**MICROFABRICATION OF ELASTOMERIC POLYMERS FOR ORGAN-ON-A-CHIP ENGINEERING AND INJECTABLE TISSUES**

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Key Words: heart, liver, soft lithography, elastomer, minimally invasive delivery, stem cell, cardiomyocyte, hepatocyte

Recent advances in human pluripotent stem cell (hPSC) biology enable derivation of essentially any cell type in the human body, and development of three-dimensional (3D) tissue models for drug discovery, safety testing, disease modelling and regenerative medicine applications. However, limitations related to cell maturation, vascularization, cellular fidelity and inter-organ communication still remain. Relying on an engineering approach, microfluidics and microfabrication techniques our laboratory has developed new technologies aimed at overcoming them.

Since native heart tissue is unable to regenerate after injury, induced pluripotent stem cells (iPSC) represent a promising source for human cardiomyocytes. Here, biological wire (Biowire) technology will be described, developed to specifically enhance maturation levels of hPSC based cardiac tissues, by controlling tissue geometry and electrical field stimulation regime (Nunes et al Nature Methods 2013). We will describe new applications of the Biowire technology in engineering a specifically atrial and specifically ventricular cardiac tissues, safety testing of small molecule kinase inhibitors, potential new cancer drugs, and modelling of left ventricular hypertrophy using patient derived cells.

For probing of more complex physiological questions, dependent on the flow of culture media or blood, incorporation of vasculature is required, most commonly performed in organ-on-a-chip devices. Current organ-on-a-chip devices are limited by the presence of non-physiological materials such as glass and drug-absorbing PDMS as well as the necessity for specialized equipment such as vacuum lines and fluid pumps that inherently limit their throughput. An overview of two new technologies, AngioChip (Zhang et al Nature Materials 2016) and inVADE (Lai et al Advanced Functional Materials 2017) will be presented, that overcome the noted limitations and enable engineering of vascularized liver, vascularized heart tissues and studies of cancer metastasis. These platforms enable facile operation and imaging in a set-up resembling a 96-well plate. Using polymer engineering, we were able to marry two seemingly opposing criteria in these platforms, permeability and mechanical stability, to engineer vasculature suitable for biological discovery and direct surgical anastomosis to the host vasculature.

Finally, to enable minimally invasive delivery of engineered tissues into the body, a new shape-memory scaffold was developed that enables delivery of fully functional tissues on the heart, liver and aorta through a keyhole surgery (Montgomery et al Nature Materials 2017).

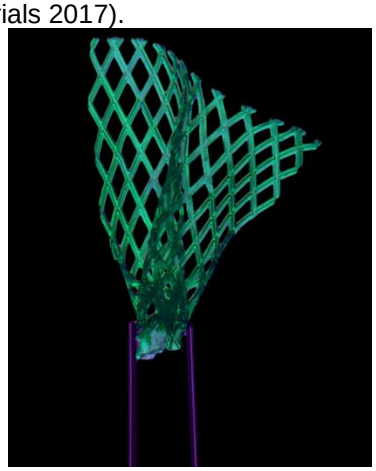

*Figure 1 . Shape memory scaffold for minimally invasive delivery of functional tissues*

## GENETICALLY ENCODED POLYMERS FOR DRUG DELIVERY

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I will highlight two technologies developed in my laboratory for the delivery of diverse drugs by genetically encoded polymer drug carriers. First, I will describe a new drug delivery system that we have developed for small molecule cancer chemotherapeutics. This methodology —attachment-triggered self-assembly of recombinant peptide polymers— can package small molecule cancer drugs with a range of hydrophobicity into soluble nanoparticles of a recombinant peptide polymer. These nanoparticles increase the solubility, plasma half-life, and tumor accumulation of the drug, which translates to improved efficacy of the nanoparticle formulation as compared to free drug. Examples of encapsulating doxorubicin, paclitaxel, and gemcitabine — three drugs with vastly different structures and physico-chemical properties— will be presented to illustrate the versatility of this new technology for drug delivery. I will also discuss an injectable delivery system based on thermally sensitive polypeptides for the sustained and tunable release of peptide drugs from a subcutaneous injection site that we have developed for treatment of type 2 diabetes.

**IMMOBILIZATION OF BIOLOGIC PHOTOSENSITIZER CONJUGATES ON NANOPARTICLES TO ENHANCE PHOTOIMMUNOTHERAPY EFFICACY**

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Light-activatable immunoconjugates have recently shown promise for photoimmunotherapy and fluorescence-guided resection in patients suffering from incurable malignancies in early clinical trials. While possessing a number of unique advantages, photoimmunotherapy and fluorescence imaging for oncological diseases can be hampered by therapeutic inefficiency resulting from inadequate photosensitizer delivery. The study suggests that successful coupling of antibody-photosensitizer photoimmunoconjugates onto polymeric nanoparticles complements the promising attributes of simple photoimmunoconjugates in two significant ways: Not only does it improve photosensitizer delivery to tumor, but also offers a forward-looking opportunity to deliver significant and diverse second agents, which can be an imaging agent or a different therapeutic agent, to further enhance the theranostic benefits of photoimmunoconjugates. This approach, based on nanoparticle engineering, achieves effective photoimmunoconjugate delivery and enhances the anti-tumor efficacy in two EGFR-overexpressing cancer cell lines *in vitro* and in a xenograft tumor mouse model. Furthermore, the selectivity, photochemical and photophysical characteristics (e.g. absorbance, fluorescence quenching, and singlet oxygen yield) of the photoimmunoconjugated nanoplatform were thoroughly investigated. This next generation photoimmunoconjugate–nanoparticle delivery approach offers a unique opportunity to monitor disease, destroy cancer cells and co-deliver a follow-up treatment more efficiently, and thus merits further investigations in preclinical and clinical settings.

**PHOTOTHERMAL THERAPY GENERATES A THERMAL WINDOW OF IMMUNOGENIC CELL DEATH IN NEUROBLASTOMA**

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Key Words: Photothermal therapy, immunogenic cell death, thermal dose window, Prussian blue nanoparticles, antitumor immune response

Nanoparticle-based photothermal therapy (PTT) has been widely investigated in cancer therapy as a rapid and minimally invasive tumor ablation technique. Over the past two decades, several reports utilizing nanoparticles for PTT of diverse tumor types *in vitro* and *in vivo* have been described. An emerging area of interest is the effect of PTT on the immune system during tumor therapy, since PTT not only causes tumor cell death, but can also release tumor antigens and endogenous adjuvants (e.g. heat shock proteins, damage-associated molecular patterns (DAMPs)) under certain conditions. These effects have the potential to increase tumor immunogenicity, which can trigger improved therapeutic responses. Engaging the immune system during PTT is important as it offers the potential for persistent treatment responses and immunological memory.
Here, we describe a thermal "window" of immunogenic cell death (ICD) elicited by nanoparticle-based photothermal therapy (PTT) in an animal model of neuroblastoma. ICD is a highly favorable cell death phenotype that initiates an adaptive immune response and is associated with improved therapeutic outcomes in cancer.[1] In studies using Prussian blue nanoparticles to administer photothermal therapy (PBNP-PTT) to established localized tumors in the neuroblastoma model, we observed that PBNP-PTT conformed to the "more is better" paradigm, wherein higher doses of PBNP-PTT generated higher cell/local heating and thereby more cell death, and consequently improved animal survival. However, *in vitro* analysis of the biochemical correlates of ICD elicited by PBNP-PTT, namely ATP and high motility group box 1 protein (HMGB1) release and increased surface calreticulin expression, demonstrated that PBNP-PTT triggered a thermal window of ICD. Specifically, the aforementioned markers of ICD were highly expressed within an optimal temperature (thermal dose) window of PBNP-PTT (63.3-66.4 °C) as compared with higher (83.0-83.5 °C) and lower PBNP-PTT (50.7-52.7 °C) temperatures, which both yielded lower expression of ICD markers (Fig. 1A). Subsequent vaccination studies in the neuroblastoma model confirmed our *in vitro* findings wherein PBNP-PTT administered within the optimal temperature window (63.3-66.4 °C) resulted in long-term survival (33.3% at 100 days) compared with PBNP-PTT administered within the higher (0%) and lower (20%) temperature ranges, and controls (0%) (Fig. 1B). Our findings demonstrate a tunable immune response to heat generated by PBNP-PTT, which should be critically engaged in the administration of PTT, both alone and when PTT is administered in combination with immune adjuvants (e.g. TLR agonists) and/or immunotherapies (e.g. immune checkpoint inhibitors) for maximizing its therapeutic benefits.

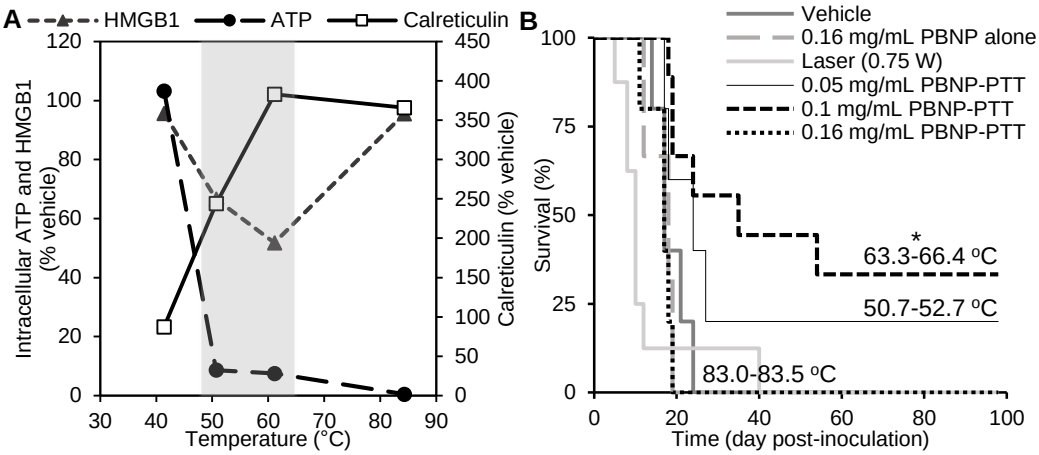


**Figure 1. PBNP-PTT exhibits a thermal window of immunogenic cell death.** A) Expression of ICD markers (HMGB1, ATP, and Calreticulin) showing increased expression between 48-65 °C (gray band) in vitro. B) Long-term survival in mice vaccinated with Neuro2a cells treated with different thermal doses of PBNP-PTT and subsequently challenged with Neuro2a cells exhibiting maximum long-term survival for animals vaccinated with PBNP-PTT-treated cells at ~65 °C compared with animals in the lower (~51 °C) and higher (~83 °C) temperature groups; * indicates $p<0.05$ as determined by a log-rank test.

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# SYNTHETIC CELLS SYNTHESIZE THERAPEUTIC PROTEINS INSIDE TUMORS

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Key Words: nanotechnology, personalized medicine, protein drugs, protocells, synthetic cells.

The existing dogma is that protein medicines need to be produced in large factories, and then injected to the patient. We propose that miniature artificial inert factories can be injected to the patient, to produce a protein of interest directly in the diseased tissue. We engineered artificial cell-like particles with an autonomous capacity to synthesize protein drugs after receiving an external signal. The protein is tuned to the patient's needs based on a predetermined DNA code we incorporate inside the particles. This approach increases treatment efficiency and reduces adverse effects to healthy tissues.
We developed a new T7-S30 based cell-free protein synthesis system, which contains all the transcription and translation machines and molecules required for protein production (Krinsky *et al*., *PloS one* 2016). This system was used to prepare liposomes that act as artificial cells, capable of producing proteins autonomously in response to a physical trigger. Functional enzymes (luciferase and tyrosinase) and fluorescent proteins (GFP) were successfully produced using the new cell-free protein synthesis system and inside the particles both *in vitro* and *in vivo*. In addition, we demonstrated the therapeutic capabilities of the protein producing particles by producing *Pseudomonas* exotoxin A, an extremely potent protein, for treating cancer. Applying the particles on 4T1 cells (a triple-negative breast cancer cell-line) *in vitro* or injecting them into a 4T1-induced tumor *in vivo*, resulted in high cytotoxicity due to the effective production of the therapeutic protein inside the vesicles (Krinsky *et al*. *Advanced Healthcare Materials,* 2017).
Synthetic cells serve as autonomous, trigger-able, artificial particles that produces a variety of proteins. This platform has promise to address a wide range of fundamental questions associated with protein synthesis in nature, as well as applicative protein delivery needs.

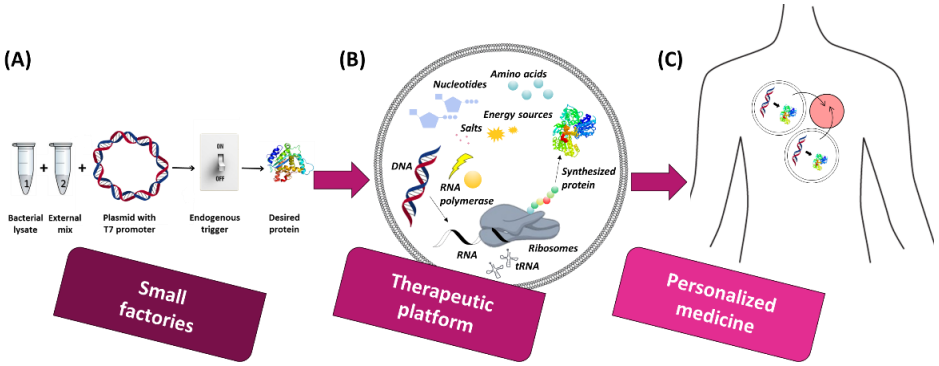


*Figure 1 - the therapeutic platform. Onsite synthesis of protein medicines inside the body is achieved by encapsulation of all the transcription and translation factors required for protein production inside a liposome. (A) Cell-free protein synthesis (CFPS) work flow. (B) Protein producing particle. (C) Personalized medicine; triggered protein production inside the patient's body at the diseased tissue.*

**SOFT TISSUE APPROXIMATION AND REPAIR USING LASER-ACTIVATED NANOMATERIALS**

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Keywords: laser sealing, tissue welding, wound healing, surgical devices

Approximation and repair of soft tissues is conventionally accomplished using sutures and staples, which are inherently traumatic. Laser-activated tissue sealing is an alternate strategy that facilitates rapid, fluid-tight approximation of ruptured tissues, but the lack of effective materials compromises efficacy. I will discuss our results on the generation, characterization and evaluation of laser-activated nanomaterials in which, gold nanorods (GNRs) are embedded within polypeptide matrices. Irradiation of these nanomaterials with near infrared (NIR) light facilitated a photothermal response, which, in turn, resulted in rapid, fluid-tight sealing and repair of soft tissues both ex vivo and in live animals. Gold nanorods were also incorporated in polypeptide fibers, resulting in the formation of laser-activated sutures which combine the advantages of conventional clinical practice (suturing) with photothermal sealing in a single surgical device. Recent studies indicate that delivery of bioactive compounds in concert with photothermal sealing using nanomaterials facilitate the acceleration of dermal wound healing and repair in live mice. Our results demonstrate that laser-activated nanomaterials can augment and / or replace sutures in several applications including soft tissue trauma, wound healing and grafts.

# NEW PHYSICAL AND CHEMICAL APPROACHES FOR THE CYTOSOLIC DELIVERY OF BIO-THERAPEUTICS AND NANOPARTICLES INTO CELLS

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Delivery of bio-therapeutics and nanomaterials into living cells is an important step not only for cell studies but also for therapy and bio-imaging. Clear examples are the intracellular delivery of various classes of nucleic acids (siRNA, µRNA, mRNA, pDNA), peptides and proteins for therapy purposes. As another example, all types of (inorganic/organic) nanoparticles are under investigation as intracellular labels for imaging purposes. Meanwhile it generally accepted that after uptake by cells, nanomaterials typically end up in endo-lysosomal vesicles in which they remain entrapped while they should escape from such compartments and arrive in the cytosolic fluids of the cells. In recent years our team undertook major efforts to understand the biophysics which play a role in (a lack of) escape of nanomaterials from endo-lysosomal vesicles. Vere recently we also discovered new chemical strategies (so named 'escape adjuvants') (1) which seems promising to 'liberate' nucleic acids (like siRNA) from endo-lysosomal vesicles into the cytosol. Furthermore we explored physical methods (either light (2,3) or ultrasound (4) driven) which directly deliver bio-therapeutics into the cytosol, thereby bypassing the endo-lysosomal routes. This lecture will explain our recent findings in this area, as reported in a serious of recently published papers (1-4). Both pharmaceutical, biological and engineering aspects of our work will be highlighted in the lecture.

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Biomaterials 2016, 83: 294-307

**HISTIDYLATED NANOVECTORS FOR MRNA VACCINE FORMULATION: INDUCTION OF A STRONG ANTI-TUMOR T CELL IMMUNITY COMBINED WITH INFLAMMATORY STATE.**

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Key Words: Cancer vaccination; N1-methylpseudo-uridine modified mRNA; Inflammation; Lipopolyplexes,

These last years, we are witnessing the emergence of new class of biopharmaceuticals based-on transcribed mRNA. They emerged as an extremely tunable vaccination platform. Formulations made of mRNA and liposomes (lipoplexes) have yielded strong T cell responses, but require induction of cytokines identical to those that have plagued clinical development of siRNA therapeutics. We have developed histidylated Lipid Polymer mRNA nanocomplexes (LPR) that combine the beneficial properties of lipid based and polymer based nanoparticles, including lowered cellular toxicities and improved colloidal stabilities. Immunization with LPR instigated extremely potent T-cell responses and showed superior effectiveness in controlling tumor growth compared to intravenous immunization with antigen mRNA electroporated dendrictic cells. Early innate responses to LPR were characterized by a type I IFN signature in the spleen. Nonetheless, conversely to LR, LPR did not depend on type I IFN responses to generate cytolytic effectors. This unique behavior of LPR enabled the generation of a less pro-inflammatory yet equally potent systemic LPR vaccine by usage of N1-methylpseudo-uridine (N1mψ) modified mRNA required to improve mRNA translatability by avoiding mRNA sensors activation. Overall, our data indicate that LPR can combine excellent immunogenicity with improved inflammatory and they could be an interesting alternative to formulations that are currently explored in early phase clinical trials..

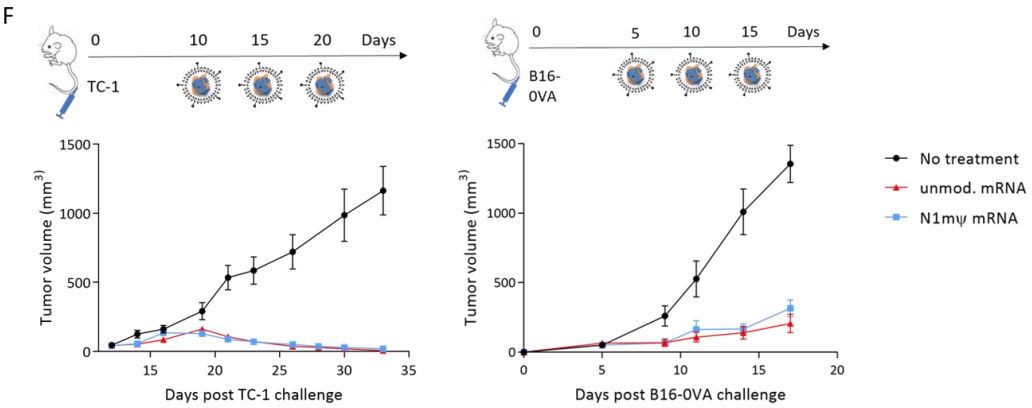


*Figure 2 – N1mψ modified mRNA does not hamper antitumor T cell immunity to LPR.* Tumor growth curves of TC-1 and *B16-OVA inoculated mice either left untreated or treated by IV immunization with respectively unmodified mRNA LPR or N1mψ modified mRNA LPR. TC-1 mice received three immunizations with E7/ mRNA LPR. B16-OVA mice received three immunizations with OVA/TriMix mRNA LPR.*

**POLYMER-NANOPARTICLE INTERACTIONS IN SUPRAMOLECULAR HYDROGELS: ENABLING LONG-TERM ANTIBODY DELIVERY**

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Key Words: polymer-nanoparticle interactions, antibody stability, controlled delivery, hydrogels

Antibody drugs are a rapidly growing set of therapeutics that increasingly prove effective for clinical applications spanning from macular degeneration treatments, to targeted cancer therapies, and to passive immunization. These antibody treatments can be engineered to target almost any cell surface moiety and their production has since been scaled to an industrial level. Despite these advances, parenteral administration of antibodies is severely constrained by high viscosities at desirable doses, poor long-term antibody stability, high required frequency of administration, and therapeutically suboptimal pharmacokinetics. Herein, we demonstrate the development of supramolecular polymer-nanoparticle (PNP) interactions between poly(ethylene glycol)-poly(lactic acid) block copolymer nanoparticles (PEG-PLA) and modified hydroxypropylmethylcellulose (HPMC-x) polymers to engineer shear-thinning, self-healing hydrogels capable of stabilizing and delivering high concentrations of antibodies over prolonged timeframes (Figure 1). The PNP interactions underpinning the behavior of these materials afford injectability and tunable mechanical properties, while also controlling antibody release kinetics. In this work, we investigate how the thermodynamics of the PNP interaction affect *in vitro* and *in vivo* antibody release kinetics, pharmacokinetics, and bioavailability. Analysis of PEG-PLA surface density, HPMC-x hydrophobicity and modification extent, and hydrogel formulation reveal explicit design handles relating PNP thermodynamics to *in vivo* antibody release kinetics via subcutaneous injection. Differences in antibody release kinetics between *in vitro* and *in vivo* experiments were examined through mathematical modelling, revealing possible mechanisms of antibody uptake from subcutaneous space to the bloodstream when compared to literature. Overall, this work presents a robust set of design parameters to tune PNP interactions to develop a new nanotechnology-based platform for long-term, controlled antibody delivery.

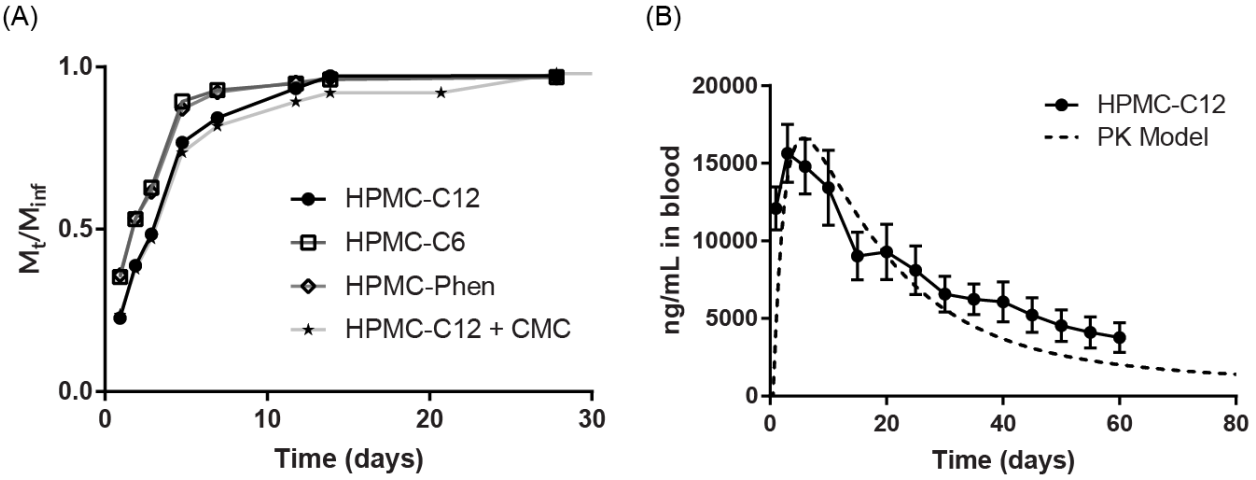


*Figure 1 – (A) In vitro experiments characterizing the delivery of a model drug, which exhibits distinct release profiles depending on functional group attached to HPMC. These functional groups exhibited varying interaction strengths with PEG-PLA nanoparticles and formed gels with different mechanical and antibody release properties. (B) Serum concentration of anti-OVA antibodies delivered from a hydrogel formulation after subcutaneous injection into C57BL6 mice. The dotted line represents a pharmacokinetic (PK) model that accounts for kinetics of antibody release from the hydrogel, uptake into the bloodstream via subcutaneous space, and decay from the bloodstream.*

**NANOMEDICINES FOR THE TREATMENT OF AUTOIMMUNE INFLAMMATION: ENGINEERING DESIGN, MECHANISMS AND DISEASES**

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The complexity of autoimmune diseases is a barrier to the design of strategies that can blunt autoimmunity without impairing general immunity. We have shown that systemic delivery of nanoparticles (NPs) coated with autoimmune disease-relevant peptide-major-histocompatibility-complex (pMHC) molecules triggers the formation and profound expansion of antigen-specific T-regulatory T-cells in different mouse models, including mice humanized with lymphocytes from patients, leading to resolution of a broad range of established autoimmune phenomena. I will highlight the engineering principles impacting biological activity, will illustrate how these nanomedicines interact with cognate T-cells and will describe the pharmacokinetic behavior and toxicological profile of this novel class of drugs, potentially useful for treating a broad spectrum of autoimmune conditions in a disease-specific manner.

**TOLERANCE INDUCTION WITH QUANTUM DOTS DISPLAYING TUNABLE DENSITIES OF SELF-ANTIGEN**

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Key Words: autoimmunity, tolerance, quantum dots, immunotherapy, theranostics

During autoimmune diseases like type 1 diabetes or multiple sclerosis (MS), the immune system mistakenly recognizes and attacks healthy tissues in the body. In MS, myelin, which surrounds and protects the axons of neurons, is attacked by inflammatory cells leading to neurodegeneration. The current standard of care for MS patients is regular injection of immunosuppressive drugs that non-specifically suppress immune function, leaving patients immunocompromised and open to opportunistic infection. New investigations aim to address this problem with immunotherapy-based strategies that promote myelin-specific tolerance. Recent reports reveal that the development of inflammation or tolerance against certain molecules is influenced by the concentration and form of self-antigen presented to immune cells (i.e. free, particle). Strategies that allow tunable delivery of self-antigen are therefore of great interest to further probe these connections. Quantum dots (QDs) were chosen as the nanomaterial to investigate these questions because they can be conjugated with a large and controllable number of biomolecules. Additionally, their size facilitates rapid drainage through lymphatics to lymph nodes (LNs), where they accumulate and can be visualized by deep-tissue imaging due to their intrinsic fluorescence. QDs could be decorated with up to 130 myelin oligodendrocyte glycoprotein (MOG) peptides, a known self-antigen of MS (**Fig 1A**). In a mouse model of MS (EAE), we treated groups with two different doses of self-antigen conjugated to QDs. Treatment resulted in a significant reduction in paralysis that was dependent on MOG dose. At a time point just before EAE symptoms arise (day 9), it was discovered that the draining inguinal LN in MOG-QD treated mice had a significantly higher number of regulatory T cells ($T_{REGS}$) than those of untreated mice (**Fig 1B**). This response was also dose dependent, as mice receiving a higher dose of self-antigen on QDs (52:1) had more $T_{REGS}$ than those injected with a lower dose (17:1). This cell population can induce tolerance to self-antigens by controlling inflammatory cells. We next tested if the level of tolerance induced was dependent on peptide dose alone or if ligand density had an effect. We treated groups with the same dose of MOG spread out over different amounts of QDs and found a trend of decreasing disease severity with decreasing ligand density (**Fig 1C**). This result inspired us to further investigate the trafficking of peptide decorated QDs and the effect that these nanomaterials have on the LNs they accumulate in. Initial studies indicate that we can track the biodistribution of MOG-QDs in naïve mice over time by flow cytometry. Strong QD signal is visible just one day post injection (*p.i.*), and peaks in the draining left inguinal LN on day 3 (**Fig 1D**). While this signal then wanes over the next 4 days, it sustains at a high level in the left axillary LN up to 1 week after injection. Interestingly, a much lower level of QD signal is seen in the right inguinal (non-draining) LN. We also quantified changes in cell populations over time following MOG-QD injection and discovered a spike in macrophages in the draining inguinal LN on day 3 *p.i.*, corresponding with peak MOG-QD signal. This is especially interesting because macrophages can engulf and present antigens to naïve T cells, which then become polarized towards regulatory or inflammatory phenotypes. Another antigen-presenting cell type, $CD11b^+$ dendritic cells, saw a steady increase over the one week study. Interestingly, a sustained drop in T cell frequency and increase in B cell frequency was seen in the draining LN beginning 3 days after injection. In future experiments, we will use this tool to investigate how antigen density and sequence affect biodistribution and immune functionality in both healthy and diseased mice. We also plan to exploit the theranostic capabilities of QDs by performing live, *in vivo* imaging experiments.

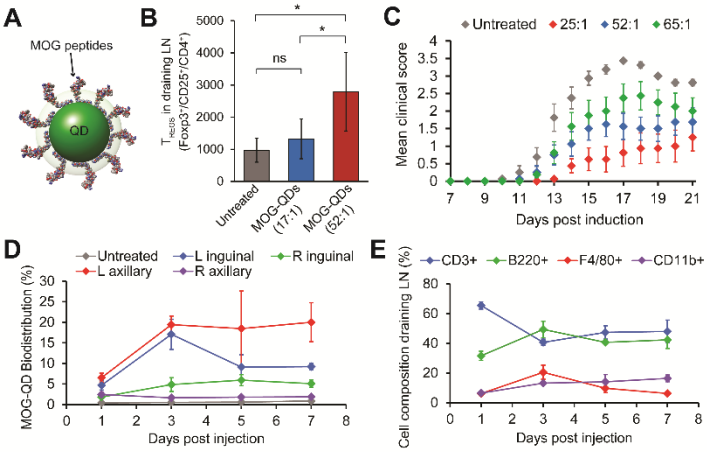


*Figure 3 – MOG-decorated QDs (A) induce $T_{REG}$ polarization in a self-antigen dose-dependent manner (B). Tolerance in EAE is correlated to MOG ligand density (C). Biodistribution of MOG-QDs (D) and the change in LN composition can be quantified (E).*

# CHIMERIC PROTEIN AND NANO-CONSTRUCT FOR TISSUE-RETAINED ENZYME TO LOCALLY SUPPRESS INFLAMMATION

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Key Words: enzyme; inflammation; self-assembly; nano-construct

There is considerable need for new retention strategies of immunomodulatory biologics for localized suppression of inflammation. We developed a chimeric protein as a well as a self-assembled nano-construct incorporating novel approaches for both retention and suppression to induce potent, confined metabolic programming. Immunosuppressive indoleamine 2,3 dioxygenase (IDO), which depletes tryptophan through the kynurenine pathway, was fused to Galectin 3 (Gal3), which binds extracellular glycans and provides tissue anchoring. Using a luciferase-Gal3 fusion reporter, tissue retention was prolonged to ~6 d whereas native luciferase is not retained and undetectable by 24 h. IDO-Gal3 injected subcutaneously controlled local LPS-challenged tissue inflammation. Furthermore, subgingival injection suppressed periodontal disease (PD) in a polymicrobial challenged mouse model. Multiplex analysis of gingival tissue revealed decreased inflammatory (IL-1β, IL-12p70, KC, IP10, MCP1, MIP2) and increased anti-inflammatory (IL-10, TGFβ3) proteins, indicating a shift toward homeostasis. Animals treated with IDO-Gal3 also showed significant decrease in bone loss commonly associated with PD, as determined by μCT analysis.

**PRECISION POLYMER ARCHITECTURES AND MOLECULAR CONJUGATES TO ENABLE THERAPEUTICS AGAINST UNDRUGGABLE TARGETS**

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Abstract: The Duvall Advanced Therapeutics Laboratory specializes in design and application of smart polymer-based technologies for: (1) intracellular delivery of biological drugs such as peptides and nucleic acids, (2) proximity-activated targeting of drugs to sites of inflammation and matrix remodeling, and (3) long-term, "on-demand" drug release from localized depots. These delivery systems are designed to improve the therapeutic index of existing drugs and/or to serve as enabling technologies for manipulation of intracellular targets currently considered to be "undruggable". To achieve optimal, finely-tuned properties for these varied biomedical applications, polymers are utilized that respond to one or more environmental stimuli including pH, matrix metalloproteinases, reactive oxygen species, and temperature. This talk will focus on the latest nanoparticle and bioconjugate strategies from our group focused on development of new molecularly-targeted breast cancer therapies.

Funding and disclosures: This work was funded by DOD and NIH.

Speaker's biography: Dr. Duvall completed his Ph.D. in BME at Georgia Tech and Emory University under the direction of Bob Guldberg and Bob Taylor in 2007. He then joined the labs of Patrick Stayton and Allan Hoffman at the University of Washington for his NIH NRSA-funded postdoctoral fellowship. Based on these foundations, the Duvall Advanced Therapeutics Laboratory (ATL) was launched in the Vanderbilt Biomedical Engineering Department in 2010, and Dr. Duvall was promoted to Associate Professor in 2016. Dr. Duvall has won awards such as the PECASE, NSF CAREER Award, AHA Scientist Development Grant, Society for Biomaterials Young Investigator Award, and BMES Cellular and Molecular Bioengineering Young Innovator Award. The ATL is funded by grants from NIH, DOD, NSF, and AHA.

**SUSTAINED RELEASE VACCINE PLATFORMS FOR ENHANCED HUMORAL IMMUNITY**

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Key Words: Hydrogel, Immunomodulation, Supramolecular, Humoral Response

Vaccines aim to modulate the immune system to elicit a sufficiently robust, yet targeted, immune response with specifiable immune cell phenotypes.[1,2] Failure of a single vaccine administration to elicit such a response likely arises from inappropriate temporal control over antigen/adjuvant presentation and immune cell activation.[2] Recent work demonstrates the potential for sustained, low-level presentation of antigens and adjuvants for just over a week to yield more potent and long-lasting immunity.[3] However, an incomplete understanding of the enormously complex and dynamic immune system dramatically limits the ability to rationally design true, single-administration vaccines.[3] Our lab has developed injectable and self-healing polymer-nanoparticle (PNP) hydrogels with the ability to deliver multiple cargo of differing compositions.[4] Due to the supramolecular interactions between the HPMC-$C_{12}$ polymer and PEG-PLA nanoparticles, these materials are prepared by simply mixing the components. This ability to streamline material preparation mitigates common challenges observed with manufacturing typical biomaterials at scale, thereby significantly enhancing translatability.[5] We have expanded this PNP hydrogel platform to allow for prolonged controlled release of an encapsulated model subunit vaccine composed of an antigen (ovalbumin) and adjuvant (poly(I:C)) (Figure 1A). The studies with this vaccine platform have yielded a deeper understanding of the effect of the kinetics of antigen/adjuvant presentation on immune activation. With a single injection of the vaccine, hydrogel formulations can elicit 10-fold higher antibody concentrations by day 35 compared to the same vaccine delivered in a typical bolus administration in PBS and had detectable antibodies past 120 days (Figure 1B). Upon an immune challenge, mice immunized with hydrogel-based vaccine formulations induced upwards of 3-fold higher antibody concentrations and higher affinity antibodies systemically. Cell phenotyping experiments indicate an increase in germinal center B cells in hydrogel formulations. These data, along with the boost in the humoral immune response, suggest that prolonged vaccine exposure from the PNP hydrogels leads to an increase in vaccine interaction with immune cells, allowing for somatic hypermutation and clonal selection of B cells. The unique properties of PNP hydrogels make this system ideally suited for controlling release of antigen and adjuvants to elucidate what molecular levers must be pulled, how hard, and when, to produce robust long-term immunity.

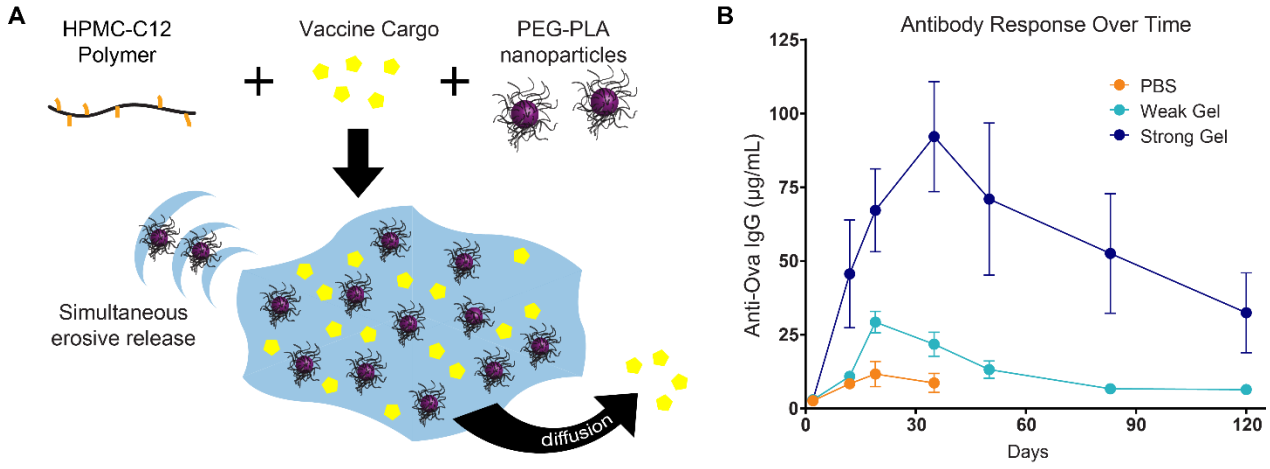


*Figure 4 – (A) Schematic of the polymer-nanoparticle hydrogel with vaccine cargo. (B) Serum concentrations of anti-ovalbumin antibodies after single administration of vaccine.*

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**DIFFERENTIAL UPTAKE OF NON-FOULING PARTICLES BY PRIMARY HUMAN NEUTROPHILS**

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Key Words: poly(ethylene glycol); drug delivery; neutrophils; phagocytosis; plasma proteins

The advent of targeted drug carriers has opened many new avenues for the delivery of therapeutics directly to the site of disease, reducing systemic side effects and enhancing the efficacy of therapeutic molecules. However, the packaging of therapeutics into particulate carriers for delivery comes with its own set of challenges and barriers. Among these, a great deal of research effort has focused on protecting carriers from clearance by phagocytes by altering carrier surface chemistry. Many groups have explored the use of polyethylene glycol (PEG) chain coatings to mitigate unwanted phagocytosis, as PEG is highly hydrophilic and is well-known for its anti-fouling propertiesNotably, very few papers have explored the effects of PEG on uptake by freshly obtained primary human phagocytes in physiological conditions, creating a disconnect between the prevailing literature and ultimate applications. In this work, we investigate the effect of PEGylation on uptake by primary human neutrophils *in vitro*, and compare these effects to several cell lines and other model phagocytic cells systems in evaluating the effects of surface chemistry on phagocytosis. We find that primary human neutrophils preferentially phagocytose PEGylated drug carriers, and that this effect is linked to factors present in human plasma. These findings have major implications for the efficacy of PEGylation in designing long-circulating drug carriers, as well as the need for thorough characterization of drug carrier platforms in a wide array of *in vitro* and *in vivo* assays.

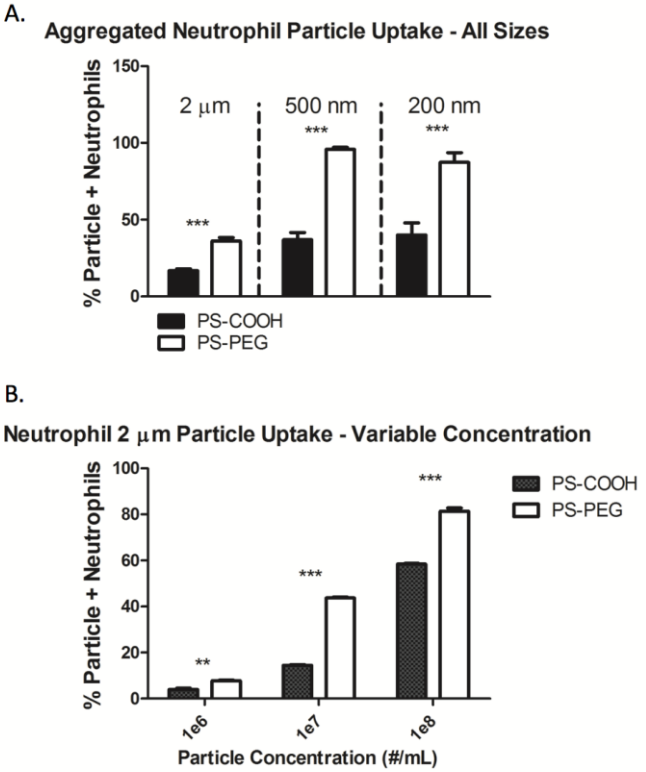


*Figure 5 – (A) Aggregated particle uptake by primary human neutrophils for carboxylated and PEGylated 2 μm, 500 nm, and 200 nm polystyrene particles. (B) Particle uptake by primary human neutrophils for carboxylated and PEGylated 2 μm polystyrene particles at concentrations of 1E6/mL, 1E7/mL, and 1E8/mL*

# COMBINATORIAL NANOCONSTRUCTS FOR BIOMEDICAL IMAGING AND DRUG DELIVERY

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Key Words: Nanoparticles, Theranostics, Drug delivery, Rational Design.

Over the last 15 years, a plethora of materials and different formulations have been proposed for the realization of nanomedicines. Yet, drug loading efficiency, sequestration by phagocytic cells and tumor accumulation are sub-optimal. This implies that radically new design approaches are needed to push forward the clinical integration of nanomedicines, overcoming well-accepted clichés. Combinatorial nanoconstructs are particle-based nano-scale systems designed for the 'smart' delivery of therapeutic and imaging agents.[1-4] The Laboratory of Nanotechnology for Precision Medicine (nPMed) at IIT synthesizes combinatorial nanoconstructs, made out of polymers, with different sizes, ranging from a few tens of nanometers to a few microns; shapes, including spherical, cubical and discoidal; surface properties, with positive, negative, neutral coatings; and mechanical stiffness, varying from that of cells to rigid, inorganic materials, such as iron oxide. These are the 4S parameters – size, shape, surface, stiffness – which can be precisely tuned in the synthesis process enabling disease- and patient-specific designs of multifunctional nanoconstructs. The role of manipulating these 4S parameters over different temporal and length scales will be elucidated in the context of future nanomedicines using in silico, in vitro and in vivo assays.

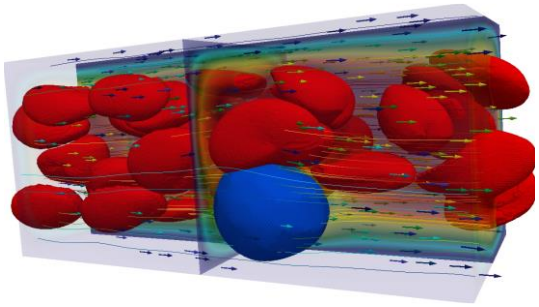

*Figure 6 – 3D in silico modeling of deformable particle and cell transport in capillary flow.*

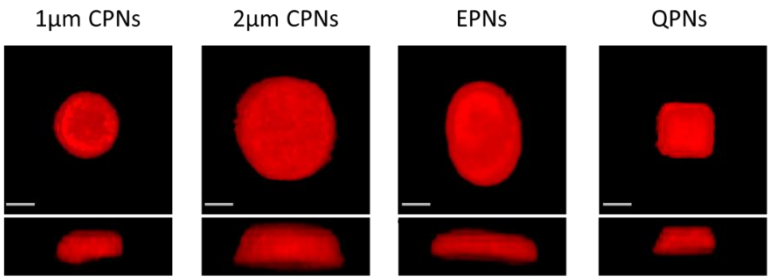


*Figure 2 – Combinatorial nanoconstructs with different size and shape made out of PLGA and PEG and loaded with Rhodamine B (red fluorescence).*

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**EVALUATING THE IMPACT OF PERFUSION ON NANOMATERIAL UPTAKE RATES AND CYTOTOXICITY USING MICROFLUIDIC *IN VITRO* & *IN SILICO* CELL CULTURES SYSTEMS**

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Key Words: microfluidics, nanoparticle uptake rates, regeneration assay

In the last decade, the application of nanomaterials (NMs) in technical products and biomedicine has become a rapidly increasing market trend. As the safety and efficacy of NMs are of utmost importance, new methods are needed to study the dynamic interactions of NMs at the nano-biointerface. However, evaluation of NMs based on standard and static cell culture end-point detection methods does not provide information on the dynamics of living biological systems, which is crucial for the understanding of physiological responses. To gain a deeper understanding of nanomaterial – cell interactions under perfused conditions, we here present a combinatorial *in vitro* & *in silico* approach to describe shear-force dependent uptake of nanoparticles on vascular endothelial cells. Additionally, we present a microsensor-integrated microfluidic system capable of monitoring the enhanced cytotoxic effects of nanodrugs on lung cells following chronic and acute exposure scenarios. Result of our study demonstrate that both active uptake rates and cytotoxicity of nanomaterials are strongly modulated by flow velocity and local shear-force conditions.

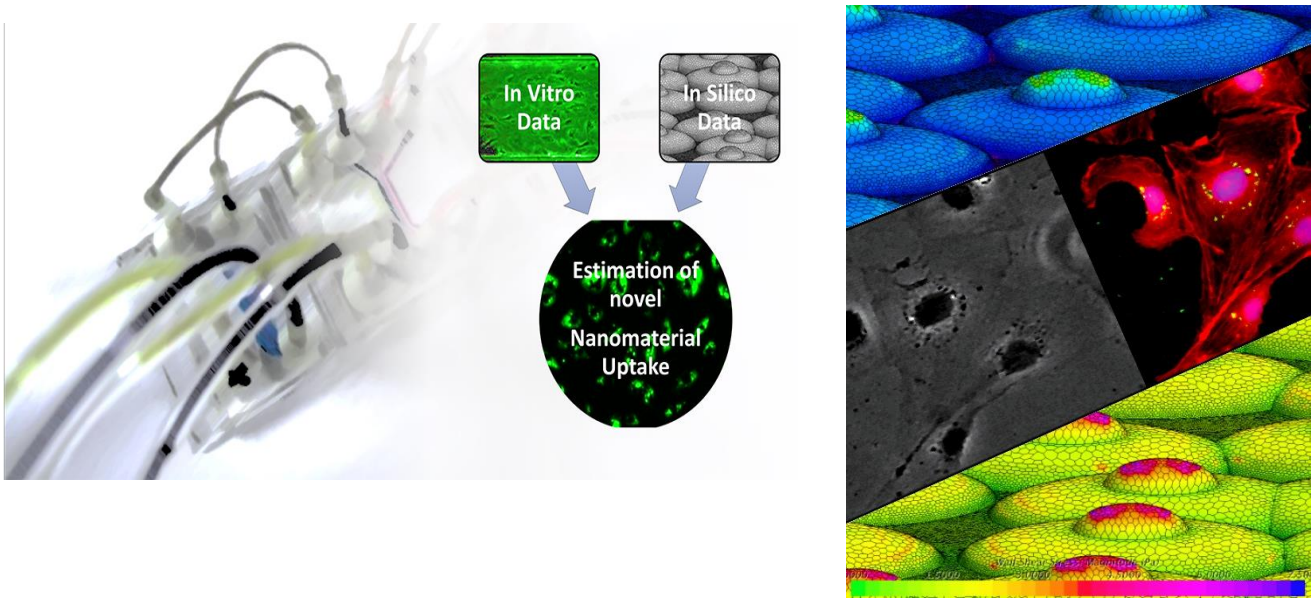


*Figure 7 – Concept of in vitro & in silico approach to study nanomaterial uptake rates*

**TISSUE MICROPROCESSING: SHAPING SUB-NANOLITER VOLUMES OF LIQUIDS ON TISSUE SECTIONS FOR MULTI-MODAL ANALYSIS**

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**GENERATION, DETECTION AND APPLICATIONS OF IN VITRO OXYGEN GRADIENTS**

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Key Words: Hypoxia, Cancer, Oxygen gradient, Microfluidics

Oxygen homeostasis is critical for the functioning of multicellular organisms. Deficiency of oxygen or hypoxia can lead to several pathological conditions such as ischemia, tumorigenesis and drug resistance. Most studies utilize specialized $O_2$ incubators to generate singular oxygen concentrations that vary significantly from the physiological conditions where hypoxic gradients exist within the tissue e.g. in solid tumors. Current microfluidic technology using polydimethylsiloxane-based (PDMS) devices enables generation of such oxygen concentration gradients, but yield low-to-moderate spatial resolution, involve tedious device assembly and are not feasible for practical research or pharmaceutical screening. We have developed a novel and simplistic approach of reproducibly and rapidly generating stable biomimetic oxygen gradients with high spatial resolution and integrated detection capability. The microfluidic split and recombine strategy utilizing $O_2$-rich and $O_2$-depleted media allows generation of prolonged dissolved oxygen (DO) gradients while an underlying platinum based sensor layer (PtOEPK) allows real-time detection of DO gradients generated. Deposition of an approximately 5-7µm thick three-sided glass coating prevents multi-directional diffusion of ambient oxygen through PDMS maintaining the gradient stability for hours or days. Two variations of the gradient devices have been developed, one offering the ability to generate continuous gradients within a single channel while another containing multiple outlet chambers each maintaining a specific concentration of DO (Figure 1).

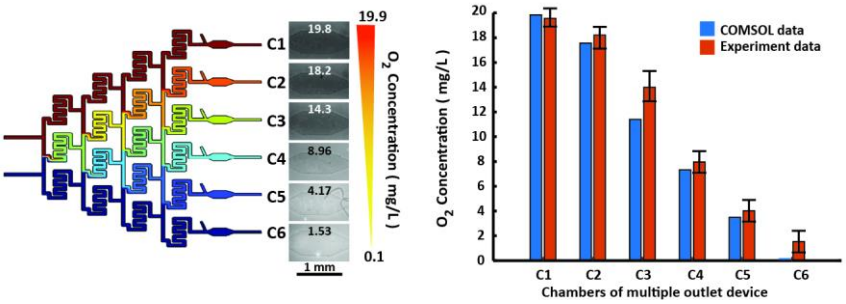
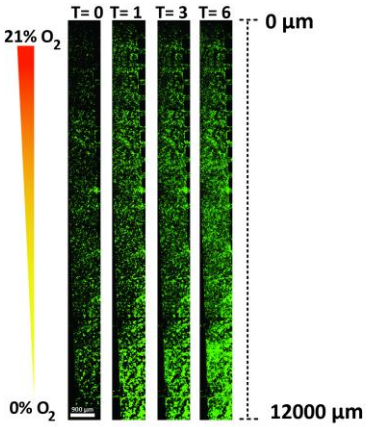



*Figure 1. Left: Layout and modeling of the gradient geometry. Right: Simulation vs empirical O2 concentrations in each chamber.*

Gradient generation was validated by introducing two aqueous solutions of known DO concentrations (0% and 21%) and observing the luminescence of the sensor layer. The gradient values obtained from experimental results closely matched when compared with those obtained through COMSOL simulations. Device functionality for cell based hypoxia studies was first demonstrated through viability analysis of immortalized mammary epithelial cells (MCF-12A) in hypoxic environments. We observed increased cell mortality with increasing hypoxic stress, with approximately 10% live cells being viable at 0% oxygen conditions. Since the chambers are physically separated, the cells are not able to migrate to oxygen rich areas providing a convenient way to monitor hypoxic effects on cells. To further evaluate the platform applicability for more complex cell functions, ER stress of breast epithelial cells (MDA-MB-468) was monitored under oxygen gradients from 0-21% $O_2$. As shown in Figure 2, cells demonstrated a 4-fold increase in ER stress levels after 6 hours and these levels also followed a gradient pattern within the channel.




Thus, our proposed technique enables convenient generation and simultaneous detection of biomimetic oxygen gradients in vitro for relatively long periods. The multi cell-outlet design mimics the functionality of several specialized O2 incubators at once. To further explore wide-scale applicability of this platform, the activity of hypoxia-activated prodrugs (HAP) in cancer cells under hypoxic gradient is currently being investigated.

# A MODEL FOR THE BLOOD-BRAIN BARRIER AND ITS APPLICATION IN MODELING METASTASIS TO THE BRAIN

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Key Words: Blood-brain barrier, permeability, cancer, metastasis, microfluidic model

The blood-brain barrier (BBB) is known to be one of the least permeable portions of the vascular system, serving to protect the central nervous system from agents deleterious to the brain while also preventing or limiting the passage of drugs to treat neurological diseases. Despite this, the brain is recognized to be one of the primary sites of metastasis, especially for lung and breast cancers. While animal experiments have proven useful, realistic models of the BBB using human cells are limited and remain a subject of intense research. Work over the past several years has led to several promising systems, although it has proven difficult to achieve levels of permeability comparable to those observed in vivo.

We have recently sought to develop several systems, first with a mixture of primary rat and human cells (Adriani et al., 2017), and more recently, a model based entirely on human cells. This latter system relies on self-assembly of three critical cell types: human iPSC-derived endothelial cells, primary brain pericytes, and primary brain astrocytes. When suspended in a fibrinogen and thrombin gel solution and introduced to a microfluidic platform, the cells organize into a microvascular network in direct physical contact with pericytes and astrocyte end-feet. Over 7 days, the network forms and attains a relatively stable state that permits perfusion from the side channels of the device.

Once formed, the vascular network is characterized by immunofluorescent imaging, RT-PCR analysis, and functional measures such as permeability to fluorescent probes of various molecular weights. In comparing the networks as they increase in cellular complexity from endothelial cells alone, to ECs and pericytes, and finally, ECs, PCs and astrocytes, the expression of basement membrane and junctional proteins is seen to increase. Simultaneously, the network permeability is observed to progressively fall, indicating improved barrier function. Permeability values for a 10 kDa FITC-dextran are found to be comparable to those measured in the rat brain.

Preliminary experiments have been conducted to determine the rates of extravasation of circulating tumor cells past the model BBB. These experiments introduce breast cancer cells (MDA-MB-231) into the microvascular network with the flow of medium where they adhere to the endothelium or arrest due to physical trapping. Once immobilized, the cells are observed over time to quantify their tendency to undergo transendothelial migration. Results show that as the CTCs are increasingly invasive as the system complexity increases (mono-culture to tri-culture), opposite to what would be expected if tumor cells follow the trend of permeability. Studies are currently underway to understand the cell-cell interactions that give rise to this counterintuitive behavior.

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Acknowledgements:
This work was supported by an Ermenegildo Zegna Founder’s scholarship, an MIT-POLITO grant (BIOMODE - Compagnia di San Paolo), the Cure Alzheimer's Fund, the Japan Society for the Promotion of Science, the US National Science Foundation (CBET-0939511), the National Cancer Institute (U01 CA202177), and an MIT and POLITO MITOR project (NANOCAB).

**ACOUSTIC ENHANCEMENT OF INTRACELLULAR DELIVERY FOR EX VIVO THERAPEUTICS**

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Christina Cortez-Jugo, Department of Chemical & Biomolecular Engineering, University of Melbourne, Australia

Key Words: gene delivery, cell therapy, siRNA, nanoparticle, macromolecule, surface acoustic wave

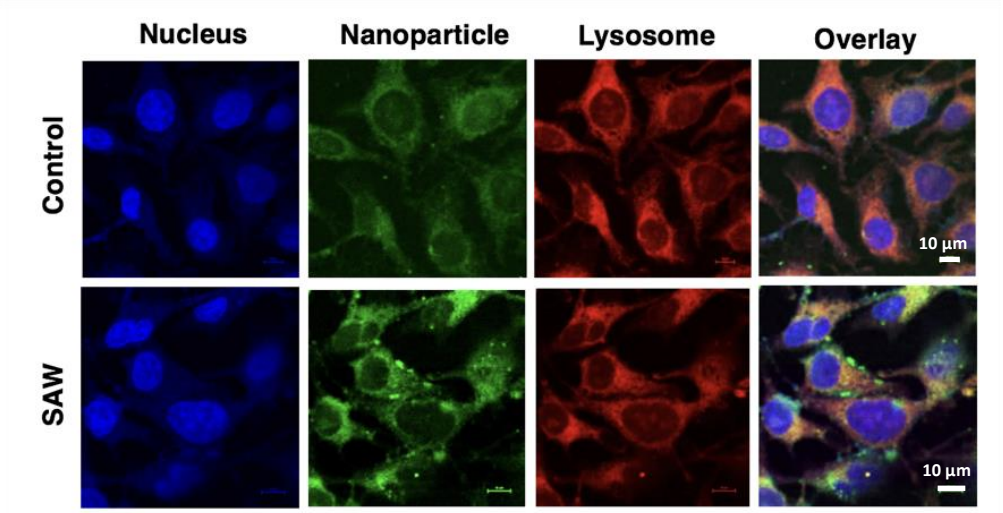


*Figure 8 – Confocal images showing distribution of internalized FITC-labelled gold nanoparticles in HEK293-T cells under with and without (control) surface acoustic wave (SAW) excitation.*

Recent advances in gene editing and therapy have highlighted the potential of *ex vivo* cell-based techniques to treat many diseases, wherein a patient's cells are harvested, engineered to insert various therapeutic agents such as nucleic acids or proteins, and re-infused. Considerable challenges however remain in the ability not just to insert these agents into cells whilst retaining high levels of cellular viability, but also to ensure that they are not lysed within the cell.

Physical methods (e.g., electroporation, sonoporation, etc.), for example, allow efficient translocation of therapeutic cargo into the cell through the formation of pores in the cell membrane. This, however, afflicts some damage to the cells, leading to apoptosis of a considerable proportion of the cells. Biochemical methods, in contrast, rely on carriers such as nanoparticles, vesicles or viruses to facilitate greater endocytotic take-up. The endocytosis pathway nevertheless results in the concentration of the internalised cargo within the endosomal regions of the cell, almost all of which ends up in the lysosome where they are degraded, Strategies that allow them to escape the endosomal recycling path in order to enter the cytoplasm are therefore required if the cargo is to target the nucleus.

We show that exposure of the cells to high frequency (>10 MHz) order sound waves are able to enhance the uptake of nanoparticles, molecules and nucleic acids by several-fold, whilst retaining very high levels (>97%) of cellular viability. This is because the high frequency excitation, unlike sonoporation, does not result in the formation of physical pores in the cell membrane. Instead, the high frequency excitation sufficiently temporarily disrupts the structure of the lipids that make up the cell membrane, thus increasing the membrane permeability sufficiently to allow the therapeutic agent to diffuse through it. The effect, is however, transient such that the organisation of the lipid structure immediately returns to its original state upon relaxation of the acoustic excitation. Such immediate recovery of the cell is the reason for the high cell viability. As this internalisation mechanism does not involve endocytosis, we observe the therapeutic cargo to be distributed throughout the cell instead of being localised within the endosomes or lysosomes (Fig. 1), thus facilitating a greater possibility for nuclear targeting and hence transfection. Indeed, with siRNA delivery into human embryonic kidney (HEK293-T) cells, we observe a two-fold knockdown in the gene expression.

**MICROPHYSIOLOGICAL MODELS OF HUMAN SKIN AND BRAIN VASCULATURE FOR DRUG TESTING**

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Key Words: vasculature, skin, brain, microfluidics, organs-on-a-chip

Organs-on-a-chip systems are biomimetic devices containing microfluidic channels and chambers populated by engineered tissues/cells that replicate key functional units of living organs. In these systems, each tissue chamber has to be connected to other tissues typically through endothelialized microchannels to accurately mimic systemic transport of drugs or soluble factors to/from tissues. We aim to establish this capability by i) restoring the physiological phenotype of endothelial cells (ECs) by recapitulating the vascular microenvironment, and ii) reproducing tissue-specific drug permeability properties within tissues of interest (e.g. blood-brain-barrier).

To generate an in vivo-like endothelial phenotype, we first developed a microfluidic system recapitulating both physiological shear stress and oxygen levels on ECs. The physiological relevance of this model was validated in terms of its responses to a broadly studied vasotoxic chemotherapeutic drug, 5-FU, and to a vasoprotective agent, Resveratrol. Next, we developed a microfluidic blood-brain-barrier (BBB) model that is capable of mimicking *in vivo* BBB characteristics for a prolonged period. We derived brain microvascular endothelial cells from human induced pluripotent stem cells (iPSCs) and co-cultured them with primary astrocytes on the two sides of a porous membrane on a pumpless microfluidic platform. This BBB-on-a-chip model exhibited significant barrier integrity as evident by continuous tight junction formation and in vivo-like values of trans-endothelial electrical resistance (TEER). We further validated the capacity of our microfluidic BBB model to be used for drug permeability studies using large molecules (FITC-dextrans) and model drugs (caffeine, cimetidine, and doxorubicin). Our BBB-on-a-chip model closely mimics physiological BBB barrier functions and can be used for human-relevant screening of drug candidates.

We next focused on the skin vasculature since the current engineered human skin constructs (HSCs) are typically maintained under static conditions, which do not allow for studying drug transport between the skin and circulation. We employed two separate strategies to address this limitation: (i) developing a skin-on-a-chip platform that can create physiologically relevant flow rates; and (ii) incorporating three-dimensional perfusable microvasculature into HSCs to recapitulate the endothelial barrier function. In the first strategy, we designed and developed a skin-on-a-chip platform that has the capability to recirculate the medium at physiological flow rates without the need for a pump or external tube connections. We demonstrated that the platform can be used to maintain HSCs for three weeks with proliferating keratinocytes and intact skin barrier function. In the second strategy, we micropatterned spatially controlled and perfusable vascular networks in HSCs using both primary and iPSC-derived ECs. The 3D-printing technology enabled us to control the geometry of the micropatterned vascular networks. We further verified that vascularized human skin constructs can form a robust epidermis and establish an endothelial barrier function, which allows for the recapitulation of both topical and systemic delivery of drugs. These new vascularization methods now allow us to connect our 3D skin constructs with other microphysiological tissues of interest, such as heart and liver, towards building more comprehensive drug screening platforms.

# BIOMINERALIZED MATERIALS AS BONE ECM MIMETICS: FROM UNDERSTANDING MOLECULAR MECHANISMS TO NEW THERAPEUTIC INTERVENTIONS

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Key Words: Biomineralization, bone tissue repair, osteoporosis

Bone extracellular matrix is a heterogeneous composite material consisting, of an inorganic (or mineral) phase, an organic phase and water. In an effort to mimic the mineral environment of bone tissue, we recently employed biomineralization and created mineralized biomaterials (1). Our studies showed that these biomineralized materials induced osteogenic differentiation of stem cells, including human pluripotent stem cells (which includes both embryonic stem cells and induced pluripotent stem cells), even in growth medium devoid of any osteogenic inducing molecules (2,3). By employing these mineralized materials, we have studied the molecular mechanism through which calcium phosphate minerals support osteogenesis with an emphasis on phosphate metabolism (4). Our studies show that extracellular phosphate (resulting from the dissolution of calcium phosphate minerals) uptake through solute carrier family 20 phosphate transporter member 1 (SLC20a1) supports osteogenic differentiation of human mesenchymal stem cells via adenosine, an ATP metabolite, which acts as an autocrine/paracrine signaling molecule through A2b adenosine receptor. Perturbation of SlC20a1 abrogates osteogenic differentiation by decreasing intra-mitochondrial phosphate and ATP synthesis. Our studies further show that the phosphate-ATP-adenosine signaling axis not only promotes osteogenic differentiation of multipotent progenitor cells but also inhibits their adipogenic differentiation (5). When implanted in vivo, these acellular mineralized biomaterials recruited endogenous cells and induced their differentiation to form vascularized bone tissues (6) and also supported donor bone marrow transplantation (7). Leveraging these understandings, we are currently studying the pivotal role of Adenosine A2b receptor, a G-protein coupled receptor on the cell membrane, in regulating bone-specific cells and treating bone metabolic disorders. In this talk, I will discuss these results and the identification of possible drug targets for osteoporosis.

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## DECODING MECHANISMS THAT REGULATE RE-EPITHELIALIZATION

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During normal wound healing, keratinocytes are the first dermal cell type to respond to the injury, covering the wound bed to establish a barrier for immune defense and provide structural and mechanical support for dermal regeneration. Failure of this re-epithelialization process results in the development of chronic wounds, which are associated with substantial medical costs. During re-epithelialization, keratinocytes can utilize multiple mechanisms to fill the space, including migration, proliferation, and hypertrophy. Additionally, individual keratinocytes are influenced by numerous factors in the wound microenvironment, including substrate mechanics and growth factors to direct these cellular decisions. To determine which individual cell behaviors represent the most promising targets to engineer re-epithelialization, we have examined collective and individual responses of HaCaT keratinocytes to changes in substrate mechanics and growth factors and utilized computational modeling to predict the hierarchy of factors driving wound closure. Our results suggest that migrational persistence is the key parameter for effective wound closure. We have further examined biomaterials-based methods to direct migrational persistence, and identified a mechanism by which immobilization of EGF induced strong migrational persistence through the activation of PLCg1 specifically in keratinocytes on the leading edge. Ongoing work is examining this process in more detail to determine the mechanism responsible for leading edge-specific activation of PLCg

**SPATIAL PATTERNING OF LIVER PROGENITOR CELL DIFFERENTIATION MEDIATED BY CELL CONTRACTILITY AND NOTCH SIGNALING**

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Keywords: tissue engineering, microarray, progenitor cells, biomechanics, liver

Liver progenitor cell differentiation and bile duct formation are driven by spatially-dependent and temporally-sequenced cell–cell and cell–factor interactions coordinated by several biochemical signaling pathways, namely Notch and TGFβ. The regionalization of biliary differentiation and morphogenesis near the portal region of the liver has suggested that spatially segregated microenvironmental signals govern this process. Our recent work utilizing biomaterial substrates of defined stiffness suggests that mechanical cues play a previously-unrecognized role in liver progenitor differentiation. Here, we used a cell microarray platform that enables the simultaneous analysis of these biochemical and biomechanical microenvironmental cues to define the mechanisms of action and functional overlap of these pathways.

We used bipotential mouse embryonic liver (BMEL) progenitor cells cultured by standard techniques (M. Weiss and H. Strick-Marchand, Institut Pasteur). To present Notch ligand to cells, we printed Fc-chimeric recombinant DLL4 on a polyacrylamide hydrogel substrate together with collagen I and Protein A/G. We integrated this cell microarray platform with traction force microscopy (TFM) by adding fiducial beads to the polyacrylamide hydrogel and imaging bead displacement before and after cell dissociation.

In order to determine the effect of activating Notch signaling on liver progenitor differentiation, we presented Fc-DLL4 to cells in arrays, inducing biliary differentiation restricted to the edges of patterns as measured by expression of OPN (Figure 1A). Addition of an inhibitor of Notch signaling prevented peripheral biliary differentiation (Figure 1A). Immunofluorescence analysis of expression of the biliary transcription factor SOX9 showed restriction to the island periphery while expression of the hepatocyte transcription factor HFN4A was central (Figure 1B). Further, we observed that SOX9 expression increased on stiff substrates while that of HNF4A increased on soft substrates (Figure 1B), which implicated biomechanical stimulation as the gradient-forming cue. Finite element modeling (FEM) simulations suggested a radial gradient of mechanical stresses in the circular patterns, which we confirmed experimentally using TFM (Figure 1C). We next sought to modulate cell contractility and measure the resulting change in fate trajectory. Peripheral biliary differentiation intensified by viral transduction with constitutively-active $RhoA^{L63}$ (MOI=200), an inducer of cell contractility (Figures 1D and 1E). Conversely, treatment with blebbistatin (25 μM), which inhibits phosphorylation of myosin II, abrogated peripheral differentiation (Figures 1D and 1E).Liver progenitors require both Notch signaling and sufficient cell contractility in order to differentiate into bile duct cells. Ongoing work is focused on characterizing Notch family signaling using *in situ* hybridization as well as examining the mechanistic links between Notch and mechanotransduction pathways.

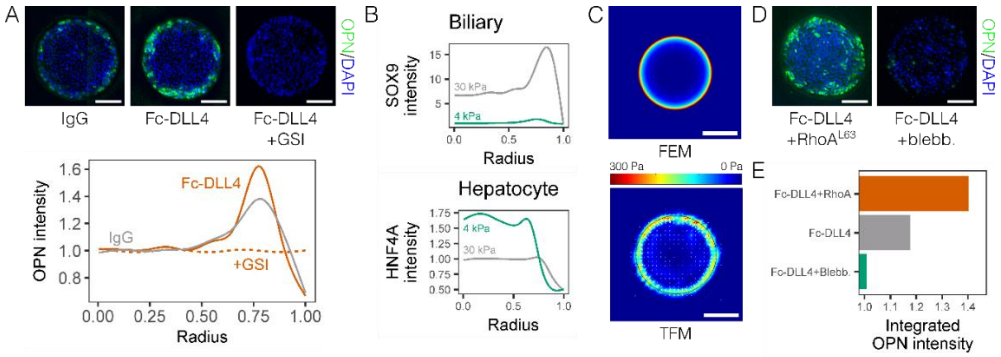


*Figure 9: Biliary differentiation of liver progenitors requires both Notch signaling and cell contractility. (A) Liver progenitors presented with ligand (Fc-DLL4) express OPN peripherally while those treated with Notch inhibitor (γ-secretase inhibitor X, GSI) do not. n=6 islands per condition. (B) Expression of the hepatocyte transcription factor HNF4A is central while that of the biliary transcription factor SOX9 is peripheral. Polyacrylamide substrate stiffness was either 4 kPa or 30 kPa. n=6 islands per condition. (C) Both FEM simulations and experimental TFM of liver progenitor islands show high cell-generated stresses at the periphery. (D) Induction ($RhoA^{L63}$) and inhibition (blebbistatin) of contractility show that it is both necessary and sufficient for biliary differentiation. (E) Means of data from (D) with n=6 islands per condition. Scale bars are 200 μm.*

**LIPID TARGETS IN PREVENTION OF CLOTTING: TRANSLATING *IN VITRO* CONCEPTS TO *IN VIVO* APPLICATION**

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Key Words: 12-lipoxygenase, platelets, hemostasis, bleeding, thrombosis

Bioactive lipids have been shown to play both pro- and anti-clotting regulatory roles in platelet function resulting in modulation of hemostasis and thrombosis. While much is known about COX-1 regulation and the role of its free fatty acid metabolites in regulation of the platelet, less is known about how 12-LOX and its fatty acid eicosanoids mediate these essential functions. Nearly 33% of deaths annually are associated with cardiovascular disease and platelet activation is essential to arteriothrombotic clots leading to myocardial infarction and stroke. Therefore a greater understanding of the role of 12-LOX in this process is needed and may represent a novel target for prevention of thrombosis. Our group has developed a highly selective 12-LOX inhibitor to target 12-LOX in the platelet and determine its potential role in platelet activation and thrombotic risk. Here, we show for the first time the *in vivo* utility of inhibiting 12-LOX. In human platelets run through a microfluidics system at arterial shear, treatment with the 12-LOX inhibitor ML355 was shown to be more effective at decreasing platelet adhesion to collagen compared to aspirin. *In vivo*, platelet accumulation at the site of injury in a number of thrombotic models in the mouse was prevented in the presence of ML355. Importantly, bleeding, a common side effect of platelet inhibition, was not affected, supporting 12-LOX as an important enzyme in regulation of hemostasis and thrombosis *in vivo* (Adili et al. Arterioscler Thromb Vasc Biol 2017,). These observations, coupled to the earlier observation by our group that inhibition or ablation of 12-LOX was effective in preventing immune-meditated thrombosis in human platelets and mouse models (Yeung et al. Blood 2014), raised the question of whether inhibition of 12-LOX might be a viable treatment of immune-mediated thrombocytopenia and thrombosis (ITTs). To address this question, transgenic mice expressing human immune receptor FcgRIIa but not ALOX12, were retro-orbitally injected with a fluorescent antibody for the platelet receptor □-GPIX to induce ITT-like symptoms. Blood was collected at several time points to assess platelet count and the mice were sacrificed after 4 hours to determine the degree of thrombosis in vascular beds such as the lungs. While induction of ITT resulted in over 80% platelet loss within an hour and significant thrombosis in the lungs within 4 hours, animals lacking 12-LOX showed protection from both of these pathologies. Hence, targeting 12-LOX with ML355 demonstrates that 12-LOX is a viable antiplatelet target for arteriothrombotic events while exhibiting limited bleeding.

**TISSUE GUIDED HYDROGEL DESIGN**

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Improved *in vitro* models are needed to better understand cancer progression and bridge the gap between *in vitro* proof-of-concept studies, *in vivo* validation, and clinical application. Many methods exist to create biomaterial platforms, including hydrogels, which we use to study cells in contexts more akin to what they experience *in vivo*. Our lab has multiple approaches to create such biomaterials, based on combinations of poly(ethylene glycol) (PEG) with peptides and zwitterions. In this presentation, I will discuss our findings in using these cell culture environments to understand the role of the extracellular matrix (ECM) in controlling cancer cell innate drug response via adaptive signaling, and how these tissue models might be used for regenerative medicine applications.

# SYNTHETIC BUILDING BLOCKS FOR HIERARCHICAL TISSUE ENGINEERING

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Key Words: Tissue regeneration, hydrogels, cell guidance, biofabrication

We focus on designing biomaterial systems for hierarchical tissue engineering. Polymeric molecular and nano- to micron-scale building blocks are assembled into soft 3D biomimetic constructs, which allow studying and controlling cell/material interactions that are difficult or impossible to investigate with current material systems.[1] These biomaterial platforms can then be applied to build *ex vivo* tissue models for drug testing or investigating pathologies, to grow tissue or organs *ex vivo* for transplantation *in vivo*, or for *in vivo* tissue regenerative therapies. The hybrid artificial biomaterial matrices consist of a polymeric crosslinked network and colloids to create macroscopic structures with new properties. Microgels and fibers are produced by adapted technologies based on fiber spinning, microfluidics, and in-mold polymerization. To arrange the building blocks in a spatially controlled manner, we rely on self-assembly mechanisms and assembly by external fields (e.g., magnetic). A better understanding of cellular processes in contact with synthetic biomaterials will supply information about the parameters, which are most important to make viable and functional regenerative materials for clinical use in a modular manner. The different methods are applied to embed and grow cells in micro-containers to form mini-tissues for transplantation or load specific biological molecules to control temporal release [2], and produce structural magnetic elements that can be aligned for cell guidance. Due to their size, the micro-objects can be injected, with or without a surrounding hydrogel. Their internal structure and degradation properties enable temporal control of tissue formation. To obtain anisotropic matrices after injection, rod-shaped elements are rendered magneto-responsive by the incorporation of superparamagnetic iron oxide nanoparticles. Due to their anisometric shape, the elements align parallel to a low external mTesla magnetic field, after which a surrounding hydrogel can crosslink to fix their unidirectional orientation after removal of the magnetic field. Fibroblasts and nerve cells sense the mechanical anisotropy, induced by a minimal amount of oriented structures, resulting in directed cell growth inside 3D hydrogels.[3] Modification of the guiding elements with adhesive peptides enhances the overall cell alignment, reduces ECM production, and increases nuclear shuttling of mechanosensitive proteins, such as YAP/TAZ.[4] Neurons inside the Anisogel show spontaneous electrical activity proving neuronal functionality and importantly, electrical signals propagate along the anisotropy axis of the material.[5] The developed hybrid hydrogel can be applied as a low invasive, injectable material to repair complex, sensitive tissues, such as the spinal cord.

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**SCALABLE AND PHYSIOLOGICALLY RELEVANT MICROENVIRONMENTS FOR HUMAN PLURIPOTENT STEM CELL EXPANSION AND DIFFERENTIATION**

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Key Words: human pluripotent stem cells; 3D culture system; microenvironment

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), are attractive cell sources for various biomedical applications including cell therapies, tissue biofabrication, drug screening and toxicity tests. These applications require large numbers of high quality cells. However, the scalable and cost-effective culturing of high quality hPSCs and their derivatives, especially for clinical applications, remains a challenge. *In vivo*, majority of human cells including the hESCs reside in 3D microenvironments that have plenty of cell-cell and cell-ECM (extracellular matrix) interactions, sufficient supply of nutrients, oxygen and growth factors, and no or minimal hydrodynamic stresses. The current hPSC culturing methods, however, provide culturing conditions that are very different from these physiological microenvironments, leading to low culture efficiency and difficulty to culture cells at large scales. For instance, we and others showed hPSCs typically expanded 4-fold in 4 days to yield around 2.0x10^6 cells/mL with current 3D suspension culturing. These cells occupy ~0.4% of the bioreactor volume. To our best knowledge, the largest 3D suspension culture volume demonstrated to date for hPSCs is less than 10 liters.

We here report a novel technology that can overcome the limitations of current hPSC culturing methods and provide physiologically relevant cell culture microenvironments. With this technology, hPSCs are processed into and cultured in microscale alginate hydrogel tubes (or AlgTubes) that are suspended in the cell culture medium in a culture vessel (Figs. 1A and B). The hydrogel tubes create cell-friendly microspaces that allow cells to interact with each other and expand. Meanwhile, they protect cells from hydrodynamic stresses in the culture vessel and confine the cell mass less than 400 µm (in radial diameter) to ensure efficient mass transport during the entire culture (Figs. 1A and B). Additionally, this technology is simple, scalable, defined and compatible with the current Good Manufacturing Practices that make it commercially viable. We showed that, under optimized culture conditions, the AlgTubes offered paradigm-shifting improvements in cell viability, growth, yield, culture consistency and scalability over current hPSC culturing technologies. We demonstrated long-term culturing (>10 passages) of multiple hPSC lines without uncontrolled differentiation and chromosomal abnormalities. Cultures between batches and cell lines were very consistent. hPSCs in AlgTubes had high viability, growth rate (1000-fold/10 days/passage in general) and yield (~5x10^8 cells/mL microspace). The expansion per passage (e.g. up to 4200-fold/passage was achieved) and volumetric yield are much higher than current 3D suspension culturing. The high yield and high expansion fold have high impact on large-scale cell production since they significantly reduce the culture volume and time, numbers of passaging operations, and the production cost. hPSCs could be efficiently differentiated into various tissues cells. In addition, AlgTubes-based scalable bioreactors could be readily built. Our comparative study showed the AlgTubes did not significantly alter hPSCs' gene expression profiles, but significantly reduced cell death, resulting in high cell expansion and yield.

In summary, the AlgTube technology combines physiologically relevant culture microenvironments, high performance, high scalability, cGMP compliance and commercial viability, and has potential to address the hPSC manufacturing challenge. This research well demonstrates the using of microfluidics to create cell niche to address a significant biomedical problem.

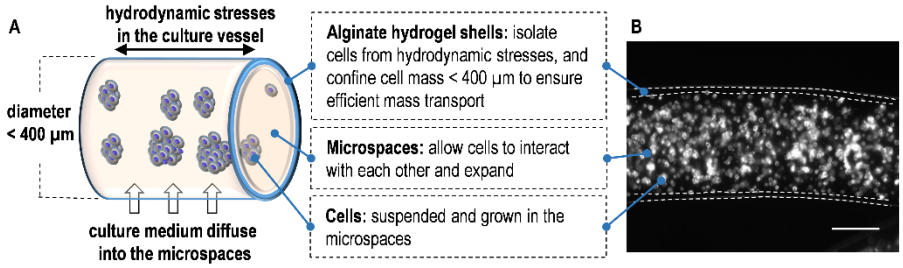


*Figure 10- The AlgTubes cell culture system.*

# NEW STRATEGIES FOR ENHANCING TUMOR IMMUNOTHERAPY BY EXPLOITING THE TUMOR MICROENVIRONMENT

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