more than half of all current drug targets. Yet, a major barrier to their characterization or exploitation in academic and industrial settings is that most biochemical, biophysical and drug development strategies require these proteins in a water-soluble state. This barrier, of membrane protein solubility, is reflected in the severe underrepresentation of membrane proteins in structural and interactome databases. The peptidisc is a recently developed membrane mimetic offering a "one-size-fits-all" approach to the problem of membrane protein preparation in aqueous solution. Herein, we describe a streamlined method – termed PeptiQuick – that combines membrane protein purification and reconstitution into a single chromatographic step. Additionally, functionalization of the peptidisc scaffold, with biotin or fluorescent tags, greatly facilitates downstream applications, such as antibody library screening and protein-protein binding assays. Through this workflow, we eliminate the need to biotinylate the target and for complicated means of protein detection. We showcase the PeptiQuick workflow and its downstream applications in Biolayer Interferometry and Yeast Display technologies.

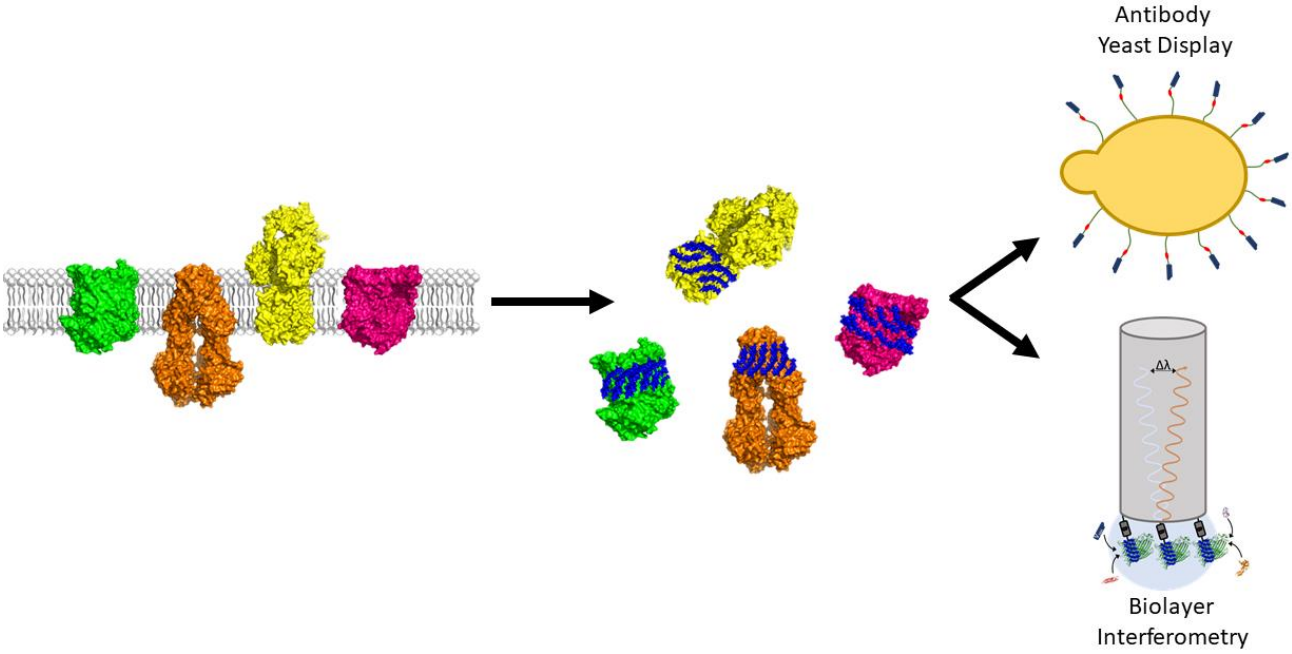

Figure 1: Schematic overview of peptidisc reconstitution with yeast display antibody discovery and biolayer interferometry applications.

Poster # 35

Influence of nitrogen source and metabolism on virulence of *Pseudomonas aeruginosa*

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Effective antimicrobial agents are essential for the prevention and treatment of life-threatening bacterial infections. Though antimicrobial resistance arises naturally in bacteria by random mutagenesis, its emergence is accelerated by the overuse of antibiotics in medicine and agriculture [1]. *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that may adopt adaptive lifestyles to cope with nutrient depletion and antibiotic exposure *in vivo* [2-4]. Motility adaptations are of particular interest to us since swarming and surfing motilities are directly associated with increased resistance and virulence in addition to evasion of environmental stressors [3]. More specifically, we seek to understand the influence of nitrogen source and metabolism on rapid surface motility as well as virulence in an *in vivo* model of chronic, high density infection [5]. The results of this study may improve our understanding of therapeutic challenges afforded by resistant pathogens and suggest alternative approaches for treatment development, particularly in the context of our abscess model.

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Poster # 36

Microfluidic approaches for the production of monodisperse, superparamagnetic microspheres in the low micrometer size range

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The preparation of small, monodispersed magnetic microparticles through microfluidic approaches has been consistently challenging due to the high energy input needed for droplet break-off at such small diameters. In this work, we show the microfluidic production of 1–3 µm magnetic nanoparticle-loaded poly(D, L-lactide) (PLA) microspheres. We describe the use of two approaches, using a conventional flow-focusing microfluidic geometry (**Figure 1**). The first approach is the separation of target size satellite particles from the main droplets; the second approach is the direct production using high flow rate jetting regimes. The particles were produced using a polymeric thiol-ene microfluidic chip platform, which affords the straightforward production of multiple chip copies for single-time use, due to large feature sizes and replica molding approaches. Through the encapsulation of magnetite/maghemite nanoparticles, and their characterization with scanning electron microscopy (SEM) and vibrating sample magnetometry (VSM) measurements, we show that the resulting particles are monosized, highly spherical and exhibit superparamagnetic properties. The particle size regime and their magnetic response show potential for in vivo intravenous applications of magnetic targeting with maximum magnetic response, but without blocking an organ's capillaries.

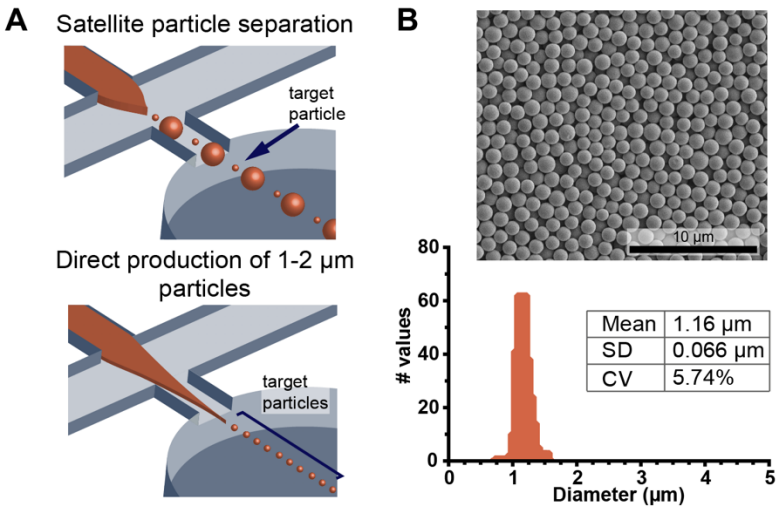

Figure 1. A) Schematic illustration of microfluidic approaches taken for the production of 1-2 µm magnetic PLA microspheres. **B)** Distribution and electron micrograph of the microspheres.

Poster # 37

Genes Regulating Biofilm Formation as Novel Biofilm-specific Drug Targets

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Antimicrobial resistance is continuously increasing worldwide and threatens to reverse the advancements of anti-infective therapies, the keystone of modern medicine. Aggravatingly, antibiotic treatment regularly fails to cure patients suffering from infections caused by adaptively resistant microbial communities, referred to as biofilms. Even though at least two thirds of all clinical infections are associated with biofilms, there are no biofilm-specific therapies on the market or in clinical trials. This project aims to identify the genes regulating biofilm formation in the remarkably antibiotic resistant, nosocomial pathogen *Pseudomonas aeruginosa* to provide novel biofilm-specific targets for the design of potent drugs.

A genome-wide screen using the transposon-sequencing (Tn-Seq) pool of *P. aeruginosa* strain PA14 was performed to identify genes involved in early and late stages of biofilm formation on hydroxy apatite, a substrate present in bone tissue and teeth. Tn-Seq mutants growing under planktonic but not under biofilm conditions will have lost the ability to form biofilms; thus the inactivated genes in these mutants are potential candidates required for biofilm formation. I will discuss regulatory genes involved in biofilm formation that also correspond to the known resistome of antimicrobials.

Random insertion of promiscuous, mariner-based transposons into the genomes of *P. aeruginosa* strains PAO1, PA14 and LESB58 resulted in the generation of Tn-Seq pools that each contained more than 200,000 mutants. In each respective genome ~90% of all genes were successfully mutagenized, as determined by amplification of transposon-genome junctions and subsequent high-throughput sequencing. My *in vitro* Tn-Seq screen of *P. aeruginosa* PA14 in synthetic cystic fibrosis medium identified ~500 genes to be involved in biofilm formation including dozens of regulatory and hypothetical genes.

The *P. aeruginosa* Tn-Seq pools are enabling us to comprehensively address the gene network regulating biofilms and contribute to the functional annotation of the genome.

Poster # 38

Three-dimensional microscopy of administered substances in cleared tissues

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3D optical microscopy of whole organs and animals can provide insight into biological structure and function [1]. 3D microscopy requires tissues to be rendered transparent with solvents and detergents that remove lipids from tissues. Unfortunately, this imaging approach is limited because the chemical treatments required to prepare tissues for 3D microscopy remove uncrosslinked molecules and most injected substances. We have developed a fluorescent peptide tag and conjugation scheme using maleimide-cysteine that allows for labeling of most biochemicals.

Here, we have used a fluorescent peptide tag that crosslinks into tissues that allows small molecules, lipids, polymers and carbohydrates to be imaged in 3D. We used this tag to retain and visualize organic nanoparticles in tumour tissues. This allowed us to distinguish between intact and degraded liposomes by using one tag for the lipids, and another tag for the encapsulated cargo and examining the 3D distribution of each tag. This simple labelling technique opens the door to investigating the transport of administered substances via a wide range of biological processes.

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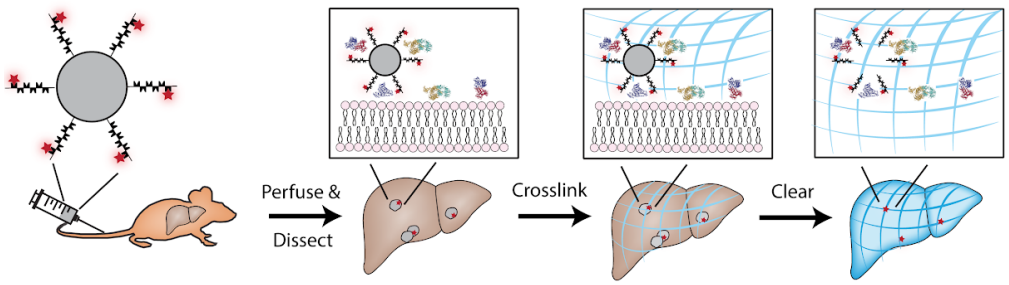

Figure 1: Nanoparticles conjugated with the tag can be injected into an animal and crosslinked along with proteins during tissue clearing. Lipids are removed but crosslinked proteins and tags are retained even when the nanoparticle is removed.

Poster # 39

High Content Imaging of Endocytosed Nanoparticles and F-Actin for a New Perspective in Cytoskeletal Mechanics

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Cellular physiological and disease processes are highly influenced by physics. Mechanics in particular is central to the proper function of cells and tissues[1]. Cells are capable of sensing mechanical forces and respond to them via physical, and chemical changes. For example, brain cells live in an extracellular matrix (ECM) with stiffness less than 1 kPa, whereas the matrix surrounding bone cells is >3000 kPa, while tumor cells can adapt to stiffer ECM, and increased interstitial pressure[2]. Focal adhesions connected to the cytoskeleton are the sensors used by cells to interact with the surrounding matrix and to produce the biochemical responses that change the cell's own mechanical properties in response to the local environment. Cancer cells display markedly different mechanical properties (Young's modulus and contractility) compared to healthy ones that are highly correlated to the mechanics of the cytoskeleton. Tumor cells are more compliant as they have evolved to be able to detach from the tumor site and migrate to a secondary site, where metastasis is formed[3]. Cell migration is an important target in oncology, and one that could be addressed with nanomedicine[4]. The formulation of a comprehensive physics-based model to correctly describe the active behavior of the cytoskeleton and the energetic cost associated with it and how this cost is balanced by biochemical activity (*e.g.* ATP hydrolysis), could, in principle, foster the engineering of more effective nanomedicines targeting the physical mechanisms associated with cell motility and plasticity.

In an effort to understand the connection between mechanical perturbations produced by nanoparticles (NPs), molecular responses, and regulatory changes that influence cell behavior, we studied the effect of peptide coated AuNPs on cytoskeletal organization and cell motility through high content imaging analysis, and real-time cell tracking microscopy[5]. We characterized the actin filament response to the presence of endocytosed NPs for up to three weeks, by quantifying the number and intensity of filaments. On the same cells we quantified morphological descriptors and followed the evolution of those quantities at different time points. The observed parameters describe the effect of NPs on actin polymerization and cell contractility.

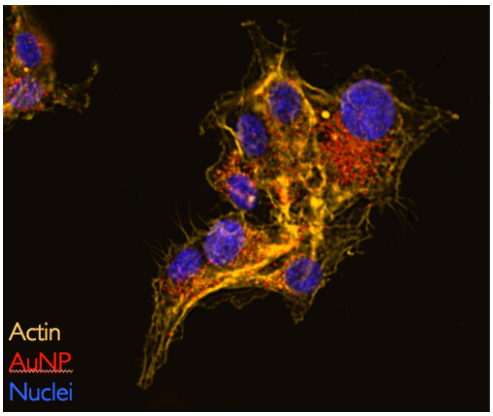

Figure 1 Glioblastoma cells are incubated with cationic NPs and their effects on the cytoskeleton studied by fluorescence microscopy.

The next step is to determine a generalized model describing the above effects. To this end, we will collect real time data to extend a mathematical model, developed by the Bacca group[6], to describe more fully the activity of molecular motors and/or cytosolic forces inside the cell.

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Development of Novel Cell Proliferation Reagent for Mass Cytometry

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Proliferation via clonal expansion is a hallmark of adaptive immunity and carboxylfluorescein diacetate succinimidyl ester (CFSE) is one of the most commonly used tools by immunologists to study this parameter. CFSE is a lipophilic fluorescent dye that works by attaching to intracellular components so that the fluorescent signal, which is measured with a flow cytometer, is only diluted upon cell division. Due to spectral overlap, the number of parameters that can be assessed with flow cytometry is rather limited. The latest innovation in single cell phenotyping is mass cytometry by time of flight (CyTOF), where the fluorescent tags are replaced by isotopically pure non-radioactive metals. Due to minimal spillover between isotope probes, mass cytometry can analyze upwards of 40 parameters with minimal need for compensation. Since CyTOF is a new technique, there are no metallated versions of some common flow cytometry reagents; CFSE is an example.

In this project, lipophilic metal complexes were developed, referred to as CFSE-equivalent reagents for CyTOF (CERCs), which stably incorporate heavy metals into cells. These compounds are able to monitor proliferation by measuring metal dilution within a labeled cell population over time with a mass cytometer. CERCs were synthesized in-house and fully characterized. A straight-forward staining process for the complexes was developed and optimized by measuring metal incorporation and retention into various cell types using ICP-MS, CyTOF and ITLCs. The stability of the staining was further validated *in vivo* using CERC-stained splenocytes injected intravenously into healthy mice followed by serial SPECT/CT imaging [**Figure 1a**]. A live cell imaging platform and MTT assay were used to show that CERCs have no impact on cell proliferation or respiration at the concentrations required for CyTOF. Finally, CERC performance as a proliferation marker was validated *in vitro* using dual CFSE/CERC stained human PBMCs and HEK293 cells and *in vivo* using similarly stained murine splenocytes in an OTii vaccination model; in all cases the fluorescent and heavy metal signals diluted simultaneously over time when the same sample was measured using both a flow and mass cytometer, respectively [**Figure 1b-c**].

CERCs are non-toxic, user-friendly compounds that can be used to measure cell proliferation over time with a mass cytometer. CERCs are a useful addition to the immunologist's toolbox.

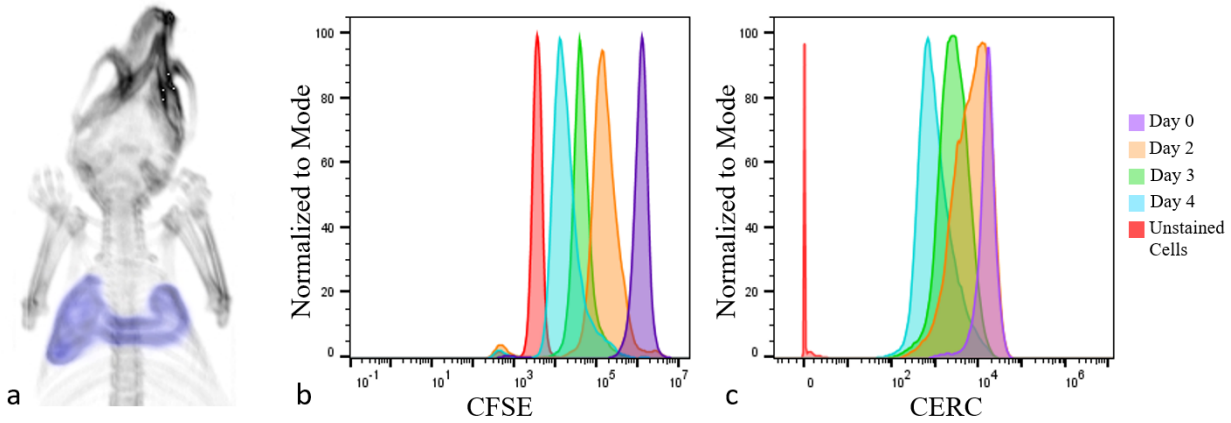

Figure 1: SPECT/CT rendering of CERC-stained splenocytes immediately after intravenous injection (a). Simultaneous dilution of CFSE and CERC in dual stained cells upon proliferation when analyzed by both flow (b) and mass cytometry (c).

Poster # 41

Functional characterization of four novel crocodylian cathelicidin peptides

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Cathelicidins are one of the major classes of host defense peptides in vertebrates and they play an important role in innate immune defense against infections. These peptides often display a broad antimicrobial activity against bacteria, fungi and even viruses. Cathelicidins also have antibiofilm and immunomodulatory activities, which make them attractive candidates for novel therapeutics. Crocodylian reptiles are ancient creatures with a potent but largely unexplored immune system that allows them to survive in microbially challenging environments. In this work, we used bioinformatic approaches to identify novel cathelicidin peptide sequences from the genome of four crocodylian species. According to phylogenetic data and physico-chemical parameters, four cathelicidin sequences were selected and chemically synthesized as linear peptide chains. The synthetic peptides were then tested for their ability to kill Gram-positiveand Gram-negative bacteria strains. Using a bioinformatics approach to screen crocodylian genomes, 18 new crocodylian cathelicidin peptides were identified with a diverse range of peptide length, charge and hydrophobicity. According to phylogenetic data and physico-chemical parameters, four of them were selected for further characterization and they were chemically synthesized to >95% purity. All the synthetic crocodylian cathelicidins displayed antibacterial activity against both Gram-positive (*Staphylococcus aureus* C623) and Gram-negative bacteria (*Pseudomonas aeruginosa* PAO1, *Salmonella enterica* ser. Typhimurium ATCC 14028 and *Escherichia coli* O157:H7) with MIC values ranging from 0.5 to 16 μM. These results demonstrate that crocodylian cathelicidins have direct antibacterial activity towards several pathogenic bacterial species and suggests they may play a role in preventing bacterial infections in crocodylians. In addition, other relevant activities such as antibiofilm activity and immunomodulatory functions of crocodylian peptides were explored to help clarify the role of cathelicidins in crocodylian innate immunity.

Hyperthermia and Radiation Therapy as Modulators of the Accumulation and Efficacy of Nanomedicine-Encapsulated Chemotherapies in Breast Cancer

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Nanomedicines are proven drug delivery vehicles for the treatment of solid cancers. However, their efficacy can sometimes be limited by the low percentage of administered nanomedicines that accumulate within some tumors. This study employs externally applied hyperthermia or external beam radiation therapy in order to modulate the tumor microenvironment and impact the accumulation and efficacy of chemotherapeutics encapsulated within nanomedicine drug delivery vehicles. The ability of hyperthermia to trigger intravascular release of chemotherapeutics from thermosensitive liposomes is also investigated.

Several approaches are employed and various biological parameters measured in order to elucidate differences in accumulation and determine the underlying causes. This study employs a theranostic pair of liposomes to determine *in vivo* fate of the nanoparticles and relate that to efficacy. Furthermore, several thermosensitive nanomedicine formulations were developed. Computed tomography imaging was used to measure perfused volumes within tumors and track nanoparticles at the whole tumour level. A wick-in-needle technique was used to measure interstitial fluid pressure while flow cytometry determined nanoparticle distribution at the cellular level. Drug cytotoxicity was measured using traditional methods. Western blot and polymerase chain reaction assays determined protein expression in response to hyperthermia.

These studies demonstrate that both hyperthermia and radiation therapy are able to modulate interstitial fluid pressure within the tumor, thereby increasing nanomedicine accumulation and improving efficacy. Additional benefits of hyperthermia include increasing blood flow and vascular permeability. We further demonstrate that hyperthermia sensitizes most cancer cells to a variety of chemotherapies by degrading breast cancer type susceptibility (BRCA) proteins and increasing heat shock protein expression. We establish that for several thermosensitive formulations, the application of hyperthermia significantly increases drug concentrations within the tumor and improves efficacy compared to free drug or non-thermosensitive nanomedicines. This body of work reinforces the need for the nanomedicine field to develop appropriate patient stratification metrics as well as a multitude of nanomedicine-based approaches to best treat a diverse patient population.

How physiologically relevant conditions affect mechanisms underlying susceptibility of *Pseudomonas aeruginosa* to antimicrobial agents

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Abstract:
Conventional antibiotic assays do not reflect the biochemical composition found in human infections, and thus are not very predictive of clinical antibacterial activity. For this reason, our research assessed the ability and mechanism of antibiotics and antimicrobial peptides to treat *Pseudomonas aeruginosa* in physiologically relevant conditions compared to standard laboratory conditions. Testing susceptibility in media relevant to the lungs of cystic fibrosis patients or the blood of sepsis patients, five antibiotics showed differential inhibitory and antibiotic:peptide synergistic effects against *P. aeruginosa* when compared to standard laboratory media. To unravel the potential causes for this altered susceptibility, RNA-Seq was used to explore changes in gene expression of *P. aeruginosa* grown or treated with azithromycin (AZM) in host mimicking conditions compared to standard media. Changes in expression of more than 60 resistome genes, genes involved in stringent responses, and genes associated with cell surface modifications was observed. Particularly, dysregulation of the *phoPQ* two component system in physiologically relevant media has been indicated to have an important effect on AZM susceptibility in these conditions. Dysregulation of these genes may disrupt Lipopolysaccharide modification and prevent *Pseudomonas* from blocking antibiotic uptake in host conditions. A TnSeq library was constructed in *P. aeruginosa* to explore essential genes for growth and antibiotic susceptibility in these host-like conditions. Preliminary analysis has identified 96 putative genes specifically essential for AZM treatment in host conditions, many of which were also dysregulated in these conditions compared to MHB. This technique in combination with RNA-Seq analysis, will give a more complete picture of the underlying changes important for antimicrobial effectiveness against *Pseudomonas aeruginosa* in the host. This research is important for understanding the mechanisms that underlie bacterial susceptibility to antimicrobials in clinically relevant conditions and could help to change standard approaches to drug testing and lead to more predictive drug discovery routes.

Poster # 44

Arriving to Modeling Frameworks For Assessing Potential Nanoparticle Cytotoxicity Risks

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Introduction – The complexity and the heterogeneousity of available data on potential risks of nanoparticles, in addition to interdependency of relevant influential attributes make it challenging to arrive at a generalization of nanoparticle toxicity behaviour. Lack of systematic approaches to investigate these risks further adds uncertainties and variabilities to the body of literature and limits generalization of proven evidences. Arriving to predictive modeling frameworks of potential cytotoxicity of engineered nanoparticles is critical in environmental and health risk analysis.

Methodology – We used decision trees together with feature selection algorithm (Gain ratio) to analyze a set of published nanoparticle cytotoxicity samples (2896 samples) which specified nanoparticle-, cell- and screening method-related attributes.

Results and Conclusion – Among several influential attributes, we show that cytotoxicity of nanoparticles is primarily predicted from the nanoparticle chemistry. Importantly, cytotoxicity screening indicator was found as an important determinant of the viability results. Representative branches of a decision tree developed in our study is presented in Figure 1. Our study indicates that following rigorous and transparent methodological approaches, in parallel to continuous addition to this dataset developed using our approach will offer higher prediction power and accuracy and reveal further hidden relationships. Results obtained in this study helps focus future studies to help in the development of nanoparticles that are safe-by-design.

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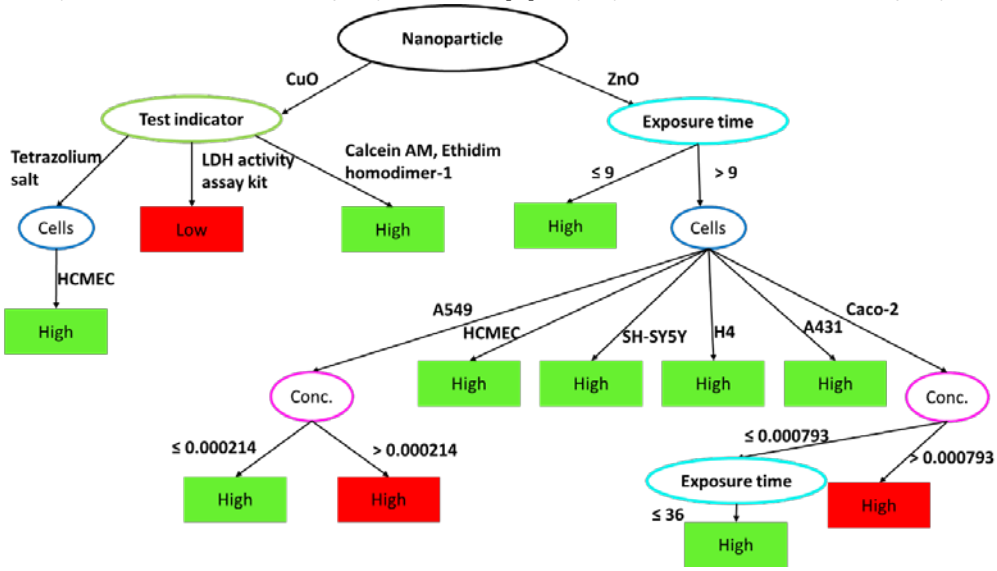


Figure 1: Representative branches of a decision tree learnt from 9 features nanoparticle type, nanoparticle diameter, nanoparticle concentration, cells, cell age, cell source, cell type, exposure time to nanoparticles and test indicator used to determine cell viability). The label "Low" in red stands for low cell viability (for <=50%), i.e. high cell toxicity. The label "High" in green indicates a high cell viability (for > 50%), i.e. low cell toxicity.

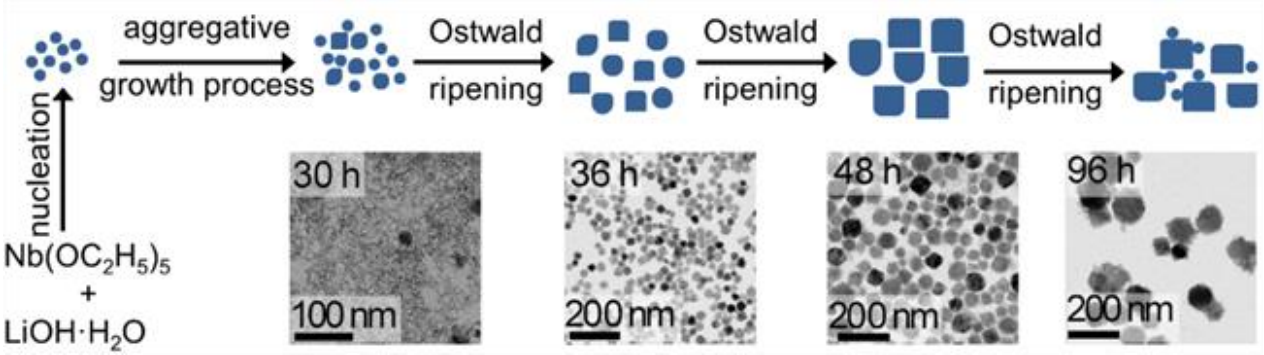
Poster # 45

Size-Controlled Synthesis of Second Harmonic Active Lithium Niobate Nanocrystals through Solution-Phase Methods

Rana Faryad Ali, Byron D. Gates*

Affiliations: Simon Fraser University, Department of Chemistry, 8888 University Drive, Burnaby, BC V5A 1S6, *contact: bgates@sfu.ca

We advanced the development of solution-phase approaches for the preparation of lithium niobate ($LiNbO_3$) nanoparticles with an average, tunable size from 30 to 100 nm. This solution-phase process results in the formation of crystalline, uniform nanoparticles of $LiNbO_3$ at a reaction temperature of 220 °C with an optimal reaction time of as short as 30 h. Advantages of these methods include the preparation of single-crystalline $LiNbO_3$ nanoparticles without the need for further heat treatment or without the need for using an inert reaction atmosphere. The growth of these nanoparticles began with a controlled agglomeration of nuclei formed during a solvolysis step. The reactions subsequently underwent the processes of condensation, aggregation, and Ostwald ripening, which remained the dominant process during further growth of the nanoparticles. These processes did produce single-crystalline nanoparticles of $LiNbO_3$, suggesting an oriented attachment process. Average dimensions of the nanoparticles were tuned from 30 to ~100 nm by either increasing the reaction time or changing the concentration of the lithium salts used in the solvothermal process. The nanoparticles were also confirmed to be optically active for second harmonic generation (SHG). These particles could enable further development of SHG based microscopy techniques.



Tunable Functionalization of Silica Coated Iron Oxide Nanoparticles Achieved through a Silanol–Alcohol Condensation Reaction

Henry Kang, Melissa Radford, and Byron D. Gates*

Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6

Self-assembled structures prepared from colloidal nanomaterials can lead to a range of new properties or the creation of materials that simultaneously exhibit multiple properties. One of the materials that has benefited from this approach to preparing new nanoscale materials has been the formation of core-shell type structures. Specifically the integration of magnetic and plasmonic properties into a single particle has been one such platform that has been sought for a range of applications, including the ability to manipulate these materials under applied magnetic fields while also tuning their optical properties. Through the research presented here we introduce a new approach to preparing these types of assemblies through using the alcohol condensation reaction to tune the surface chemistry of the core material. The core, iron oxide nanoparticle was coated with a thin shell of silica. Our method utilized the alcohol condensation reaction to adjust the chemistry of these surfaces, such as to derive thiol functionalized surfaces. This approach is in contrast to the use of (3-mercaptopropyl)trimethoxysilane, which has been widely used for obtaining a similar functionality on the surfaces of silica particles. The utilization of alcohol based reagents decreases the stringency under which the reagents must be handled, while also increasing the diversity of functionality that can be readily obtained from widely available reagents. Additional surface chemistries were also demonstrated through the alcohol condensation reaction, achieving surfaces functionalized with carboxylic acids or alcohols. The thiol functionality was, however, the primary focus of this study as a demonstration of an alternative approach to preparing core-shell particles of gold nanoparticles assembled onto the silica capped iron oxide cores. The desired assemblies were prepared with a tuneable coverage of gold nanoparticles. The final assemblies were verified using a range of electron microscopy techniques. Transmission electron microscopy (TEM) techniques were utilized to verify the composition, structure, and morphology of the materials within these assemblies. For example, energy dispersive X-ray spectroscopy (EDS) coupled with TEM system was utilized to distinguish the different compositions of the species within these assemblies. Surface coverage of gold nanoparticle within these assemblies was also verified through a series of tomographic analyses. The 3D, reconstructed images of these assemblies demonstrate the density of the gold nanoparticles within the assemblies. Electron microscopy analyses were also utilized for evaluating the outcome of a range of control experiments used to provide evidence for the specificity and success of this approach to preparing complex assemblies, as well as to tune the conditions used in their preparation. This new approach to tuning the surface chemistry of nanoparticles could be easily extended to other chemistries by adjusting the alcohol-based reagents used in the condensation reaction.

Poster # 47

A Mechanically Driven Magnetic Particle Imaging (MPI) & Phase-Weighting Implementation

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[1]Department of Physics, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada

Magnetic particle imaging (MPI) [1] is a tracer-based medical imaging modality in which distributions of superparamagnetic nanoparticles (SPNs) are inferred from their response to time-varying and spatially-inhomogeneous magnetic fields. It provides fast and sensitive mapping of particle distributions, and is expected to yield sub-millimeter resolution. We describe and demonstrate an approach to magnetic particle imaging in which particle excitation and field free point (FFP) manipulation are decoupled from one another [2]. The prototype instrument we describe uses rotating arrays of permanent magnets to scan the FFP through the field of view, and current-driven oscillating magnetic fields to elicit non-linear magnetization responses from superparamagnetic nanoparticles. The additional degrees of freedom enabled by this decoupling suggest new strategies for studying and exploiting contrast mechanisms, optimizing image quality and resolution, and device-size scaling. Narrow-band phase sensitive detection of these responses at one or more harmonics of the excitation field provides a rich source of information from which images can be reconstructed. Images generated from data acquired using this instrument are presented, demonstrating native resolutions of order one millimetre if the magnitude of the detected signal is employed (second panel from right in **Figure 1**). The resolution of these images can be substantially improved at the expense of signal-to-noise ratio (SNR) using 'phase-weighting' or 'relaxation-weighting' protocol [3]. MP phase-weighted images with resolutions of order a few hundred microns are obtained and presented (third panel from right in **Figure 1**).

[1] B Gleich et al. (2005) Nature **435** 1214; [2] H Bagheri et al. (2018) APL **113** 183703 ; [3] H Bagheri et al. (2019) JMMM, Under Revision

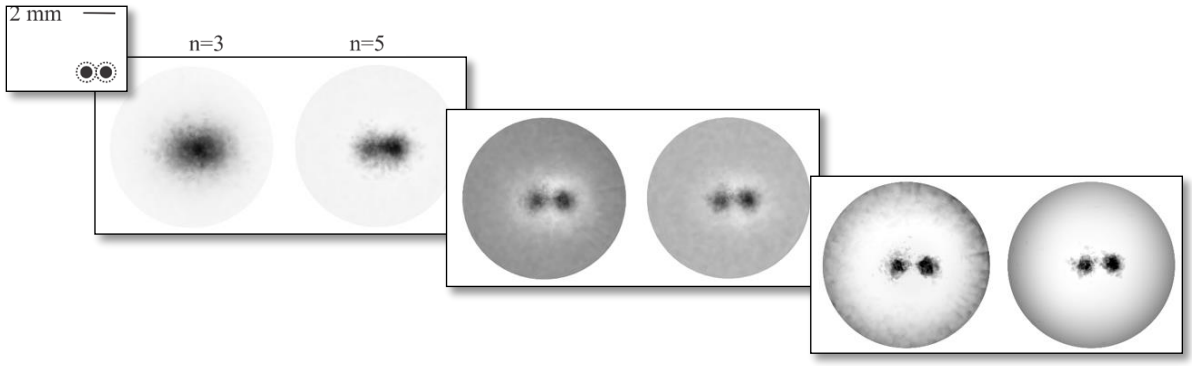

Figure 1: From right to left: phantom describing the spatial distributions of SPNs, MPI images acquired using the magnitude of the third (n=3) and fifth (n=5) harmonics, phase-weighted images acquired using the complex response of the third and fifth harmonics, high-pass filtered phase-weighted MP images acquired using the complex response of the third and fifth harmonics [3].

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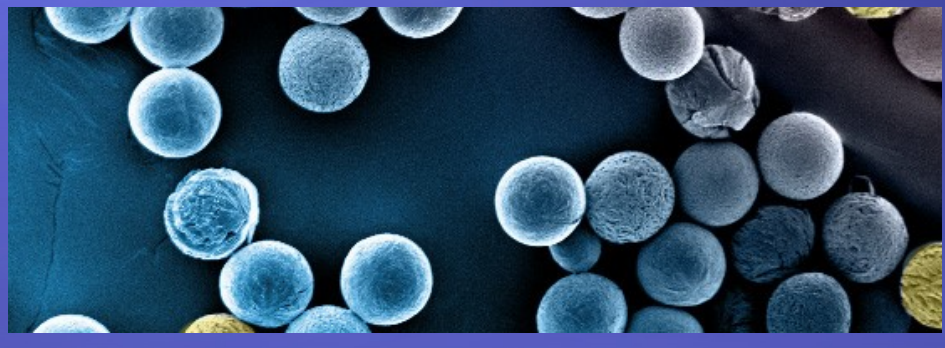

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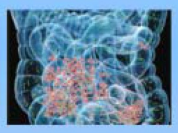

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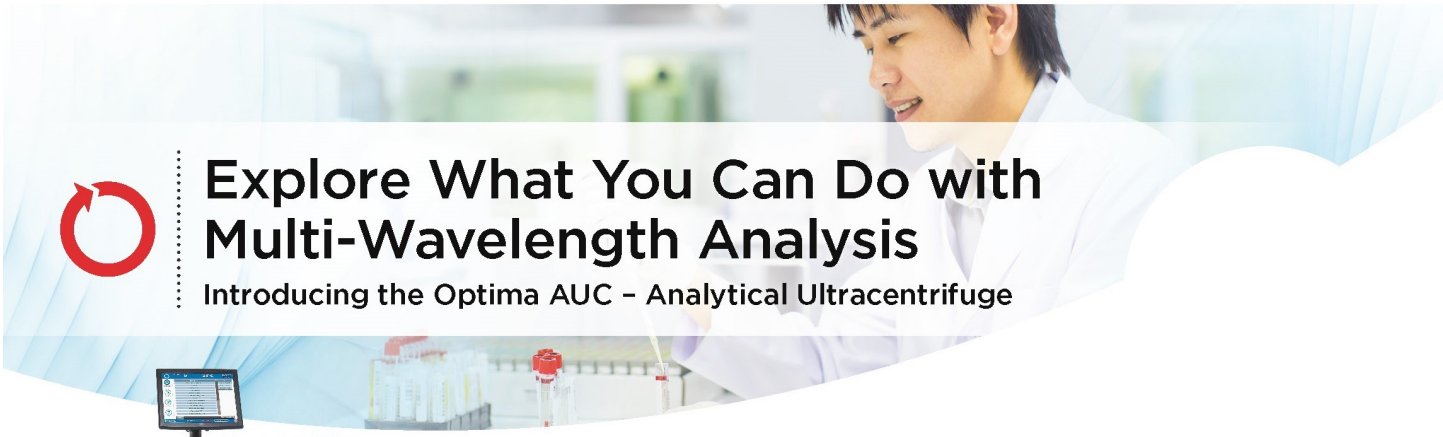

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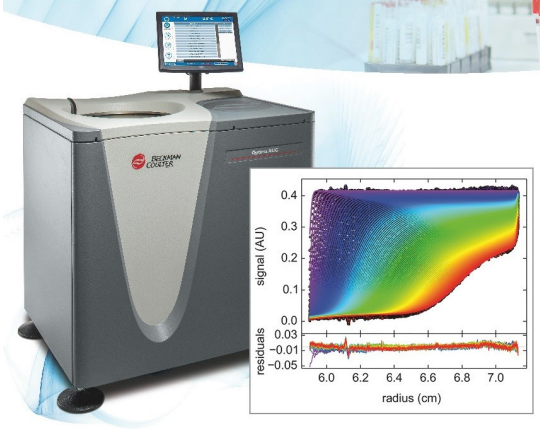

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