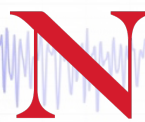
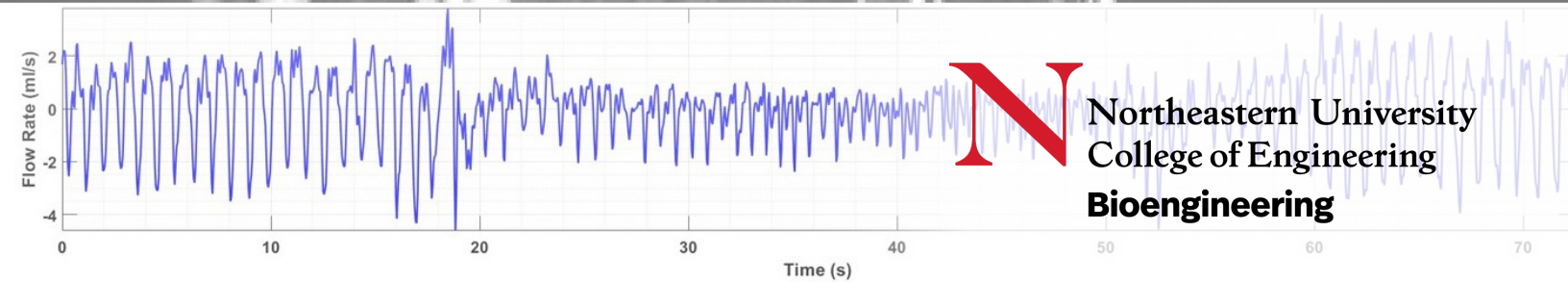
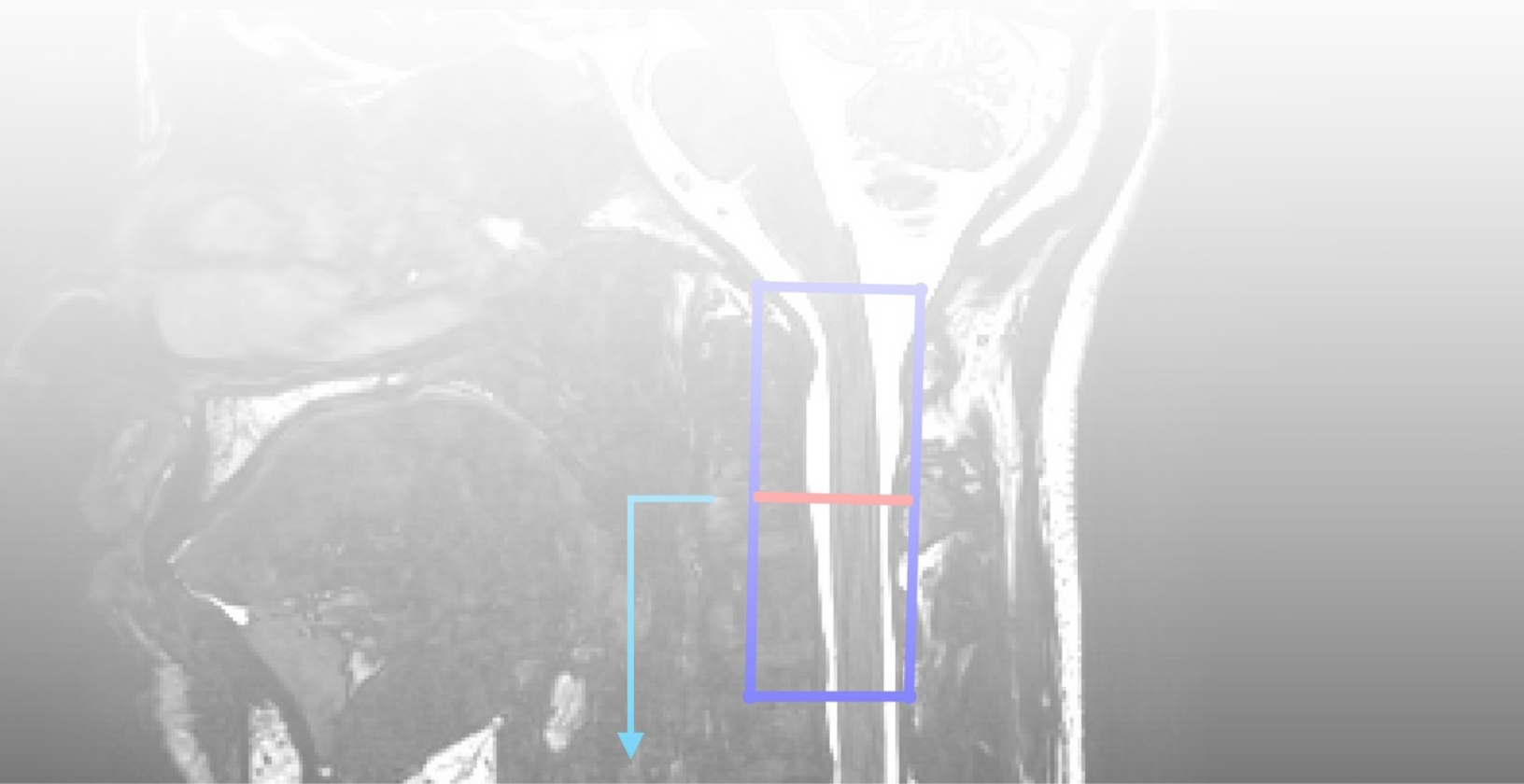


6th Annual

BIOENGINEERING RESEARCH SYMPOSIUM

Wednesday, June 18th 2025



Northeastern University
College of Engineering
Bioengineering

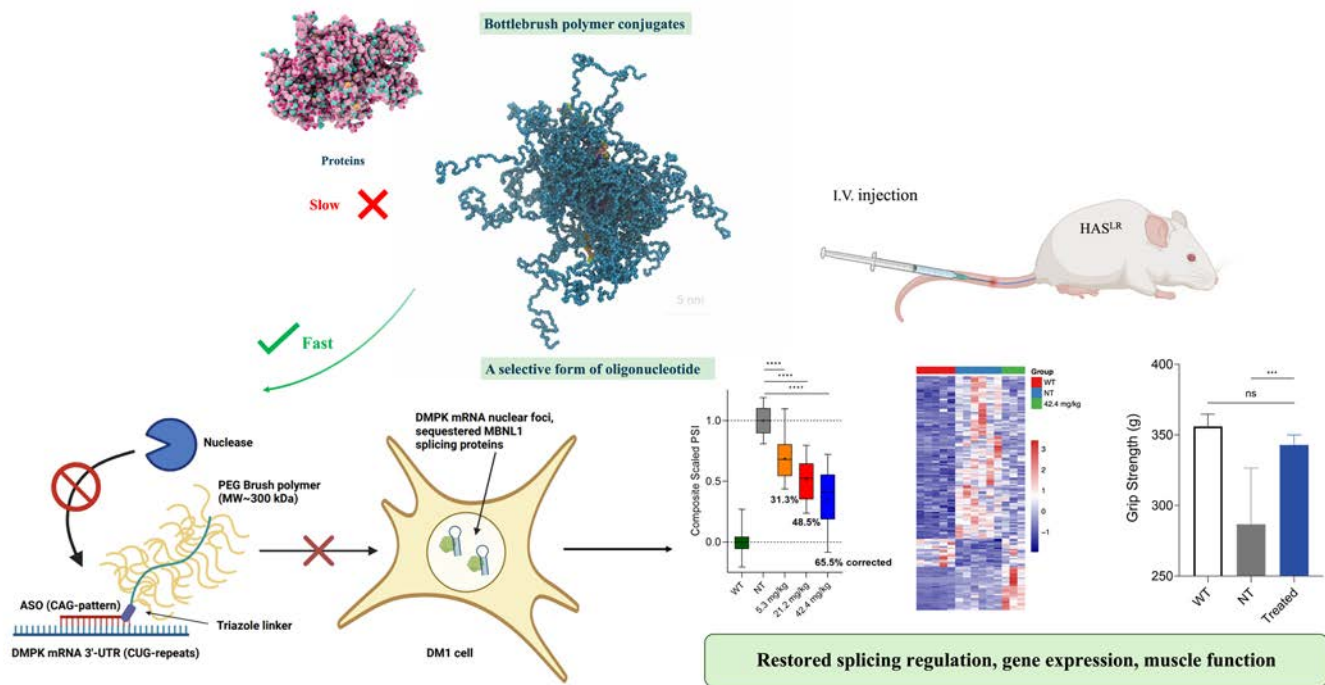
Cover design: The figure shows reduced stroke volume during the Valsalva maneuver, with recovery in the post-Valsalva and Relaxation phases.

Mahsa Karamzadeh, PhD Candidate; Frank Loth Lab.

6th Annual

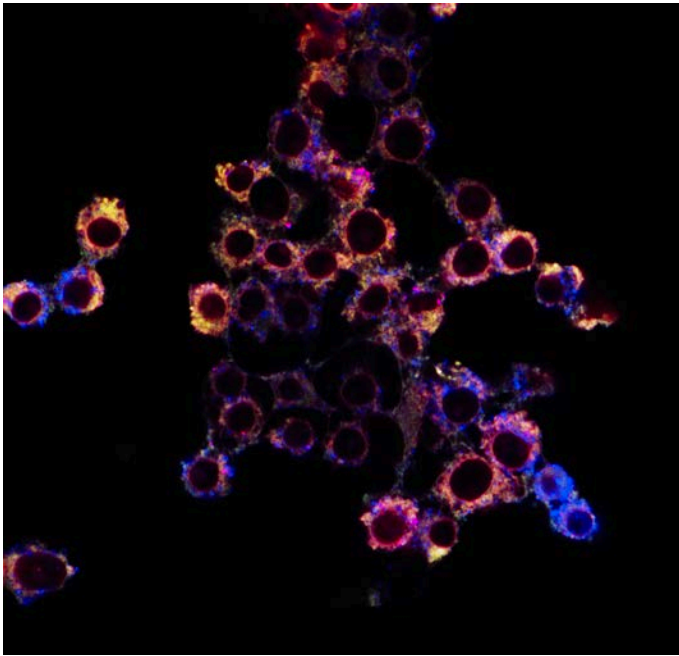
BIOENGINEERING RESEARCH SYMPOSIUM

Wednesday, June 18th 2025



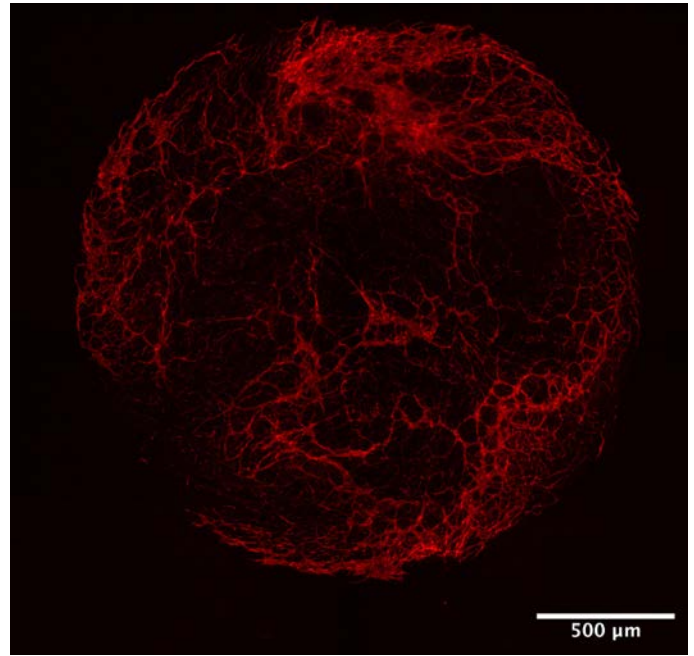
This figure illustrates the therapeutic strategy of using bottlebrush polymer-conjugated antisense oligonucleotides (pacDNA) to target toxic CUG repeats in DM1. After intravenous injection in HSALR mice, the conjugates improve nuclease resistance, enhance tissue delivery, and help restore splicing regulation, gene expression, and muscle strength.

Yao Li, PhD Candidate; Ke Zhang Lab.



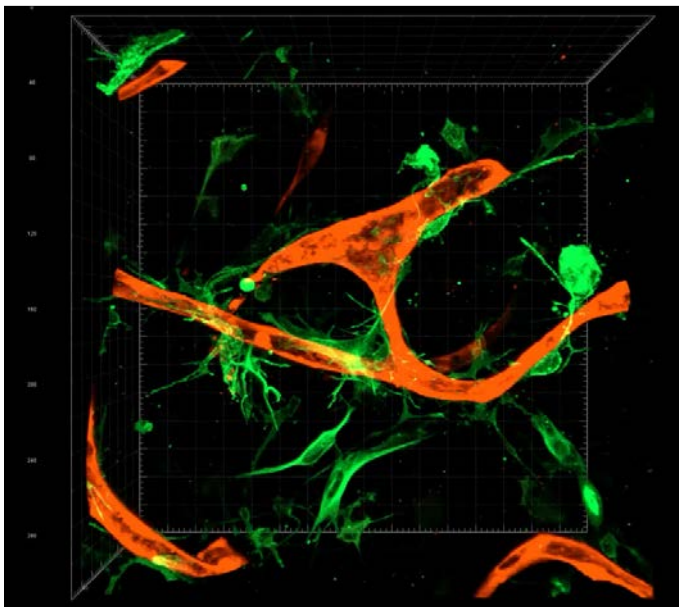
The Figure titled “Web of Macrophage” illustrates the intricate interplay between Raw 264.7 cells and Microbubbles. This process encompasses attachment, internalization, and lysosomal activation occurring concurrently, resulting in distinct color signals at varying intensities.

Krunal Sanjay Patel, PhD Student; Tao Sun Lab.



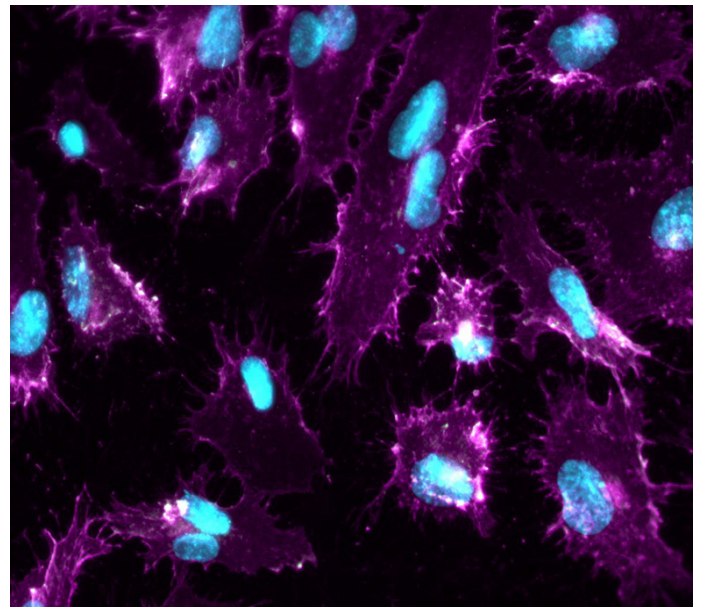
hPSC-derived vasculature in a 3D fibrin gel dot. dsRED fluorescence depicts VECAD signal post-differentiation from an hPSC line modified with a VECAD promoter-mOrange reporter. Scale bar 500um.

Stefanie DeFronzo, PhD Candidate; Guohao Dai Lab.



Neurons (green) surrounding vasculature (red) in an in vitro 3D neurovascular niche model.

Abigail Ecker, PhD Candidate; Guohao Dai Lab.



VE-Cadherin Expression in HUVECs. Immunostaining of human umbilical vein endothelial cells (HUVECs) reveals VE-cadherin localized at the cell periphery, indicating the formation of adherens junctions. This pattern suggests that the cells are establishing tight junctions, which are critical for maintaining endothelial barrier integrity.

Aadhira Nair, MS Student; Amir Vahabikashi Lab.



Axolotls are highly regenerative organisms being studied with the goal of developing translational therapies for humans. The Genome Technology Lab at Northeastern is utilizing nanopore long-read RNA sequencing to investigate the intricacies of how gene, isoform, and RNA modification expression is altered during axolotl limb regeneration.

Connor Powell, PhD Student; Miten Jain Lab.

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Poster Presentation Abstracts - Morning	19
Poster Presentation Abstracts - Afternoon	39
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5-Minute Thesis Competition Abstracts	65

Schedule of Events

9am - 5pm | Wednesday, June 18th | Curry Student Center Ballroom

8:30 - 9:00am

CSC Ballroom

Breakfast

9:00 - 9:30am

CSC Ballroom

Keynote Address

Sara Rouhanifard, *Associate Professor of Bioengineering*

"inCu-click: Enabling Copper-Catalyzed Click Chemistry Inside Living Cells."

9:30 - 10:30am

CSC Ballroom

Oral Presentations - Morning Session

Oleksandra "Sasha" Fanari, *PhD Candidate*

"Comparative Analysis of RNA Modifications for Immortalized and Primary Human T Cells."

Benjamin Goykadosh, *PhD Candidate*

"A Novel Mechanism for Long Distance Calcium Signaling without Gap Junctions."

Catherine "Katya" Karpova, *PhD Candidate*

"Development of Cardiac Specific Endothelial Cells to Support Vascularized Cardiac Organoids."

10:30 - 11:30am

CSC Ballroom

Poster Presentations - Morning Session

11:30am - 12:30pm

CSC Indoor Pit

Lunch

12:30 - 1:30pm

CSC Ballroom

Poster Presentations - Afternoon Session

1:30 - 2:30pm

CSC Ballroom

Oral Presentations - Afternoon Session

Sahana Gangadharan, *PhD Candidate*

"Characterizing Chemosensory Neuronal Responses through Development in Both Sexes."

Ashlyn McCann, *PhD Candidate*

“Improving Tomographic fNIRS Contrast and Depth Sensitivity Using
Actiation-Prior-Guided Reconstruction.”

Krunal Sanjay Patel, *PhD Student*

“Labelling Macrophages with Lipid Microbubbles for Multi-Modal Imaging
and Immunomodulation.”

2:30 - 3:00pm

CSC Indoor Pit

Coffee Break

3:00 - 4:00pm

CSC Ballroom

5 Minute Thesis Competition

4:00 - 4:30pm

Break

4:30 - 5:00pm

CSC Ballroom

Awards Ceremony



inCu-click: Enabling Copper-Catalyzed Click Chemistry Inside Living Cells

Sara Rouhanifard

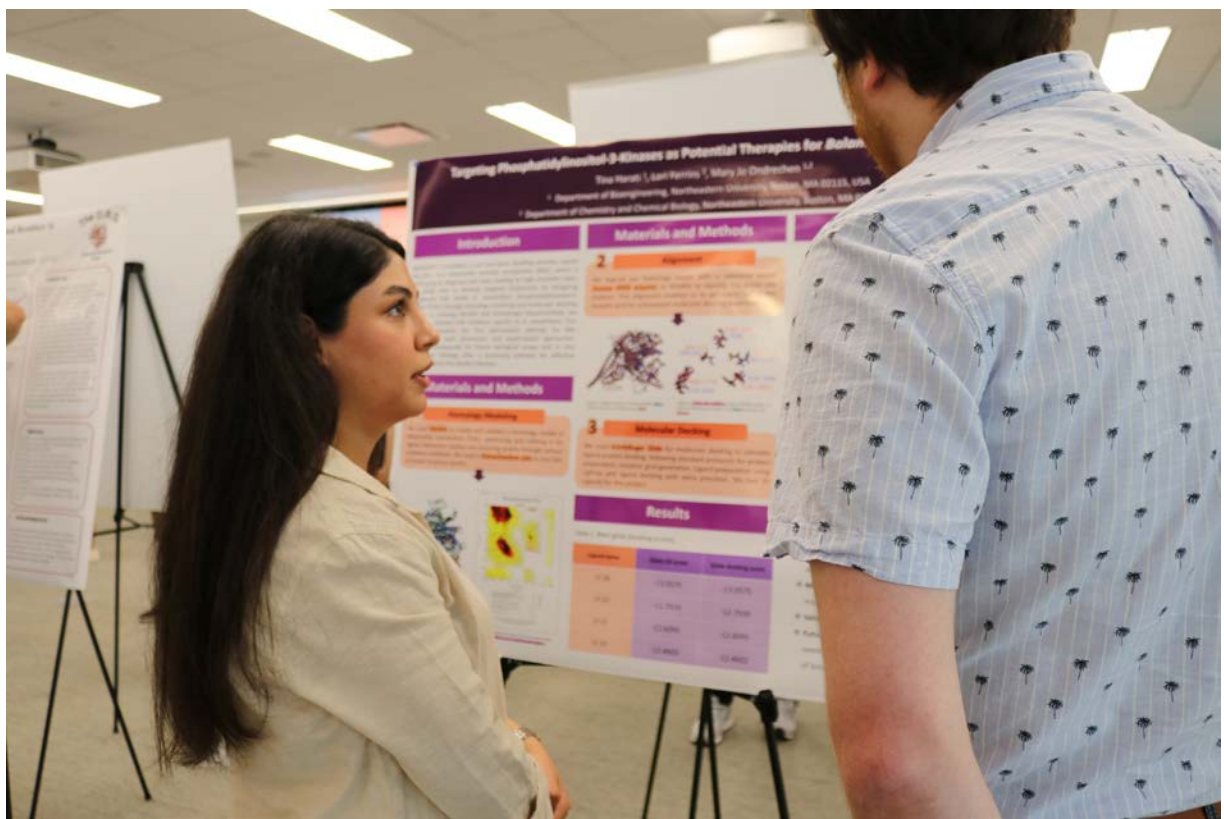
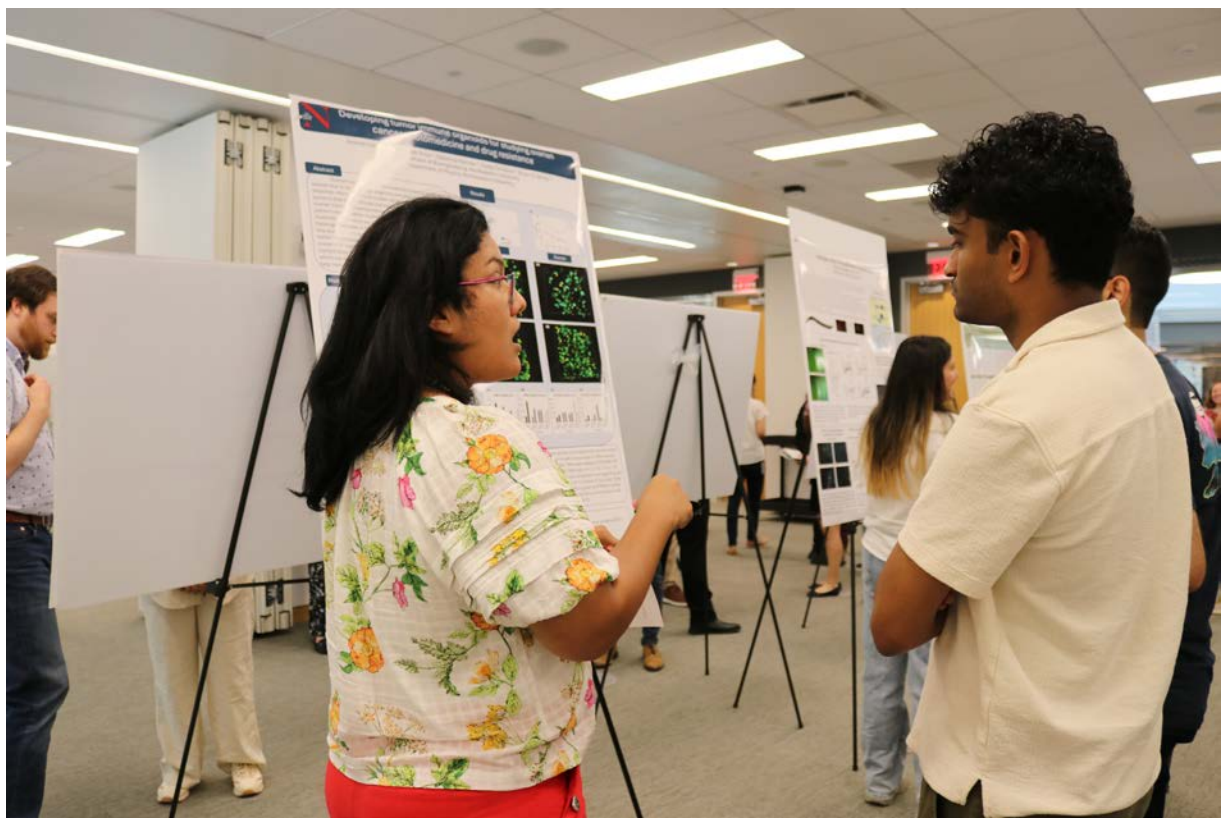
Associate Professor of Bioengineering

Abstract: Copper-catalyzed azide–alkyne cycloaddition (CuAAC) is a cornerstone of bioorthogonal chemistry, prized for its rapid kinetics and compatibility with diverse molecular probes. However, despite its widespread use in extracellular and fixed-cell labeling, live-cell CuAAC has remained elusive due to the toxicity of copper at concentrations required for efficient catalysis. Addressing this challenge requires a bioengineering solution—one that reimagines the molecular toolkit to enable precise and safe chemistry inside living systems.

In this talk, I will present inCu-click, a bioengineering solution that enables CuAAC-based labeling within live cells. At the core of our approach is BTT-DNA, a DNA-conjugated copper ligand that achieves efficient catalysis at low, non-toxic copper levels. This engineered molecule enhances intracellular copper localization, supports nucleic acid-templated proximity, and enables delivery into cells—making it possible to label and track intracellular molecules in real time without compromising cell viability.

By integrating chemical design with cellular delivery and molecular tracking, inCu-click represents a bioengineering platform for minimally invasive, high-resolution interrogation of biomolecular dynamics. This strategy opens the door to spatiotemporally precise studies of cellular function, with broad applications across molecular biology, synthetic biology, and therapeutic development.

Biography: Sara Rouhanifard joins the Bioengineering department in January 2019 as an Assistant Professor. She earned her PhD at Albert Einstein College of Medicine in biochemistry. Her research interests include developing chemical approaches to track and quantify important RNA processing events and modifications in single cells; DNA: protein interactions that drive differences in gene expression; understanding differences in RNA expression and the impacts on disease and development.



Bioengineering Research Symposium, 2024

Oral Presentations Abstracts

MORNING SESSION

Comparative analysis of RNA modifications for immortalized and primary human T cells

Oleksandra Fanari¹, Dylan Bloch¹, Yuchen Qiu¹, Michele Meseonznik¹,
Meni Wanunu^{1,2}, and Sara H. Rouhanifard^{1,3,*}

¹Dept. of Bioengineering, Northeastern University, Boston, MA

²Dept. of Physics, Northeastern University, Boston, MA

³*Lead contact*

Immortalized cell lines, like Jurkat cells, are widely used to model primary human cells, but their RNA modification landscape remains unclear. A longstanding hypothesis suggests that immortalization disrupts RNA modification machinery, making these cells suboptimal for studying modifications.

To investigate this, we compared pseudouridine (psi) modifications in human mRNA from primary and immortalized Jurkat T-cells using Direct RNA Sequencing (DRS). RNA modifications are known to influence immune cells' biology, yet pseudouridine's role in T-cell biology remains unexplored.

We found 87% of shared ψ -sites at different occupancy levels, with 13% of sites being unique to primary or immortalized cells. Gene ontology analysis of conserved ψ -sites identified roles in cellular housekeeping functions, including RNA modification-related processes, suggestive of a potential feedback loop regulating psi deposition. Additionally, RNA-modification enzymes showed comparable gene expression in primary and immortalized cells.

These findings suggest largely conserved ψ -sites and machinery between immortalized and primary T cells, though site-specific differences may have functional relevance.

A novel mechanism for long distance calcium signaling without gap junctions

Benjamin M. Goykadosh¹, Vasuretha Chandar¹, and Harikrishnan Parameswaran¹

Dept. of Bioengineering, Northeastern University, Boston, MA

Long-range calcium (Ca^{2+}) signaling governs vital processes like airway contraction, insulin production, and activation of transcription factors. Currently, this long-range Ca^{2+} signaling is attributed to diffusion of a signaling molecule, IP3, through gap junctions. However, recent studies have shown that IP3 diffuses 30 times slower than previously reported and is therefore insufficient for long-distance Ca^{2+} propagation. Here, we identify a novel mechanism in which mechanically induced intercellular forces drive intercellular calcium signaling. Using an *in vitro* informed computational model, we demonstrate that intercellular forces trigger regenerative cytosolic Ca^{2+} oscillations in neighboring smooth muscle cells (SMCs). We show that SMCs communicate extracellular matrix (ECM) stiffness over distances eight times their cell-length via Ca^{2+} signaling to neighboring cells. These oscillations increase in frequency with applied force, allowing synchronized calcium dynamics between cells and facilitating long range Ca^{2+} communication. Specifically, this enables cells adhered to a stiff ECM (13 kPa) to amplify Ca^{2+} oscillation frequency and contractility in neighboring cells on a soft ECM (0.3 kPa). Our findings propose a new paradigm for calcium signaling, wherein mechanically induced IP3 release serves as a regenerative mechanism for long-range calcium propagation. These findings provide critical insights into how localized ECM remodeling produces systemic effects, offering a framework to understand early stages of disease progression in asthma, fibrosis and cancer.

Keywords: Calcium Signaling, Asthma, Cellular Communication, Biomechanics

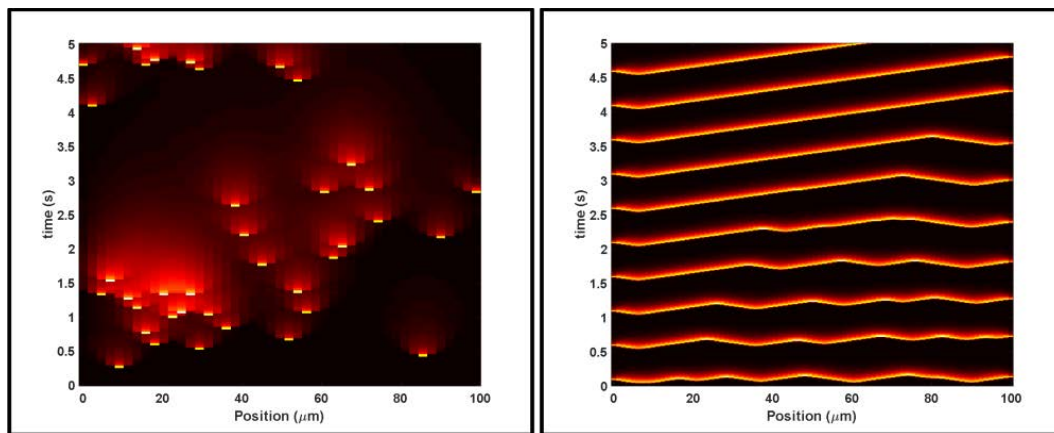


Figure 1: Ca^{2+} firing patterns as a function of IP3 concentration. At low levels of [IP3], transient sparks and flashes of Ca^{2+} can be seen, but no sustained waves (**Left**). At higher concentrations of IP3, the system is capable of sustaining long-range, regenerative waves (**Right**).

Development of Cardiac Specific Endothelial Cells to Support Vascularized Cardiac Organoids

Catherine S. Karpova, Guohao Dai

Department of Bioengineering, Northeastern University

Keywords: Cardiovascular, Endothelial Cells

Cardiovascular disease (CVDs) is the leading cause of death worldwide, as it claims the lives of 17.9 million people annually. This is because the myocardium is unable to regenerate on its own. Thus, there is a high demand for the development of an accurate human cardiac tissue model. It would allow for CVD modeling, drug testing, tissue repair, cell therapy and would eliminate the need for animal testing. Human pluripotent stem cells (hPSCs) derived cardiomyocytes (CMs) have shown great promise for the development of engineered cardiac tissue. Groups have demonstrated protocols that successfully differentiate hPSCs into CMs. However, current models have two major limitations: lack of vasculature and CM maturity. To address these limitations, we are creating a platform that would allow cardiac-specific ECs (CECs) to develop from hPSCs alongside the CMs. ECs aid in the development and maturation of CMs through direct cell-cell contact and angiocrine signals. MEOX2 and TCF15 are critical CEC transcription factors that control an array of genes responsible for fatty acid uptake in CECs. The upregulation of MEOX2 and TCF15 will facilitate the development of CECs, which will improve developed vasculature, CM functionality and CM maturation, forming a more accurate cardiac tissue model.

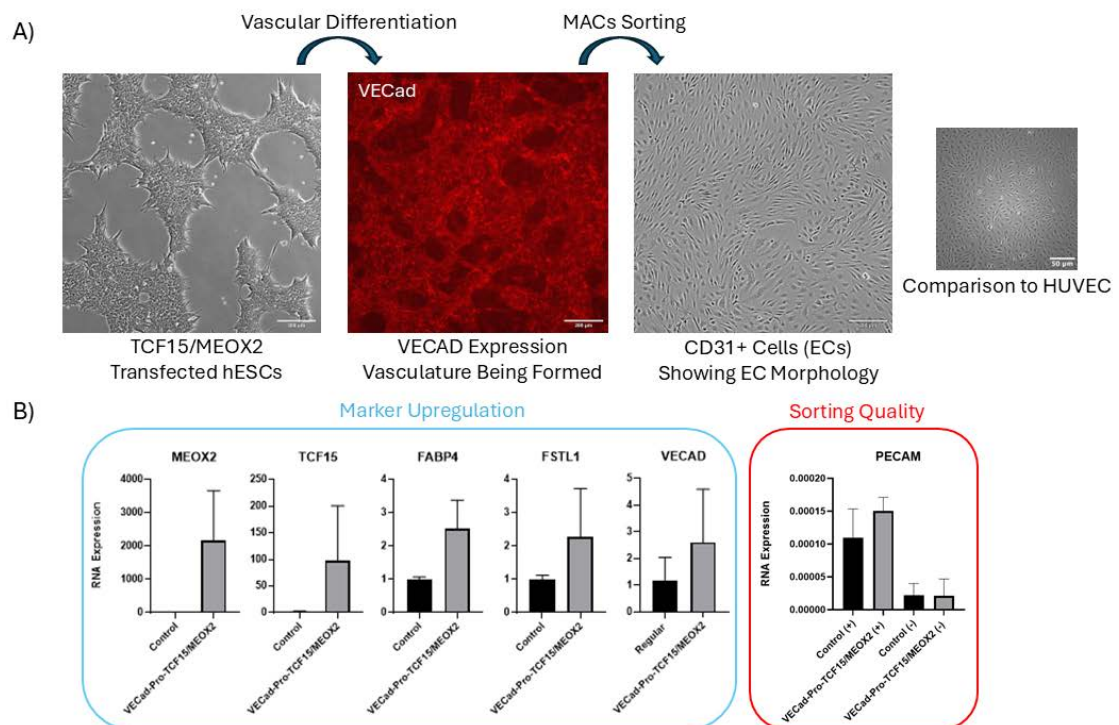


Figure 1: A) Live imaging throughout the development of CECs B) qRT-PCR data showing the upregulation of MEOX2 and TCF15 in the developing CECs

<i>Number</i>	<i>Presenters</i>	<i>Lab</i>
1	Stefanie DeFronzo	Dai
2	Aishwariya Dutta	Haase
3	Abigail Ecker	Dai
4	Narges Mirdamadi	Oakes
5	Aadhira Nair	Vahabikashi
6	Pratiksha Pawar	Haase
7	Abhishek Rampelli	Konry
8	Amrita Rhoads	Libby
9	Sogand Shayanfard	Oakes
10	Andrew Stein	Jain
11	Xinyi Yao	Bellini
12	Fan-Yu Yen	Fang
13	Connor Powell	Jain
14	Yuntao Li	Yaseen
15	Zixi Chen	Joy

Poster Presentations Abstracts

MORNING SESSION

Optimization of a Cas9-mediated endoglin knockout hPSC line for vascular disease modeling

Stefanie DeFronzo¹, Guohao Dai¹

¹Department of Bioengineering, Northeastern University

Hereditary hemorrhagic telangiectasia (HHT) is a vascular disorder caused by mutations in the endoglin gene (*ENG*), leading to dysregulated angiogenesis and malformed blood vessels. Current in vitro models rely on assembled vascular cell types rather than a fully self-derived human system, limiting their ability to replicate disease pathology. To address this, we aim to optimize a complete *ENG* knockout (KO) in human pluripotent stem cells (hPSCs) using a Cas9-mediated approach. Preliminary work using lentiviral Cas9 and multiple guide RNAs successfully, but partially, disrupted *ENG* expression in endothelial cells and hPSCs (Figure 1). To combat this issue, we will employ a non-lentiviral transfection method using a single plasmid containing dual gRNAs to improve editing efficiency and achieve complete loss of *ENG* expression (Figure 2). The resulting KO line will be differentiated into 3D vascular networks and assessed for hallmark HHT features, including disrupted endothelial junctions, impaired mural cell interactions, and leaky or malformed vessels. Gene expression, vascular function, and morphological metrics will be compared to healthy and patient-derived hPSC controls. Successful optimization of this *ENG*-KO hPSC platform will provide a powerful tool for modeling HHT and screening therapeutic interventions in an hPSC-derived physiologically relevant system.

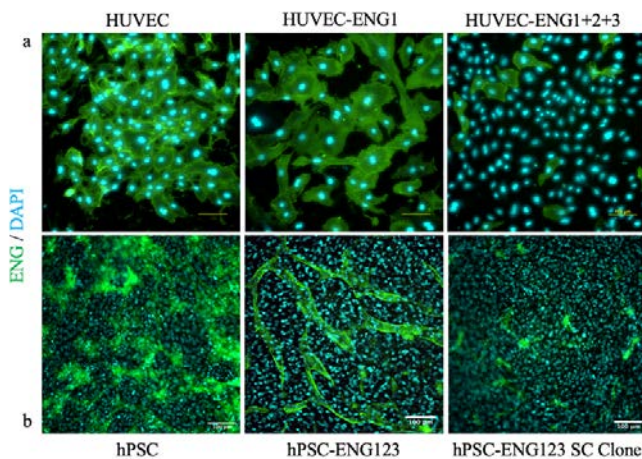


Figure 1. Cas9-Mediated Lentiviral Knockout of Endoglin in HUVECs and hPSCs. Fluorescent images of *ENG* in control HUVECs, HUVECs with one *ENG* gRNA, and HUVECs with three *ENG* gRNA. Scale bar 100um (a). *ENG* staining in control hPSCs, hPSCs with three *ENG* gRNA, and a single-cell clone of hPSCs with three gRNA. Scale bar 100um (b). *ENG* stain in green, DAPI in cyan.

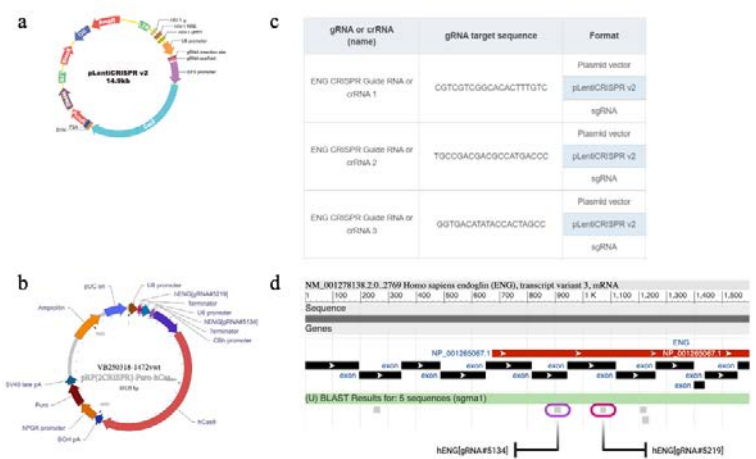


Figure 2. Optimizing CRISPR/Cas9 KO of *ENG* in hPSCs. Lentiviral vector (a) and gRNA sequences (c) of originally used method to generate *ENG*-KO HUVECs and hESCs. Non-viral Cas9 and gRNA co-expressing plasmid design for new method of *ENG* KO in hPSCs (b). Two new gRNAs (#5134 and #5219) depicted in the NCBI Blast of human *ENG* compared to the original gRNA locations (d).

Key words: stem cells, vascular tissue engineering, vascular disease

Microenvironmental regulators of leukemia expansion as potential therapeutic targets

Aishwariya Dutta¹, Shenglin Mei², Karin Gustafsson³, David Scadden³, Charles Lin⁴, and Christa Haase^{1,*}

(1) Department of Bioengineering and Physics, Northeastern University, Boston, USA

(2) Department of Biomedical Engineering, Virginia Tech, Virginia, USA

(3) Department of Stem Cell and Regenerative Biology, Harvard University, Boston, USA

(4) Wellman Center for Photomedicine, Mass General Research Institute, Boston, USA

**Correspondence: c.haase@northeastern.edu*

To investigate the microenvironmental factors contributing to early acute myeloid leukemia (AML) development, we conducted longitudinal intravital microscopy in a HoxA9/Meis1 mouse model of AML, which revealed pronounced spatial heterogeneities in early AML expansion. While certain microenvironments supported AML cell dormancy, others emerged as proliferation hotspots. To further explore the mechanisms driving this heterogeneous response and identify potential interactions between proliferating AML cells and their microenvironment that could be therapeutically targeted, we harvested both AML and stromal cells from these distinct microenvironments using Image-seq, an image-guided single-cell sequencing technology we previously developed [1].

Following single-cell RNA sequencing, we performed comprehensive ligand-receptor interaction analysis, revealing fundamentally different communication networks between dormant and proliferative states. Dormant AML cells interact with blood vessels and bone marrow stromal cells via signaling molecules (CXCL12, KITL, PGF, MIF, OSM). In contrast, proliferative AML cells reorganize their surroundings to create supportive scaffolding rich in collagen and adhesion proteins (Collagen IV/I, MDK, JAG1/EFN), sending growth signals to blood vessel cells through angiogenic factors (VEGFB, TNF, TGF β) and proteases (CTSG).

To validate the functional significance of these interactions and their potential as novel therapeutic targets, we will utilize an MLL-AF9 AML transplantation model [2] and perform gene knockout of critical ligands/receptors on AML cells. To this end, we have additionally identified the minimum effective radiation dose that ensures robust leukemic engraftment while preserving bone marrow microenvironment integrity and implemented relevant flow cytometry protocols for analyzing leukemic burden.

Keyword: Acute Myeloid Leukemia (AML), Bone marrow microenvironment, Single-cell RNA sequencing, Ligand-receptor interaction, Gene knockout

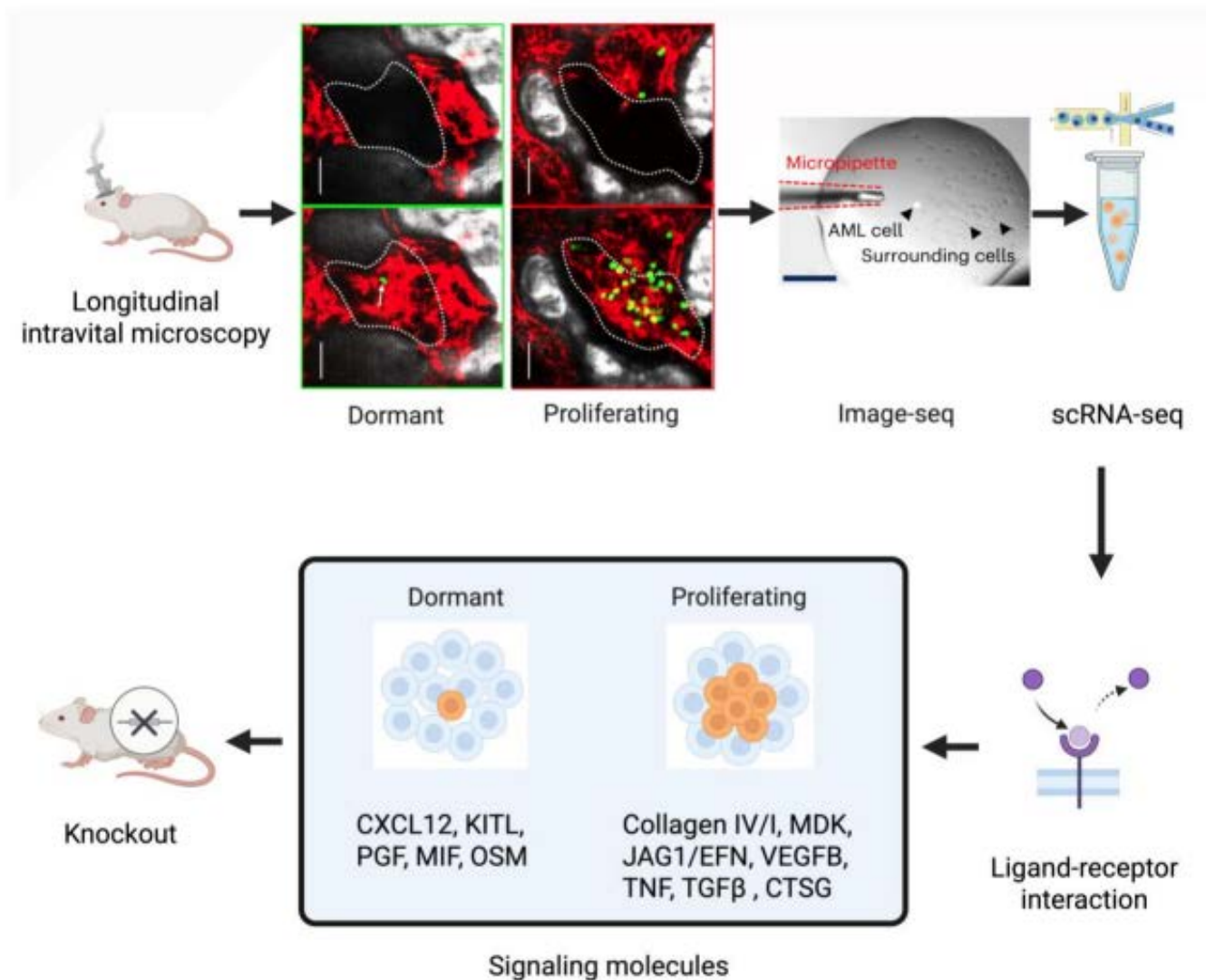


Figure 1: Longitudinal tracking and Ligand-receptor interaction of dormant versus proliferating AML cells

References:

- [1] Haase, C., Gustafsson, K., Mei, S., Yeh, S.C., Richter, D., Milosevic, J., Turcotte, R., Kharchenko, P.V., Sykes, D.B., Scadden, D.T. and Lin, C.P., 2022. Image-seq: spatially resolved single-cell sequencing guided by in situ and in vivo imaging. *Nature Methods*, 19(12), pp.1622-1633.
- [2] Long, W.Y., Shen, X., Xing, S., Xiong, G.L., Wang, H.G. and Yu, Z.Y., 2021. Establishment of AML Mouse Model by Transplantation of Hematopoietic Cells from MLL-AF9 Transgenic Mice. *Zhongguo shi yan xue ye xue za zhi*, 29(5), pp.1369-1374.

Development of an *in vitro* neurovascular niche model to study Alzheimer's Disease

Abigail Ecker, Nicole Rodriguez, Guohao Dai

Rodent models are the current gold-standard for preclinical drug efficacy assessment but are unable to accurately model certain human-specific brain diseases, such as Alzheimer's Disease (AD): until 2021, the failure rate of disease-altering treatments for AD in clinical trials was 100%. Recently, there has been a push to develop humanized *in vitro* neurovascular niche (NVN) models to address this translation crisis and assist in pre-clinical efficacy assessment. The NVN is a brain microenvironment that contains microvasculature formed by brain microvascular endothelial cells (BMECs) supported by pericytes (PCs) and astrocytes (ACs) and neighboring neurons and microglia. Previously, we developed an *in vitro* NVN model in a microfluidic device platform (MFD) with microvascular networks supported by ACs and PCs, and neural stem cells (NSCs) which directly differentiate into physiologically-active neurons inside the model. Here we present our efforts to increase the survival, proliferation, and maturation of neurons within this model by adding different neuron-specific media supplements such as neurotrophin-3 (NT3), B27+insulin (B27i), and a combination of small molecules meant to accelerate neuronal maturation, nicknamed GENToniK (GT). Additionally, we present our efforts to install an AD-specific single-point mutation into stem cells that can be differentiated to neurons, effectively developing an AD cell line that can be incorporated to model an AD-NVN for future exploration.

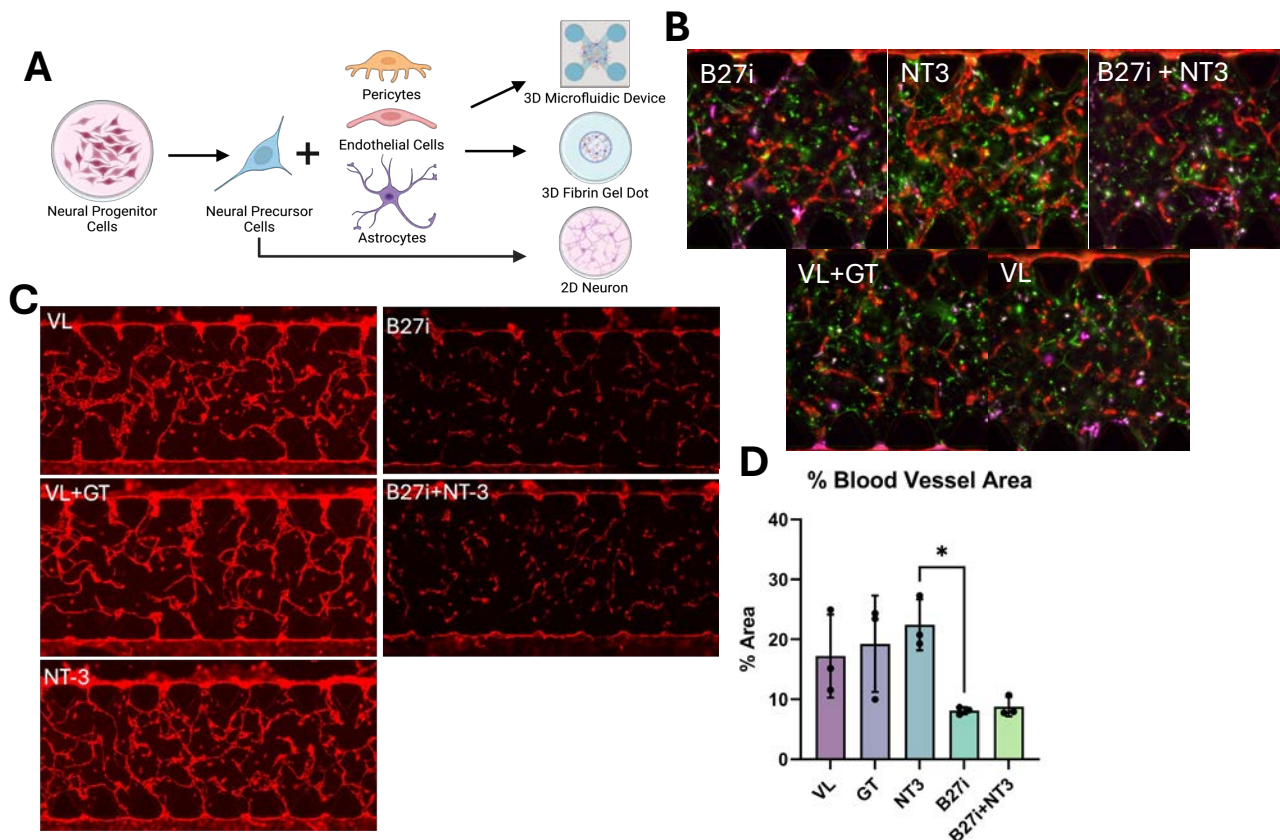


Figure 1: Neuron-specific media supplement impact on an *in vitro* NVN model. A: schematic overview of experiment seeding. B: B27 + insulin, NT3, combo, GT, and VasculiLife control all supported formation of TuJ1+ neurons. Vasculature is shown in red, neurons in green, and ACs stained for GFAP in magenta. C: B27i hinders vasculature formation, while NT3 and GT conditions support vessel formation. D: Quantification of percent vessel area calculated from red area over total area within MFD gel channel. Statistics performed using one-way ANOVA, *p<0.05.

Prediction of Total and Regional Deposition Efficiency of Surfactant Particles in the Preterm Neonate Respiratory System

Narges Mirdamadi¹, Tianyi Lee¹, Chiara Bellini¹, Robert DiBlasi^{2,3}, Jessica Oakes¹

- (1) Department of Bioengineering, Northeastern University, Boston, MA, USA
- (2) Department of Respiratory Care Therapy, Seattle Children's Hospital, Seattle, WA 98105, USA
- (3) Center for Respiratory Biology and Therapeutics, Seattle Children's Research Institute, Seattle, WA 98101, USA

Neonatal Respiratory Distress Syndrome (NRDS) in preterm infants is caused by insufficient surfactant, leading to alveolar collapse and impaired breathing. Aerosolized surfactant therapy offers a non-invasive treatment, but delivery efficiency is low. This study introduces both one-dimensional (1D) and three-dimensional (3D) Computational Fluid and Particle Dynamics (CFPD) models to simulate aerosol transport and deposition in neonatal airways using realistic lung geometries.

The 1D model uses an implicit finite volume advection-diffusion scheme to simulate surfactant dynamics in a conduit-alveoli system, accounting for axial flow, radial exchange, and deposition mechanisms including impaction, sedimentation, and diffusion. Model predictions were validated against existing adult experimental and computational data, confirming its reliability. In parallel, 3D airway geometries reconstructed from neonatal MRI/CT scans were used to simulate airflow using the Navier-Stokes equations and particle transport via a modified Maxey-Riley approach. Results from the 1D and 3D models showed strong agreement in deposition patterns within the upper-airways for 1–5 μm particle sizes.

Results demonstrate increased overall deposition with larger particles (up to 100% for 5 μm) but reduced alveolar delivery (38% at 1 μm to 19% at 5 μm). These findings highlight the need for smaller particles to achieve effective deep-lung surfactant delivery. Future work will include subject variability and delivery optimization.

Keywords: NRDS, Aerosolized surfactant therapy, CFPD model, 3D Airway Reconstruction, Particle Deposition, Alveolar Delivery, Delivery Optimization

Acknowledgements: Bill and Melinda Gates Foundation (INV-018835-5).

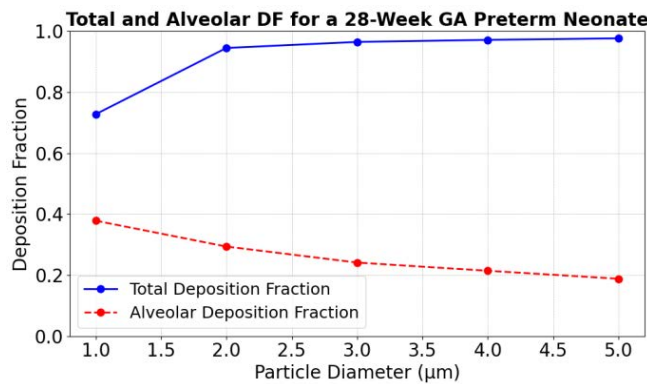


Fig. 1: Total and Alveolar Deposition fraction in the Respiratory Tract of a 28-Week Gestational Age Preterm Neonate. This figure illustrates nasal breathing deposition patterns for particle diameters between 1 μm and 5 μm , which are relevant for surfactant delivery. The simulation is based on a tidal volume of 7 mL, an FRC of 23 mL, and a flow rate of 28 mL/s.

Glaucoma-on-a-Chip: An In Vitro Model for Schlemm's Canal Endothelial Barrier Function**Aadhira Nair, Amir Vahabikashi****Department of Bioengineering, Northeastern University****Abstract:**

Glaucoma is a leading cause of irreversible blindness, driven by increased resistance to aqueous humor outflow in the trabecular meshwork and Schlemm's canal (SC) endothelium. Conventional models fail to replicate the biomechanics of this system, highlighting the need for physiologically relevant in vitro systems. To address this, we are developing a microfluidic "glaucoma-on-a-chip" device that replicates SC endothelium under controlled pressure and physiological conditions.

The device includes a biocompatible, transparent porous elastomeric membrane mimicking SC endothelium pore dimensions and densities. It features an upper chamber with inlet/outlet ports for fluid flow and a lower channel for collecting fluid. Microfluidic channels establish physiological pressure gradients (5–6.5 mmHg) across the SC endothelium, enabling studies of endothelial permeability and biomechanics. Once the SC endothelial monolayer forms, we plan to use fluorescent tracer assays to evaluate the device's performance and the effects of drugs on barrier function under controlled flow conditions. This scalable platform supports high-throughput testing of SC-targeted therapeutics, reducing reliance on animal models.

Our glaucoma-on-a-chip device represents a significant advancement in modelling the pathophysiology of glaucoma, hence offering a versatile tool for mechanistic studies and drug development against this pervasive disease.

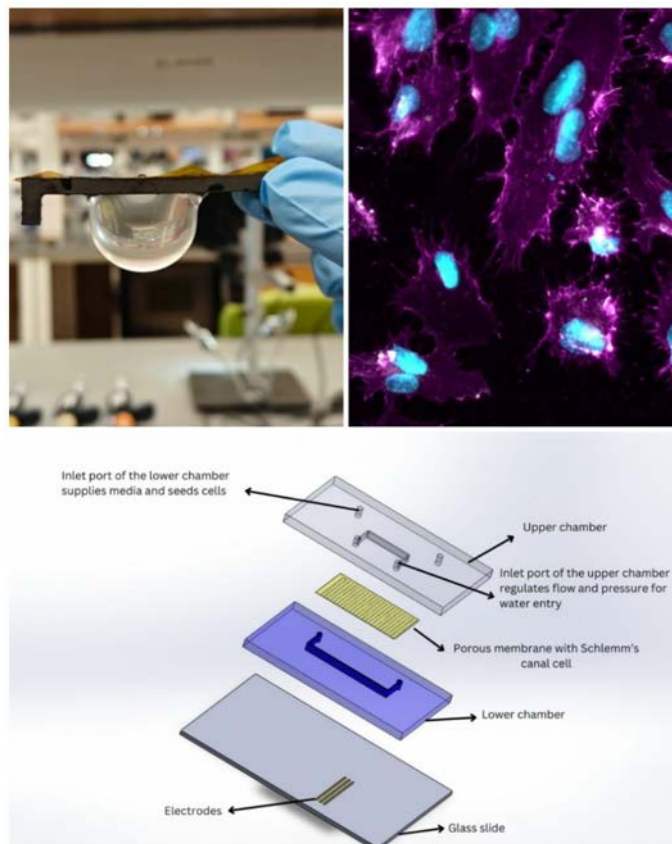


Fig 1- a) Ecoflex membrane b) Immunostained Huvecs c) Glaucoma Chip

RADIATION CONDITIONING REFINEMENT FOR MLL-AF9-CAS9 CELL ENGRAFTMENT IN A MURINE AML MODEL

Pratiksha Pawar¹, Aishwariya Dutta¹, David Sykes², Christa Haase¹

¹Department of Bioengineering, Northeastern University, Boston, USA.

²Massachusetts General Hospital, Boston, USA.

Abstract:

Acute myeloid leukemia (AML) is an aggressive bone marrow cancer that has a 5-year survival rate of less than 32% (Shimony et al., 2025). Our goal is to use CRISPR/Cas9 genome editing in a mouse model to validate candidate therapeutic targets identified through spatial transcriptomic analysis. We employ an MLL-AF9 transplantation model in which AML cells constitutively express Cas9 and GFP, enabling efficient gene editing and assessment of leukemic burden via imaging and flow cytometry (Haase et al., 2022). To support this, we identified the minimum effective radiation dose that ensures robust leukemic engraftment while preserving bone marrow microenvironment integrity. We systematically tested various irradiation doses and cell numbers to optimize engraftment conditions in C57BL/6 mice, minimizing radiation-induced inflammation and ensuring maintenance of niche architecture. Additionally, we performed kill curve assays to assess the blasticidin sensitivity of MLL-AF9-Cas9 cells, which will be used for selecting genetically modified populations. Together, these efforts have established a robust experimental platform for evaluating novel therapeutic targets against AML.

Reference:

- Haase, C., Gustafsson, K., Mei, S., Yeh, S. C., Richter, D., Milosevic, J., Turcotte, R., Kharchenko, P. V., Sykes, D. B., Scadden, D. T., & Lin, C. P. (2022). Image-seq: spatially resolved single-cell sequencing guided by in situ and in vivo imaging. *Nat Methods*, 19(12), 1622-1633. <https://doi.org/10.1038/s41592-022-01673-2>
- Shimony, S., Stahl, M., & Stone, R. M. (2025). Acute Myeloid Leukemia: 2025 Update on Diagnosis, Risk-Stratification, and Management. *Am J Hematol*, 100(5), 860-891. <https://doi.org/10.1002/ajh.27625>

Enriching immunotherapies for serial-killing cytotoxic cells

Abhi Rampelli

Department of Bioengineering, Northeastern University

While cancer immunotherapies have transformed treatment for many patients, their effectiveness remains inconsistent due to variability in cytotoxic cell potency and cancer cell resistance. A major challenge is the inability to identify the rare natural killer and T cells capable of serial killing, a process in which one immune cell eliminates multiple cancer cells. These cells play a critical role in driving durable therapeutic responses, yet current technologies cannot efficiently detect or isolate them. This project addresses that need by developing a droplet-based microfluidic platform that uses real-time intra-droplet cell counting to track immune cell function and isolate the most potent cytotoxic cells based on the number of cancer cells they eliminate. Beyond its clinical applications, this platform introduces a new analytical tool for microfluidics by enabling the profiling of single-cell interactions within droplets. It has the potential to significantly improve the precision and efficiency of immune cell therapy development while advancing the study of complex cell behaviors in confined, tunable microscale systems.

Keywords: Immuno-oncology, droplet microfluidics, high-throughput screening, functional cell sorting

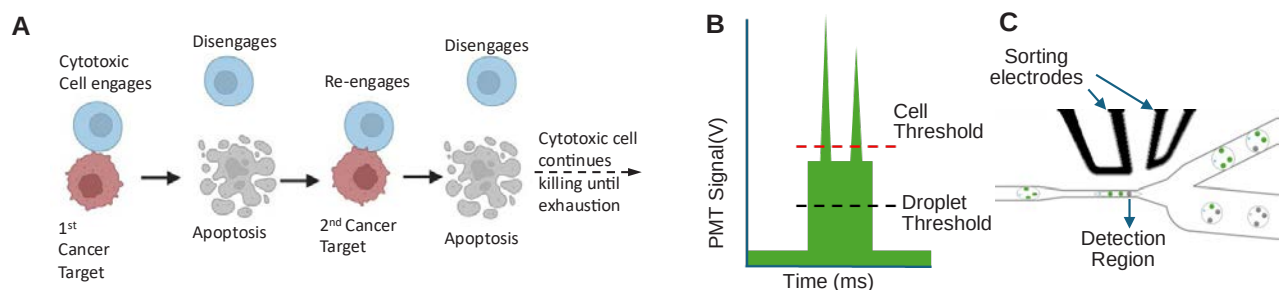


Figure 1: A) Cytotoxic cell serially killing cancer cells B) Representation of droplet and cell threshold on fluorescence signal at C) sorting junction with narrow channel for single cell detection.

DEVELOPMENT OF A KINASE IDR MUTANT LIBRARY TO INVESTIGATE ANTIBIOTIC RESISTANCE

Amrita Rhoads¹, Noam Grunfeld¹, Erel Levine¹, Elizabeth Libby¹

¹Department of Bioengineering, Northeastern University

Bacterial Hanks-type Serine/Threonine kinases and their partner phosphatases are major regulators of intrinsic antibiotic resistance in diverse bacterial species. In the highly genetically tractable organism *Bacillus subtilis*, the kinase PrkC has been shown to respond to antibiotic treatment via phosphorylation of an essential transcription factor and subsequent regulation of downstream enzymes. However, while the mechanism underlying kinase activation in response to antibiotic treatment is currently unknown, we have demonstrated it is strongly dependent on a currently uncharacterized intrinsically disordered region (IDR) of the kinase. While the cytoplasmic kinase domain of PrkC exhibits strong sequence and structural homology to eukaryotic protein kinases, relatively little is known about the adjacent juxtamembrane IDR, which we have previously found to be essential for kinase activation *in vivo*. To assess the impact of individual residues on kinase function and antibiotic resistance, we generated a library of single-residue mutants across the IDR. Kinase activity was measured with both single-cell fluorescent reporters and β -lactam susceptibility assays, allowing us to create a quantitative model of residue contribution to kinase activity and antibiotic resistance. This work will elucidate the sequence-function of IDR motifs in bacterial kinases, including key features that may present novel targets for antimicrobial development and synthetic biology applications.

Keywords: antibiotic resistance, bacterial kinase, bacterial phosphatase

TOXICOCHEMICAL PROFILING OF DUAL CIGARETTE AND E-CIGARETTE EXPOSURES AND THEIR IMPACT ON PULMONARY FUNCTION OF FEMALE APOE-/- MICE

Sanika Shingwekar*, Sogand Shayanfard*, Abir Saha, Shang Liu, Chiara Bellini, Jessica M. Oakes

*authors contributed equally

Abstract:

The growing popularity of electronic nicotine delivery systems (ENDS) has been accompanied by a significant rise in the dual use of both conventional cigarettes and e-cigarettes. While each product is independently known to impair pulmonary function and structure, and to alter inflammatory and immune responses, the consequences of their combined, long-term use remain poorly understood. Therefore, in this study, we aimed to investigate the effects of chronic dual exposure, following long-term cigarette use, by first subjecting female ApoE-/- mice to cigarette smoke for 24-weeks followed by either cigarette smoke, filtered air, e-cigarette aerosols, or combined e-cigarette aerosols and cigarette smoke for an additional 24 weeks. To assess airway hyperresponsiveness (AHR) and subsequent bronchoconstriction as exposure duration increased, we performed a methacholine challenge on conscious mice using double-chamber plethysmography (Scireq). Respiratory parameters such as specific airway resistance (SRaw), mid-tidal expiratory volume (EF50), and breathing frequency (f) were recorded across increasing methacholine doses. Actively, we measured respiratory rate (RR), tidal volume (TV), minute volume (MV), and breathing waveform patterns by whole body plethysmography in mice exposed to smoke for 1 to 6 months. Furthermore, to inform the computational aspect of our study, we directly measured key input parameters such as particle size distributions and the chemical composition of cigarette and e-cigarette aerosols. These data are critical for developing inhalation models that predict particle deposition and respiratory impact in dual-use scenarios, enabling linkage between adverse health outcomes and deposited dose.

Impact of Direct RNA Sequencing Chemistry Updates on RNA Modification Detection

Authors

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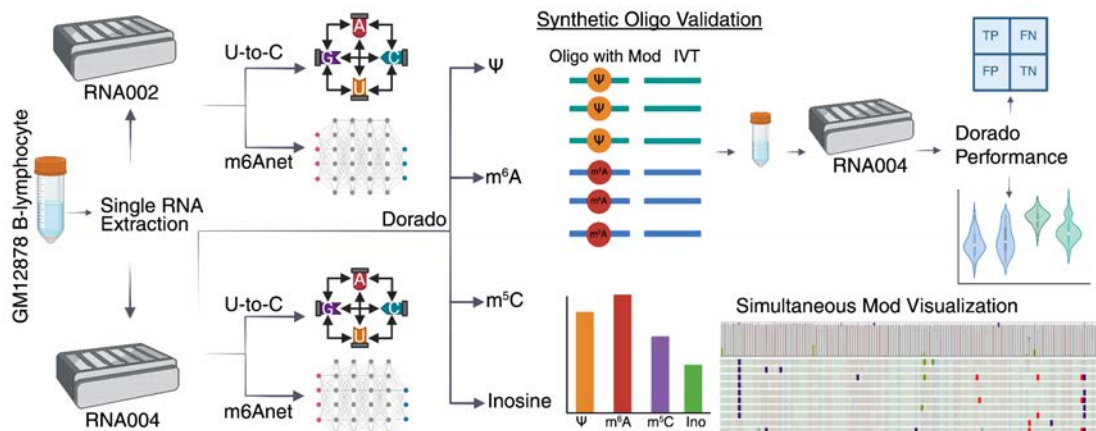
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Keywords: Direct RNA Sequencing, RNA Modification, Nanopore Sequencing, Modification Calling Performance

Nanopore technology can directly sequence intact RNA molecules, offering a unique capability to recognize native modifications. Oxford Nanopore Technologies recently updated its Direct RNA Sequencing technology from RNA002 to RNA004 sequencing chemistry. This update included an updated basecaller (Dorado) for increased sequencing accuracy, and ionic current models for *de novo* detection of four RNA modifications. Using a single RNA extraction from GM12878 B-lymphocytes, we compared RNA002 and RNA004 sequencing chemistries and evaluated Dorado's modification calling accuracy. We computed U-to-C mismatches, previously used to identify pseudouridine, and ran m6Anet for identifying N6-methyladenosine. We documented site-specific differences between RNA002 and RNA004 for both U-to-C mismatches and m⁶A occupancy. Dorado results for each respective modification showed both global and site-specific differences in occupancy when compared with RNA002. We also sequenced true-positive synthetic and corresponding true-negative IVT oligonucleotides for 6 sites (3 pseudouridine; 3 m⁶A) to validate site-specific Dorado modification calling. Dorado pseudouridine model achieved 96–98% for both accuracy and F1-score with a ~1% false positive rate. Similarly, Dorado m⁶A model achieved 94–98% accuracy, 96–99% F1-score, and a ~1–2% false positive rate. Our results demonstrate that Nanopore DRS can simultaneously detect multiple RNA modifications on individual mRNA strands.



An Experimental & Computational Framework for Dual Cigarette and E-cigarette Exposures in Apoe^{-/-} Mice

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Abstract

Despite marketing claims, e-cigarettes' health effects, particularly in dual use with conventional cigarettes, are not fully understood. This study presents an integrated framework to simulate dual cigarette and e-cigarette exposures with equivalent cotinine bioavailability in Apoe^{-/-} mice. We developed standardized exposure protocols by characterizing cotinine pharmacokinetics following cigarette smoke and e-cigarette aerosol exposures. Blood samples collected at multiple timepoints enabled quantification of serum cotinine concentrations via LC-MS/MS. Using a time-variant linear model, we estimated cotinine survival functions and pharmacokinetic parameters, revealing distinct profiles between exposure types. E-cigarette exposure resulted in higher cotinine levels compared to cigarette smoke, driven by greater nicotine intake and faster metabolism.

Through systematic optimization, we established 56-minute equivalent-AUC exposure protocols (cigarette: 2-second puffs, 2 puffs/minute, 35 mL; e-cigarette: 2-second puffs, 1 puff/3 minutes, 55 mL). Additionally, dose-response studies demonstrated linear relationships between nicotine dose and cotinine AUC for both exposure types, allowing proportional adjustment of exposure durations within the 56-minute window to simulate different dual-use scenarios.

Our approach ensures that observed outcome differences are attributable to product type rather than nicotine dose variability, while isolating nicotine's effects from other smoke or aerosol constituents. This framework provides a foundation for investigating health effects across various exposure scenarios.

Keywords: Cotinine pharmacokinetics, dual-use exposure, tobacco-related toxicology

Natural language driven meta-analysis of neuroimaging data and automated processing pipelines

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We present a proof-of-concept demonstrating the automated query, retrieval, and processing of neuroimaging datasets hosted on NeuroJSON.io [1] using natural language user prompts. Leveraging large language model (LLM) based neural networks, user prompts are converted into machine-executable commands that interfaces with the document-oriented database engines on NeuroJSON.io, enabling streamlined data search, download, and processing. Specifically, our prototype utilizes an LLM in conjunction with LangChain [2] and Pydantic [3] to create structured outputs in the JavaScript Object Notation (JSON) format. Our system retrieves diverse neuroimaging datasets based on user-specified criteria such as age, gender, and modality. We then automate image segmentation, 3-D full-head mesh generation using Brain2Mesh [5], and perform quantitative mesh-based Monte Carlo (MMC) [6] light simulations.

A Python-based script was developed and was able to successfully convert diverse user prompts into actionable structured commands, based on which, the specified datasets were downloaded, pre-processed and used for image processing. This framework offers scalable, artificial intelligence assisted neuroimaging data analysis, significantly lowering barriers in neuroimaging research. Furthermore, it can be readily extended to include diverse existing toolboxes and software packages, showcasing its flexibility and potential for widespread adoption in the neuroimaging community. Our work highlights a novel integration of LLMs with neuroimaging databases, advancing user accessibility and efficiency in large-scale neuroinformatics research.

Keywords: Data analysis pipeline, Large language model, Meta-analysis, Neuroimaging, Neuroinformatics

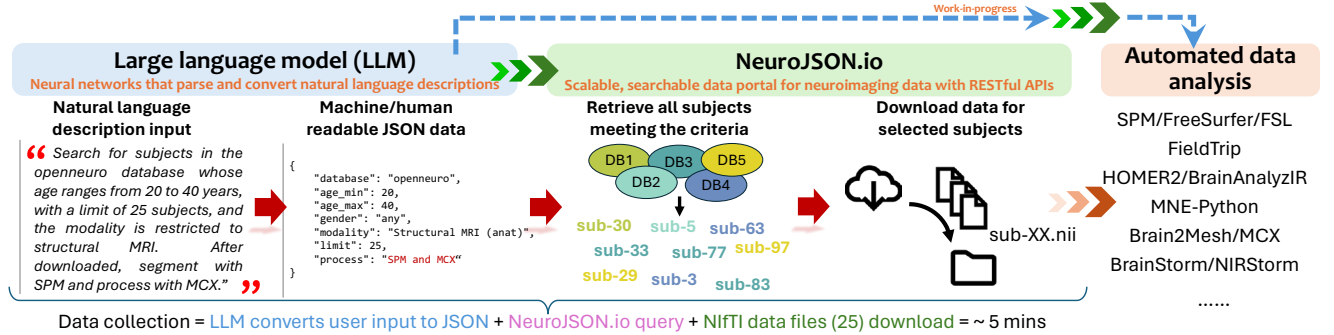


Fig. 1. LLM-powered neuroimaging data analysis using datasets from NeuroJSON.io

Reference:

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5. Tran A†, Yan S†, Fang Q*, "Improving model-based fNIRS analysis using mesh-based anatomical and light-transport models. *Neurophotonics*," 7(1), 015008 (2020)
6. Yuan Y, Yan S, and Fang Q*, "Light transport modeling in highly complex tissues using the implicit mesh-based Monte Carlo algorithm," *Biomed. Optics Express*, 12(1), 147-161, (2021)

RNA modification atlas at isoform resolution in axolotl limb regeneration

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Keywords: isoforms, methylation, nanopore, direct RNA, regeneration

Limb regeneration is a complex process composed of multiple unique stages defined as: initial formation of a wound epidermis, progenitor cell mobilization, progenitor pool expansion, resolution, differentiation, and growth. Each stage utilizes specific genetic signaling pathways, leading to significant differential gene expression between homeostatic and regenerating conditions. While many studies have described active signaling pathways during regeneration by means of functional experiments and RNA-seq data, none have explored the implication of RNA modifications in limb regeneration despite existing literature documenting RNA modifications as a result of cell stress.

We present a pilot study in which we performed direct RNA sequencing (DRS) on axolotl intact limb and 7-day post amputation (dpa) blastema samples to create a map of isoform level expression along with differential RNA modification expression between conditions (Figure 1). Recent advances in DRS have improved accuracy, allowing for refined isoform detection in addition to calling 4 modifications on native RNA molecules. This will be a first look at how RNA modifications such as: i) Adenosine-to-Inosine methylation events in coding regions and stop codons; ii) pseudouridine associated readthrough events in stop codons; and iii) N6-methyladenosine and 5-methylcytosine editing events in mRNA may play a role in early blastema formation.

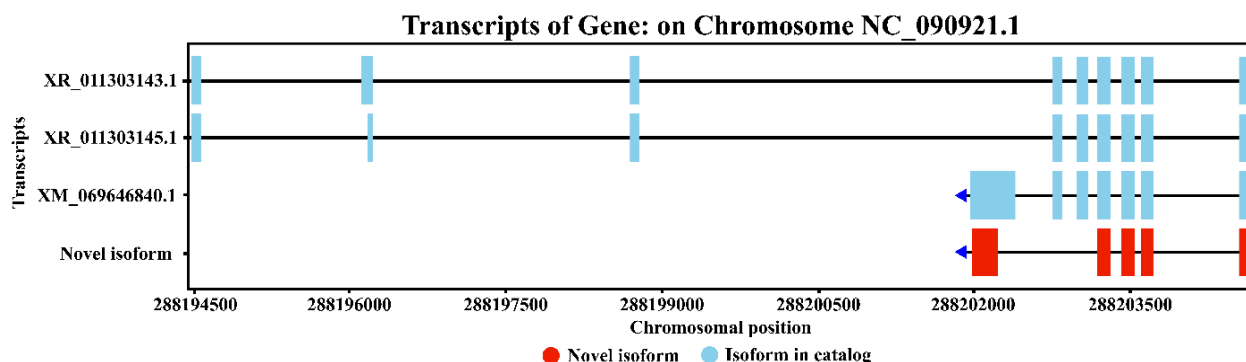


Figure 1: Isoform level analysis of cold-inducible RNA-binding protein (*Cirbp*).

Cirbp is significantly modulated in regeneration of multiple different organs in axolotl such as limb and pancreas. Our sequencing data reveals a novel isoform of *Cirbp*, not present in the current axolotl genome assembly. In addition to characterizing novel isoforms and their implications in protein translation, we will present differential RNA modification data at isoform resolution.

An integrated platform for real-time monitoring animal physiology and behavior during intravital brain imaging

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ABSTRACT

We present an integrated platform that records behavioral data and measures pupillometry in real time, thereby providing complementary information on brain alertness and detecting animal motion during intravital brain imaging. The integrated device was successfully coupled with 2-photon microscopy and widefield intrinsic signal imaging for awake mouse imaging. The results demonstrate that the device enables rapid screening of imaging trials impaired by motion artifacts. We also observed a strong correlation between cellular and microvascular activity in the cortex and pupil diameter during functional activation. The results suggest that our device streamlines imaging setup, reduces imaging time, and provides evidence that pupil size can be used as a readout of brain activity.

Keywords: Motion Detection, Pupillometry, Functional Brain Imaging, Two-Photon Microscopy, Optical Intrinsic Signal Imaging

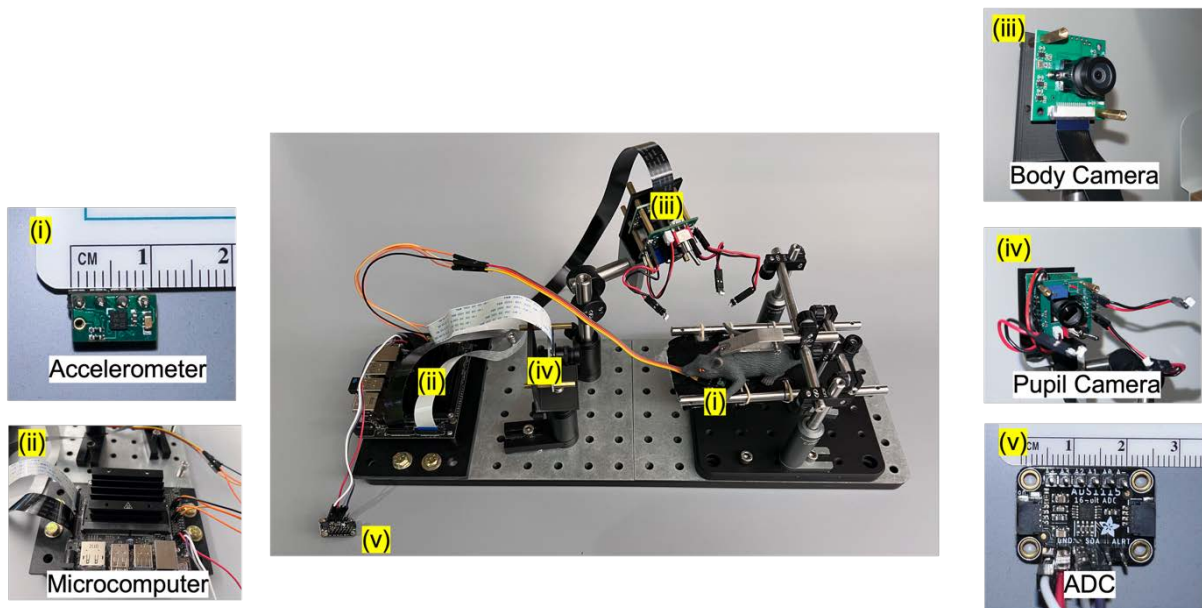


Figure 1. (A) Schematic of the integrated platform.

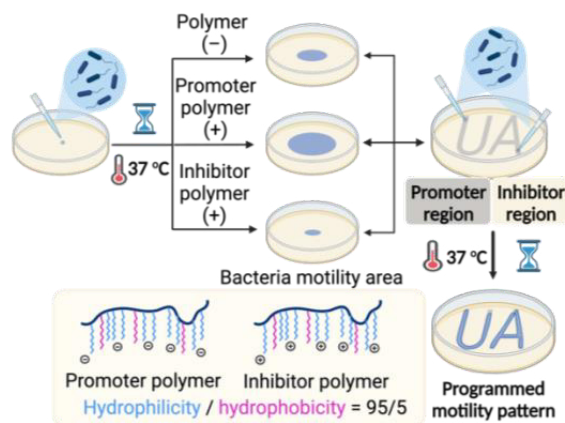
Programming Surface Motility and Modulating Physiological Behaviors of Bacteria via Biosurfactant-Mimetic Polyurethanes

Zixi Chen^{1,2}, Apoorva Vishwakarma¹, Abraham Joy^{*1,2}

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Controlling bacterial behaviors benefits both infection prevention and biotechnological applications, such as biomacromolecule and small-molecule production in manufacturing. To achieve this goal, researchers traditionally rely on complex genetic modifications or microscale physical barriers. However, these methods are time-intensive and technically demanding, limiting their broader applications. Since bacteria naturally respond to environmental stimuli, this creates opportunities for simpler extracellular approaches. Herein, we synthesize water-soluble, low molecular weight polyurethanes (~10 kDa) that mimic bacterial biosurfactants through step-growth polymerization with variable functional groups (-OH, -COOH, -NH₂). We use *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Escherichia coli* (*E. coli*) in bacterial surface motility assays to measure the influence of polyurethane compositions on bacterial surface swarming and twitching. Meanwhile, we also investigate the effects of polymers on bacterial surface physiological behaviors, including bacterial growth, messenger production, and extracellular polymeric substance (EPS) production. We find that carboxyl-functionalized polyurethanes dramatically enhance bacterial swarming—17-fold for *P. aeruginosa* and 13-fold for *E. coli*—while outperforming commercial surfactants, whereas amine-functionalized variants reduce *P. aeruginosa* swarming by 58%. By leveraging the inhibition and promotion effects from -NH₂ and -COOH-containing polymers, we achieve spatial programming of bacterial migration into designed macroscale "UA" patterns. Carboxyl polymers significantly increase EPS production for potential biomacromolecule manufacturing. This biosurfactant-mimetic polyurethane platform serves as a simpler means of controlling microbial systems while enhancing the downstream effects of EPS production. Our findings contribute significantly to combining genetic engineering methods to design more valuable material matrices that support engineered living materials (ELMs).



<i>Number</i>	<i>Presenters</i>	<i>Lab</i>
1	Kelvin Amakye	Haase
2	Bryce Axe	Ferris
3	Neda Ghohabi Esfahani	Jain
4	Tina Thuy Nguyen Hoang	Chung
5	Prathamesh Mane	Joy
6	Sanika Shingwekar	Oakes
7	Nathaniel Silvia	Dai
8	Yihan Wei	Libby
9	Yao Li	Zhang
10	Charbel Yazbeck	Oakes
11	Hannah Wilker	Bellini
12	Jingxin Wang	Haase
13	Hengli Zhang	Bajpayee

Poster Presentations Abstracts

AFTERNOON SESSION

Towards the development of a three-photon microscope with an integrated spatial light modulator for deep imaging and optogenetics within the bone

Kelvin Amakye¹, Vincent De Sars², Eirini Papagiakoumou², Christa Haase¹

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²Institut de la Vision (Inserm), Paris

Abstract

Intravital microscopy of the bone marrow has provided valuable insights into hematopoietic stem cell (HSC) biology and blood cancer progression. However, the highly scattering nature of bone tissue has limited its application to calvarium bone marrow. Nevertheless, emerging evidence suggests unprecedented heterogeneity in the behavior of individual bones¹⁻⁴. To uncover potential differences in the dynamics between these bones, we propose employing three-photon microscopy, which enables increased imaging depth compared to two-photon or single-photon approaches⁵. We propose to design a three-photon microscope with a spatial light modulator to image the tibia bone marrow in live animals while performing optogenetic manipulations to induce specific genetic changes in HSCs in vivo and concurrently monitor their crosstalk with neighboring cells. Our preliminary simulations identified the optimal microscope layout and the maximum achievable field of view. Here, we introduce the optical layout of our newly constructed three-photon microscope and present our first images. Additionally, we have constructed a cost-effective optical system using a Spatial Light Modulator, which can be integrated into the three-photon microscope. This minimally invasive tool will allow us to study cellular interactions in bone marrow microenvironments and will provide novel insights into mechanisms underlying Acute Myeloid Leukemia and HSC biology.

Keywords: Intravital Microscopy, Hematopoietic Stem Cell biology, Calvarium bone marrow, Tibia bone marrow, Three-photon Microscope, Spatial Light Modulator, Acute Myeloid Leukemia.

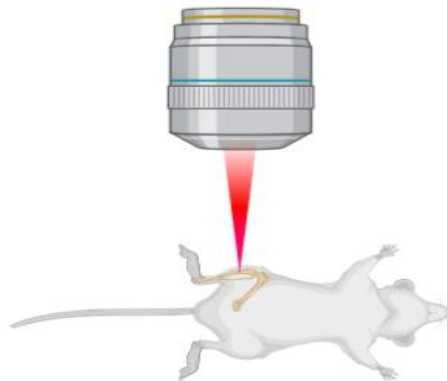


Figure 1. Illustration of tibia bone marrow intravital microscopy in a live mouse

MYELIN WATER FRACTION MRI FOR IN-VIVO QUANTIFICATION OF MYELINATION IN PRECLINICAL MODELS

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Quantitative in-vivo imaging of myelination remains challenging in preclinical models. Myelin Water Fraction (MWF) MRI-imaging provides a direct measure of myelin by isolating signals from water trapped within myelin sheaths, differentiating it from intracellular/extracellular water and cerebrospinal fluid. Unlike indirect methods such as diffusion-weighted imaging (DWI), MWF is less influenced by confounding factors like neuroinflammation.

We developed a high-resolution MWF protocol using a Multi-Slice Multi-Echo (MSME) sequence on a Bruker BioSpec 70/20 MRI, with optimized multiexponential T2 fitting (NLLS, Laplace inversion) and B1-field mapping for accurate signal interpretation. With 30 echoes per interval our approach achieved an in-plane resolution of 143 μm , facilitating precise separation of myelin water signals. In an upcoming validation study, adult Sprague-Dawley rats will either be designated as healthy controls or treated with cuprizone to model multiple sclerosis-related demyelination. Imaging results will be compared to ex-vivo histological (Luxol Fast Blue) and molecular (MBP, MOG Western blot) analyses to create correlation maps to validate our methodology.

Preliminary findings demonstrate that our refined MWF protocol accurately detect region-specific myelination. This method thus may offer a reliable tool for tracking demyelination and remyelination in preclinical studies, supporting future therapeutic investigations targeting myelin pathology.

Keywords: Myelin Water Fraction (MWF), Preclinical MRI, Myelination, Multiple Sclerosis (MS), Quantitative Imaging, T2 Relaxation, MRI Methodology, White Matter

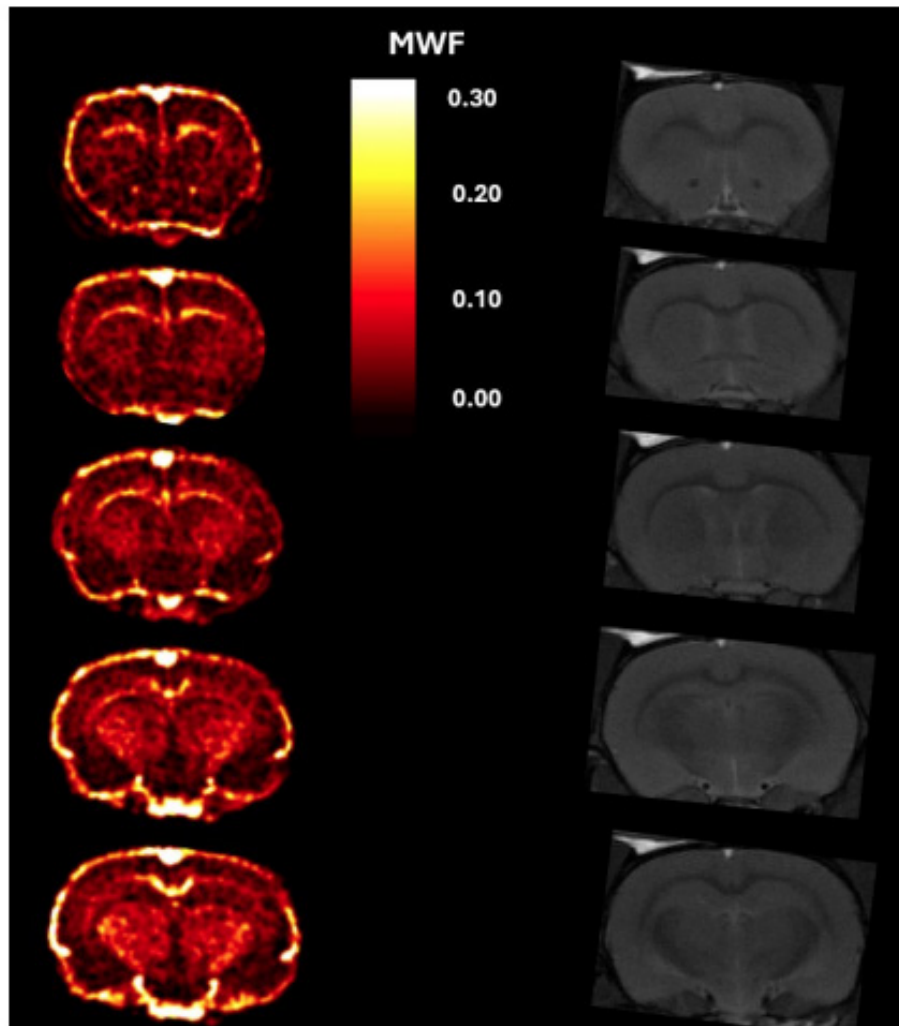


Figure 1: Preliminary MWF Map (TR = 2800 ms, TE = 7 ms) of control SD adult rat contrasted to T2-Weighted images.

Axe Continued.

Combining Nanopore Direct RNA Sequencing with Mass Spectrometry for Modification Analysis of Bacteriophage MS2

Authors

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2 University of Michigan, Department of Chemistry, Ann Arbor, MI

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Nanopore technology can directly sequence intact RNA molecules, providing a unique ability to detect native modifications. Oxford Nanopore Technologies recently updated Direct RNA Sequencing (DRS), moving from RNA002 to RNA004 chemistry, alongside an updated Dorado model with improved basecalling accuracy and the ability to call four modifications: pseudouridine (Ψ), 6-methyladenosine (m6A), 5-methylcytosine (m5C), and inosine. However, these modification calls should be validated, making benchmarking against orthogonal methods like in vitro transcription (IVT) or liquid chromatography-tandem mass-spectrometry (LC-MS/MS) essential.

We profiled RNA modifications in the MS2 bacteriophage genome using LC-MS/MS, identifying Ψ as the predominant modification (~1 site per transcript), with all others present at very low levels (<0.04 modifications per RNA). We then sequenced bacteriophage RNA using DRS and applied Dorado modification calling. While Dorado detected Ψ in agreement with LC-MS/MS, it also reported multiple high-confidence calls for m6A, m5C, and inosine, suggesting false positives.

These results demonstrate that while Dorado effectively identified Ψ , it also overcalled other modifications. These findings underscore the importance of orthogonal validation and appropriate negative controls (IVT RNA) for Nanopore-based modification detection. Continued refinement of basecalling algorithms and systematic benchmarking will be critical for achieving reliable RNA modification profiling.

Key words: Nanopore Direct RNA Sequencing, RNA modifications, LC-MS/MS validation, Dorado modification calling

Multiple secondary messenger pathways compose the preconditioning signal and modulate neuroregeneration via CREB

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Keywords: Axon regeneration, Neurons, *C. elegans*, Lesion conditioning, CREB, Signaling pathways

In mammalian lesion conditioning, a peripheral axon injury enhances central axon regeneration by driving transcriptional changes in regeneration associated genes (RAGs) that confer regenerative capacity. However, the “preconditioning” signal initiating these changes remains unclear. DLK (*dlk-1*) is the primary mediator of conventional (one lesion) regeneration. We recently demonstrated that the transcription factor cAMP response element binding protein CREB (*crh-1*) is the primary driver of conditioning. In seven neuron types, loss of CREB eliminates conditioned (two lesion) regeneration. Removing CREB significantly reduces changes in RAG transcription, indicating CREB mediates transcriptional changes required for conditioning.

We previously established cGMP and Ca^{2+} /activity inhibit and cAMP activates conditioning in the ASJ sensory neuron. Addition of CREB mutation largely abolishes the conditioning effect, indicating CREB is downstream of each pathway. In mammals, conditioning is enhanced by earlier peripheral axon injury. In mutants with disrupted cGMP and Ca^{2+} pathways, there is strong regeneration. This suggests the preconditioning effect may be modulated by the cGMP and Ca^{2+} pathways. We are further studying these pathways to assess how they mechanistically modulate CREB to drive conditioned regeneration. Understanding the conditioning mechanism could illuminate potential therapy for central nervous system and spinal cord injuries using the body's intrinsic regenerative capacity.

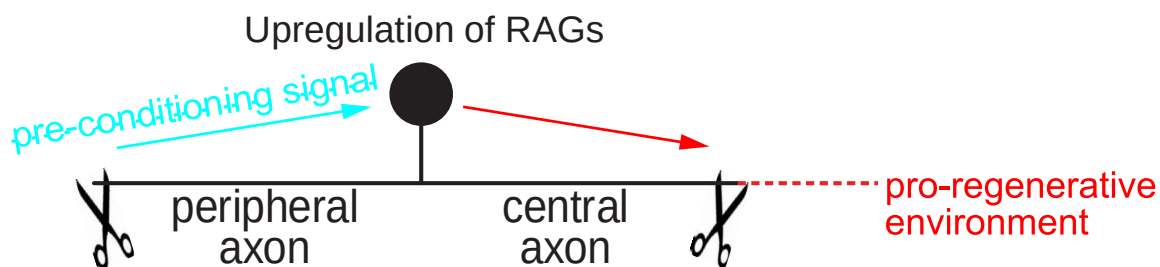


Figure 1. Diagram of a neuron cell body with the central axon concomitantly cut with the peripheral axon initiating a pre-conditioning signal.

Peptidomimetic Polyester Electrospun Patches for Hemorrhage Control

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Uncontrolled hemorrhage accounts for ~ 80% of battlefield deaths and 40% of civilian deaths. Ideal hemostatic materials for hemorrhage control would mimic, amplify, and leverage natural hemostatic mechanisms. These materials should also be biocompatible, and biodegradable to avoid cumbersome operative removal and systemic toxicity. Currently available hemostatic materials do not meet all the above requirements. Additionally, existing studies lack comparison of the effects of different chemistries on their hemostatic efficacy. This study outlines the fabrication of biodegradable hemostatic electrospun patches utilizing our previously developed peptidomimetic polyester library. It investigates the effect of electrospun patches formed using polyesters with diverse functional groups (carboxylic acid v/s primary amines) to form a hemostatic plug. Electrospun patches provide high surface area to interact with blood components and restrict them to wound site while providing a gauze-like material format. The electrospun patches provide faster hemostasis and better clot-mediated wound adhesion and closure compared to cotton gauze. The primary amine functional groups slowed blood clotting and provided lower clot-mediated wound adhesion and closure compared to carboxylic acid groups showing a clear effect of chemistry.

Keywords: Hemorrhage control, Hemostatic material, Clot contraction, Biodegradable hemostats, Electrospinning

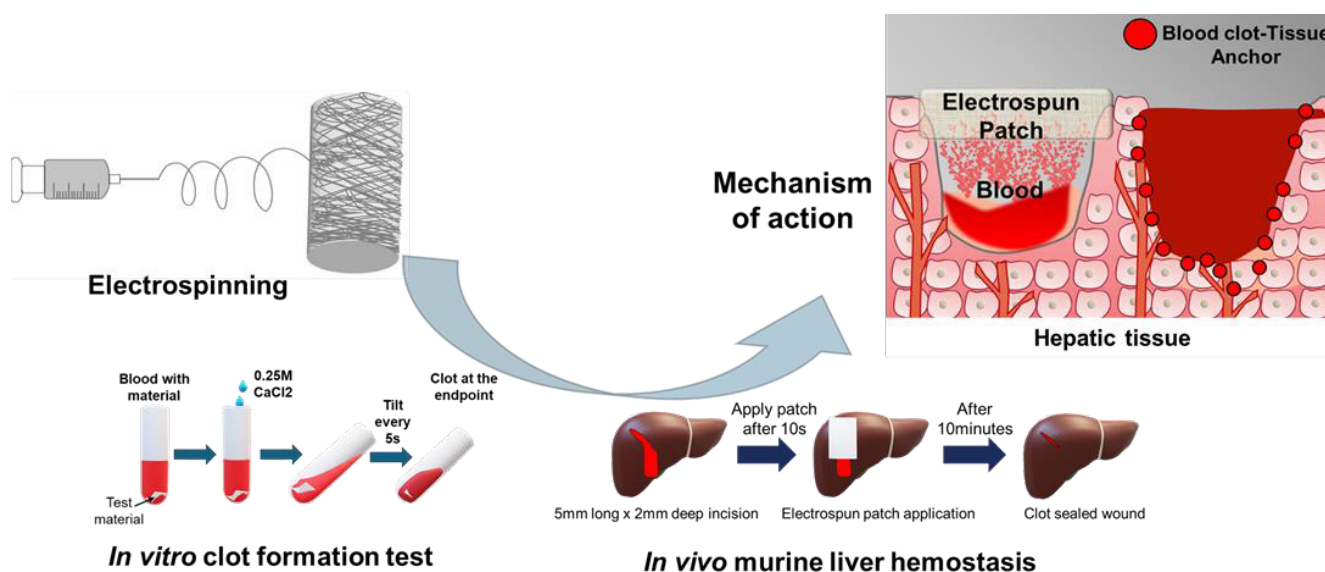


Figure 1. Electrospun patches (EPs) were fabricated using peptidomimetic polyesters which show procoagulant action *in vitro* and accelerated hemostasis *in vivo*. The EPs work by restricting the blood to wound site and forming a contracted hemostatic clot.

TOXICOCHEMICAL PROFILING OF DUAL CIGARETTE AND E-CIGARETTE EXPOSURES AND THEIR IMPACT ON PULMONARY FUNCTION OF FEMALE APOE-/- MICE

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*These authors contributed equally

Abstract:

The growing popularity of electronic nicotine delivery systems (ENDS) has been accompanied by a significant rise in the dual use of both conventional cigarettes and e-cigarettes. While each product is independently known to impair pulmonary function and structure, and to alter inflammatory and immune responses, the consequences of their combined, long-term use remain poorly understood. Therefore, in this study, we aimed to investigate the effects of chronic dual exposure, following long-term cigarette use, by first subjecting female ApoE-/- mice to cigarette smoke for 24-weeks followed by either cigarette smoke, filtered air, e-cigarette aerosols, or combined e-cigarette aerosols and cigarette smoke for an additional 24 weeks. To assess airway hyperresponsiveness (AHR) and subsequent bronchoconstriction as exposure duration increased, we performed a methacholine challenge on conscious mice using double-chamber plethysmography (Scireq). Respiratory parameters such as specific airway resistance (SRaw), mid-tidal expiratory volume (EF50), and breathing frequency (f) were recorded across increasing methacholine doses. Actively, we measured respiratory rate (RR), tidal volume (TV), minute volume (MV), and breathing waveform patterns by whole body plethysmography in mice exposed to smoke for 1 to 6 months. Furthermore, to inform the computational aspect of our study, we directly measured key input parameters such as particle size distributions and the chemical composition of cigarette and e-cigarette aerosols. These data are critical for developing inhalation models that predict particle deposition and respiratory impact in dual-use scenarios, enabling linkage between adverse health outcomes and deposited dose.

Keywords: dual exposure, specific airway resistance, breathing frequency, respiratory rate, tidal volume, particle size distributions, chemical composition

The Perivascular Niche Enforces Glioblastoma Stem Cell Dormancy in an In Vitro 3D Glioblastoma-Vascular Model

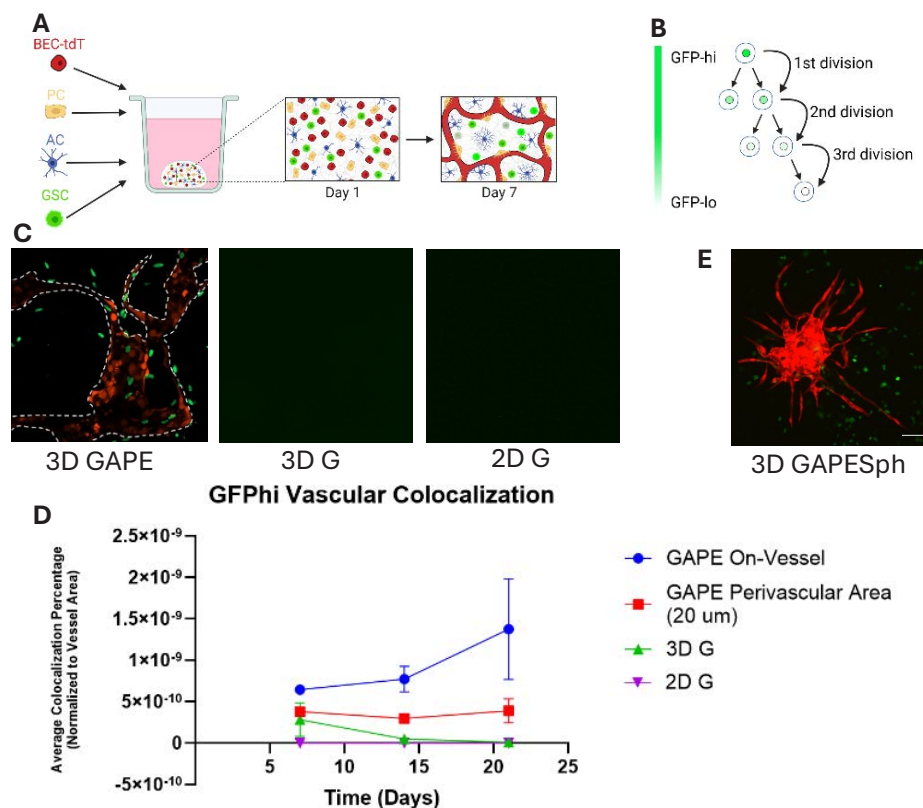
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Department of Neuroscience, Mount Sinai Medical School

Glioblastoma multiforme (GBM) is the most common primary brain tumor and its prognosis lands among the worst of any cancer diagnosis. It is relatively common, accounting for 52% of primary brain tumors. A subpopulation of tumor cells – glioblastoma stem cells (GSCs) – have high tumorigenic potential and are exceptionally robust and thus represent a major source of tumor recurrence. GSC subpopulations have been observed to harbor a subset of quiescent cells, or cells which cease proliferation but maintain functionality and self-renewal capacity. These subpopulations are observed to be present largely in perivascular areas of the brain. Identifying the molecular underpinnings of these phenomena is a critical step in advancing the understanding of glioblastoma's evasiveness and developing more effective treatments. We have devised a 3D *in vitro* brain tissue analog which utilizes human primary cells to generate a vascularized environment in disperse, and branching spheroid conformations to examine the behavior of GSCs. The GSCs are genetically modified to facilitate observation of proliferation in real time. We have discovered an association of GSC dormancy with proximity to a vascular network. This work motivates further research into the molecular mechanisms of dormancy as a potential target for novel cancer therapies.

Keywords: vascular engineering, glioblastoma, quiescence, dormancy, treatment resistance, disease modeling, glioblastoma stem cells



Localization and Quantification of Bacterial Kinase Activity during Sporulation

Yihan Wei¹, Elizabeth Libby¹

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Hanks-type Ser/Thr kinases, also known as eukaryotic-like kinases, play key roles in bacterial regulatory networks under varying environmental conditions. In response to nutrient deprivation, *Bacillus subtilis* can produce highly resilient spores for surviving under harsh conditions. The kinase YabT is specifically expressed during sporulation and is required for the proper timing and development of the bacterial spore. However, the activating signals and timing of YabT activity remains unknown.

To answer these questions, we developed a biosensor to quantify and assess the spatial localization of YabT activity during sporulation. We found that YabT activity specifically localizes to the developing spore. This is in contrast to another Ser/Thr kinase, PrkC, which is expressed in all phases of growth, including the developing spore. However, both kinases are known to be essential for the proper development of the bacterial spore. Using a combination of biosensors for each kinase and genetics, we provide in vivo evidence that PrkC may participate in a phosphorelay with YabT in the developing spore.

This suggests that bacterial Ser/Thr kinases can temporally and spatially integrate signaling during a developmental process.

Bottlebrush Polymer Conjugates for Enhanced Antisense Oligonucleotide Therapy in Myotonic Dystrophy Type 1

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Oligonucleotides are a promising genetic medicine for myotonic dystrophy type 1 (DM1), the most common adult-onset muscular dystrophy. However, poor muscle distribution of nucleic acid drugs after systemic administration has hindered drug development, and no curative treatment exists. Additionally, DM1 pathology requires drug localization to the nucleus, where pathogenic mutant RNA is trapped, posing challenges after endocytosis and endosomal escape. Here, we show that a locked nucleic acid oligonucleotide targeting mutant CUGexp RNA tracts, conjugated to a bottlebrush polymer, exhibited improved muscle distribution and potent correction of DM1-associated splicing at low nanomolar doses in a DM1 mouse model. Significant improvements in myotonia, body weight, and grip strength were observed. The conjugates were well tolerated after 12 weeks of weekly intravenous dosing. These results suggest that bottlebrush polymer bioconjugates may overcome key limitations of traditional antisense drugs for muscular dystrophies, with the potential as potent, durable, and cost-efficient DM1 therapies.

A Design of the gene therapy vector

Protein

PEG brush polymer (300-350 kDa)

Triazole linker

DMD cell

B In vivo efficacy

I.V. injection

DMD mouse

C In vitro efficacy

DMD cell

Composite Scores (P5)

Grip Strength (g)

Restored splicing regulation, gene expression, muscle function

Li Continued.

Alterations in pulmonary mechanics and alveolar-capillary barrier permeability following acute wildfire smoke exposure in apolipoprotein E-deficient mice

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Background and Purpose: Wildfires are complex natural phenomena whose smoke composition is shaped by the geographical location that coordinates the synergistic interplay of fuel type, meteorological and combustion factors. This variability creates a diverse and evolving pollutant profile, complicating the assessment of pulmonary responses to smoke exposure. As wildland fires increase in both intensity and frequency, the associated health risks from particulate matter air pollution continue to rise, posing a particularly severe threat to occupational workers who face prolonged and often unavoidable exposure while wearing little or no respiratory protection equipment. In our previous work, chronic exposure to smoldering Douglas fir smoke showed a trend toward increased lung compliance at 16 weeks, accompanied by a transient elevation in lung remodeling-associated inflammatory markers at 8 weeks, at a cumulative dose comparable to that experienced by wildland firefighters over their career. Building on these findings, the present study aims to provide a comparative analysis to elucidate the acute effects of smoldering versus flaming combustion on lung mechanics, with a particular focus on *in vivo* lung permeability. We hypothesize that exposure to flaming smoke induces a more rapid decline in lung function and greater increases in lung permeability, driven by hyperstimulation of inflammatory markers associated with emphysema-like lung remodeling.

Methods: We exposed 8-10-week-old female apolipoprotein E-deficient mice (ApoE^{-/-}) mice to 4mg/m³ of flaming 50/50 dried Eucalyptus/Manzanita smoke over 7 consecutive days, 2 hours/day. Following 24 hours of the final exposure, we assessed lung mechanics via forced-oscillation technique (flexiVent, SCIREQ). Input impedance measurements were collected during derecruitment at positive end-expiratory pressures (PEEPs) of 12, 9, 6, 3 and 0 cmH₂O and fitted to the constant-phase model to derive coefficient of tissue elastance (H), tissue resistance (G), and Newtonian resistance (R_n). In addition, a cyclic stretch injurious ventilation protocol was used to induce ventilator-induced lung injury (VILI), consisting of 5 minutes at 50 ml/kg tidal volume, a plateau pressure of 37.5 cmH₂O, zero PEEP, and a respiratory rate of 50 breaths per minute. This approach was employed to assess the onset of VILI, defined as the time (seconds) at which H doubles, which occurs earlier in injured lungs. A novel *in vivo* permeability technique was used to assess the lung permeability following smoke exposure with FITC-Dextran tracer. Instead of the commonly used intratracheal instillation, we employed a nebulization approach using an Aerogen vibrating mesh nebulizer (2.5-4 µm droplet size) integrated with the flexiVent system, operating at 120 breaths per minute with a 2:1 inspiratory-to-expiratory ratio and a tidal volume of 20 ml/kg body weight. One-hour post-nebulization, blood was collected, and the lung was lavaged to measure the content of FITC-Dextran translocated to plasma and retained in BALF, to calculate for permeability leak index (%).

Results: The current study revealed a significant decline in H, G, and R_n at all PEEP levels except at 12 cmH₂O, following just seven days of flaming smoke exposure. In addition, the onset of VILI occurred earlier in exposed mice compared to baseline. Nebulized Evans Blue dye exhibited a spatial deposition pattern in the lungs comparable to that observed with inhaled flaming smoke. Our very recent data show an increase of FITC-dextran in the blood plasma, a corresponding decrease in BALF, and an elevated lung permeability index in positive control mice, at 4-, 24-, and 48-hour post- intratracheal instillation of lipopolysaccharide. These findings highlight the promising potential of this approach for improved *in vivo* assessment of lung permeability in murine exposure models alongside lung mechanics assessment.

Conclusion: Acute exposure to flaming Eucalyptus/Manzanita smoke induces a more rapid decline in lung function compared to chronic exposure to smoldering Douglas fir smoke. The impact of acute smoldering combustion of the same fuel type and sex differences remains to be investigated. To strengthen our findings, we will also perform immunofluorescence staining for key markers of lung inflammation and decline in epithelium barrier integrity.

Keywords: Occupational health, lung injury, smoldering, flaming, permeability

Funding: NIH/NIEHS R01E5033792 and DHS/FEMA - EMW-2017-FP-00446

THE SUPERIMPOSITION OF CIGARETTE SMOKE AND MENOPAUSE: HOW CHRONIC EXPOSURE AND A VCD-MODEL OF REPRODUCTIVE SENESCENCE INTERSECT IN AORTIC FUNCTION AND MECHANICS

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During the menopausal transition, estrogen's cardioprotective effects decline, and cardiovascular disease (CVD) risk factors like hypertension develop faster in women than age-matched men. Despite clear mechanistic differences, preclinical CVD research remains overwhelmingly biased, over 70% of studies solely use male mice and <1% replicate human-like menopause. Accounting for menopause is crucial when evaluating the impact of environmental stressors that stretch across a significant portion of the lifespan. Among stressors and modifiable characteristics, cigarette smoking is the single greatest risk factor for first myocardial infarction, a severe complication from coronary artery disease (CAD). Female smokers are 25% more likely to develop CAD and have higher mortality rates than male counterparts.

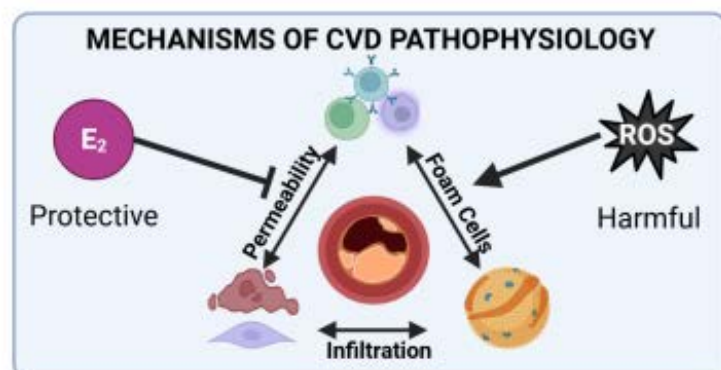
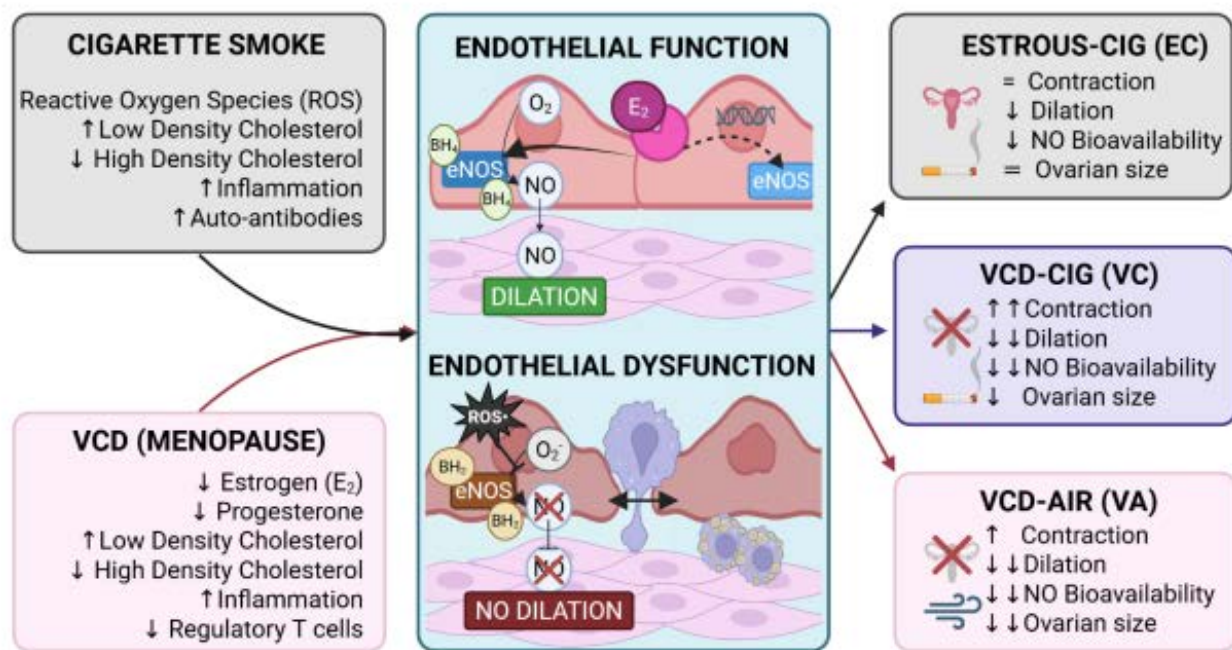
To address these limitations, we combined an established preclinical menopause model with cigarette smoke exposure to determine the superimposed effect of these two stressors on aortic function. Emulating human menopausal transition using 4-vinylcyclohexene diepoxide (VCD), a selectively ovotoxic molecule, leaves ovaries intact while gradually decreasing and eventually ceasing endocrine function.

Data showed two components of menopausal vascular phenotype: increased contractility and impaired vasodilation, suggesting vessels are overly primed for contraction and cannot effectively dilate to relieve high pressure. VCD-Cig mice were the most contractile and least dilatory of all conditions.

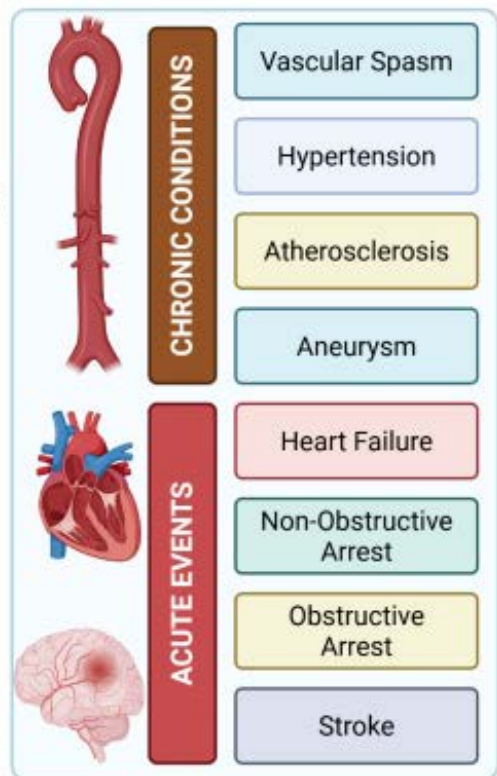
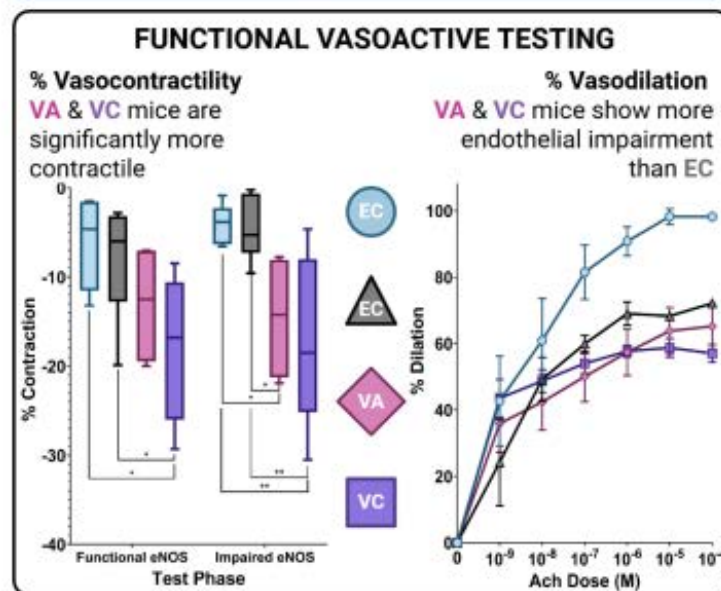
KEYWORDS: Menopause, VCD, Cardiovascular Disease, Aorta, Vasculature, Vasoactive Testing, Cigarette Smoking

Figure 1 – Experimental plan, mechanistic explanation of endothelial dysfunction and the role it plays in atherosclerosis, observed results, and functional vasoactive testing results, and the implications for chronic CVD and adverse acute events.





Menopause & cigarette smoke both impair endothelial function, initiating a cascade of CVD pathophysiology



Towards Optogenetic Manipulation of Bone Remodeling Activity to Alter Hematopoietic Stem Cell Fate

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Aging hematopoietic stem cells (HSCs) lose regenerative potential and exhibit increased myeloid cell production, contributing to immune dysfunction. However, therapeutic strategies to enhance lymphoid production remain limited. We observed an enrichment of early lymphoid progenitors in bone marrow microenvironments adjacent to bone cavities with mixed bone remodeling activity (M-type) compared to cavities dominated by bone deposition (D-type) or resorption (R-type). To determine whether altering bone remodeling activity can influence HSC fate and boost lymphoid production, we aim to reduce bone resorptive activity in R-type cavities using optogenetic tools in a newly developed mouse model.

We will then utilize Image-seq, a technology we developed for image-guided single-cell RNA sequencing, to aspirate hematopoietic cells from optogenetically manipulated bone marrow cavities. Before library preparation and sequencing, hematopoietic stem and progenitor cells (HSPCs) will be isolated from the cell aspirates using flow cytometry. We have optimized a protocol for high-purity isolation of HSCs (Lin-Ckit+Scal1+CD150+CD48-Flt3-EPCR+) and HSPCs (Lin-Ckit+Scal1+). This establishes a critical component of the experimental framework for investigating the link between bone remodeling activity and HSC fate, with the ultimate goal of uncovering therapeutic strategies for age-related lymphoid deficiencies.

Cationic Interleukin-1 Receptor Antagonist (catIL-1Ra) for Prolonged Post-Traumatic Osteoarthritis Pain Relief

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Introduction: Post-traumatic osteoarthritis (PTOA) is driven by upregulated inflammatory mediators that contribute to both disease progression and pain¹. A key cytokine elevated after joint injury is interleukin-1 (IL-1), a potent pro-inflammatory, cartilage-degrading molecule that increases in synovial fluid within weeks to months post-injury, depending on disease severity and heterogeneity^{2,3}. IL-1 receptor antagonist (IL-1Ra, a.k.a Anakinra), which blocks IL-1 signaling by competing for receptor binding, is FDA-approved for rheumatoid arthritis, which clinical therapeutic effect is limited by poor joint bioavailability and rapid clearance—even after intra-articular injection—due to fast synovial turnover and the dense, avascular cartilage matrix, resulting in a short joint half-life (~4 h) and subtherapeutic efficacy in OA⁴. In addition, its effectiveness in relieving OA-related pain remains unclear, particularly in preclinical models. To address this, cationic peptide carriers (CPCs), arginine-rich sequences that enhance drug uptake and retention in cartilage, have been utilized to help deliver IL-1Ra for full cartilage penetration^{5,6}. In our recent work, we developed a recombinant fusion protein combining CPC with IL-1Ra (catIL-1Ra), which showed improved cartilage penetration and protection in IL-1-challenged cartilage explants compared to Anakinra⁷. In this study, we administered catIL-1Ra intra-articularly in a PTOA rat model (medial meniscus transection, MMT) and evaluated its joint retention and associated pain response relative to MMT rats treated with saline or Anakinra.

Methods: *Production:* cationic IL-1Ra (catIL-1Ra) was produced in HEK293 cells (RayBiotech, Atlanta, GA). The IL-1Ra domain was fused at the C-terminus to a cationic CPC sequence, R(RRRNN)₃RRRR, separated by a short hybrid linker (EAAKGGGGG) (Fig. A). This design was computationally predicted and previously shown to maintain effective binding to the IL-1 receptor (IL-1R) ⁸. The fusion protein was purified using immobilized metal affinity chromatography (IMAC) via a nickel column, achieving >90% purity as confirmed by SDS-PAGE and Coomassie blue staining. Anakinra was obtained from commercial Kineret prefilled syringes. *Animal surgery:* All surgical procedures and subsequent *in vivo* experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Northeastern University or Rush University Medical Center. Sham or medial meniscus transection (MMT) surgeries were performed on the right knees of thirty 10-week-old male Lewis rats under isoflurane anesthesia. Rats received subcutaneous injections of buprenorphine (0.01 mg/kg) and meloxicam (2 mg/kg) for analgesia. A skin and muscle incision were made along the pre-sterilized medial side of the right hindlimb. In the MMT group, the medial collateral ligament (MCL) was dissected, and the medial meniscus was transected until complete separation was confirmed by independent movement of the dissected tissue segments. In the sham group, the MCL was exposed but not cut, and the incision was then closed. Muscle was sutured with 5-0 Vicryl, and skin was closed using wound clips, which were removed two weeks post-surgery. *In vivo evaluation:* At three weeks post-surgery, 0.17 mg of Anakinra, catIL-1Ra, or an equivalent volume of saline was administered via intra-articular (IA) injection into the right knee. The study was terminated two weeks post-injection, at which point the right knees and ipsilateral L3–L5 dorsal root ganglia (DRGs) were collected for further analysis. Pain behavior was assessed using hindlimb weight-bearing measured by an incapacitance meter (IITC) at predetermined time points (Fig. B). Weight bearing was calculated as Weight Bearing% = Weight Bearing on Right Leg / (Weight Bearing on Right Leg + Left Leg) * 100%. Each data point was averaged from 12 individual measurements, and observers were blinded to treatment groups to eliminate bias. Blood serum collected at selected time points was analyzed for IL-1Ra concentration using ELISA. *Statistics:* Data is presented as mean ± standard error of the mean. Statistical analysis of weight-bearing data was performed using two-way ANOVA followed by Tukey's post hoc test. Serum IL-1Ra concentrations were compared using unpaired t-tests between treatment groups. A *p* value < 0.05 was considered statistically significant; # vs MMT-saline, * vs MMT-Anakinra.

Results: During the first three weeks post-surgery, sham rats (*N* = 5) maintained balanced weight-bearing around 50%, indicating no lateralized pain (Fig. C). In contrast, MMT rats (*N* = 13) initially exhibited post-surgical muscle pain, but after recovery and with the progression of PTOA, stabilized at approximately 45% weight-bearing after 1.5 weeks with a level significantly different from the sham group (Fig. C). Following IA injection with saline using a 31G syringe, sham rats maintained their baseline performance near 50% throughout the experiment (data not shown). MMT rats were arranged into treatment groups receiving saline, Anakinra, or catIL-1Ra (*N* = 4–5 per group). At 4 hours post-injection, the Anakinra-treated MMT group showed a transient return to sham-like weight-bearing levels (data not shown), while the MMT-saline and catIL-1Ra groups remained unchanged (Fig. E). Anakinra-treated rats maintained a non-significant trend toward improved weight-bearing over saline for one week (Fig. D). In contrast, the catIL-1Ra group, despite no early improvement at 4 hours, exhibited delayed and sustained pain relief, with significant improvement on days 2 and 3, and a trend toward reduced pain from 8 hours up to 10 days post-injection (Fig. E). Correspondingly, serum IL-1Ra levels showed that while Anakinra was undetectable since day 2, catIL-1Ra remained detectable until day 3, aligning with the observed analgesic effect (Fig. F).

Discussion/Significance: This study evaluated the pain-relieving potential of Anakinra in PTOA and demonstrated a prolonged effect with its cationic form, catIL-1Ra, in MMT rats. A single intra-articular injection of 0.17 mg Anakinra produced a transient, non-significant improvement in weight-bearing that peaked at 4 hours and declined thereafter. In contrast, catIL-1Ra did not improve weight-bearing at 4 hours, likely due to delayed distribution, but showed a significant pain relief effect from days 2 to 3, maintaining symmetric weight-bearing levels, which effect diminished by two weeks post-injection. Compared to Anakinra, catIL-1Ra achieved higher IL-1Ra retention in the joint for at least 3 days, supporting sustained release. These results suggest catIL-1Ra enhances joint residence time and provides prolonged pain relief. Ongoing studies are investigating joint histopathology, including the synovium and ipsilateral DRGs, as well as bulk RNA sequencing of DRGs.

Acknowledgements: NIAMS R01 AR075121, NIBIB EB028385. **References:** ¹Miller+, Cytokine 2014; ²Irie+ Knee 2003; ³Barbosa Inflamm. Res. 2020; ⁴Chevalier+ A&R 2009; ⁵Mehta+ OAC 2023; ⁶Vedadhavami+ ART 2022; ⁷Boyer+ OAC 2025

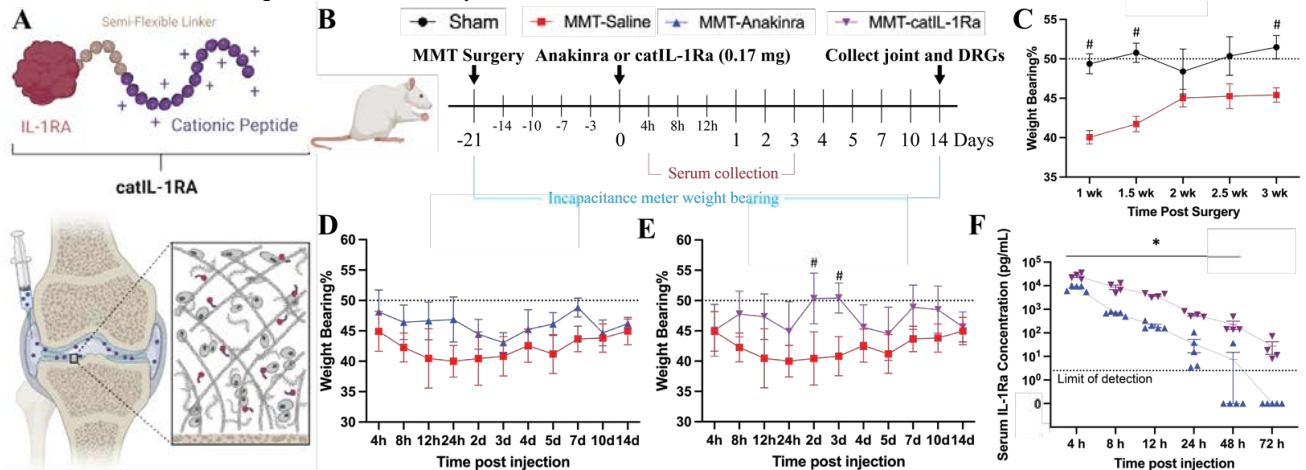


Figure. A) (Top) Schematic of the cationic IL-1Ra (catIL-1Ra) fusion protein, composed of IL-1Ra linked to a cationic peptide carrier (CPC) via a semi-flexible linker. (Bottom) Illustration of catIL-1Ra penetrating the full thickness of cartilage, facilitated by electrostatic interactions with negatively charged glycosaminoglycans, enabling sustained intra-cartilage delivery of IL-1Ra. **B)** *in vivo* experimental timeline **C–E)** Weight-bearing performance of sham and MMT rats treated with saline, catIL-1Ra, or Anakinra across the experimental timeline. **F)** Serum IL-1Ra concentrations measured over a 3-day period post-administration. Data are presented as mean ± SEM. Statistical significance was defined as *p* < 0.05; # indicates comparison vs. MMT-saline, * indicates comparison vs. MMT-Anakinra.

Oral Presentations Abstracts

AFTERNOON SESSION

Characterizing chemosensory neuronal responses through development in both sexes

Sahana Gangadharan¹, Jinmahn Kim², Mahdi Torkashvand², Maedeh Seyedolmohadesin², Sina Rasouli², Vivek Venkatachalam²

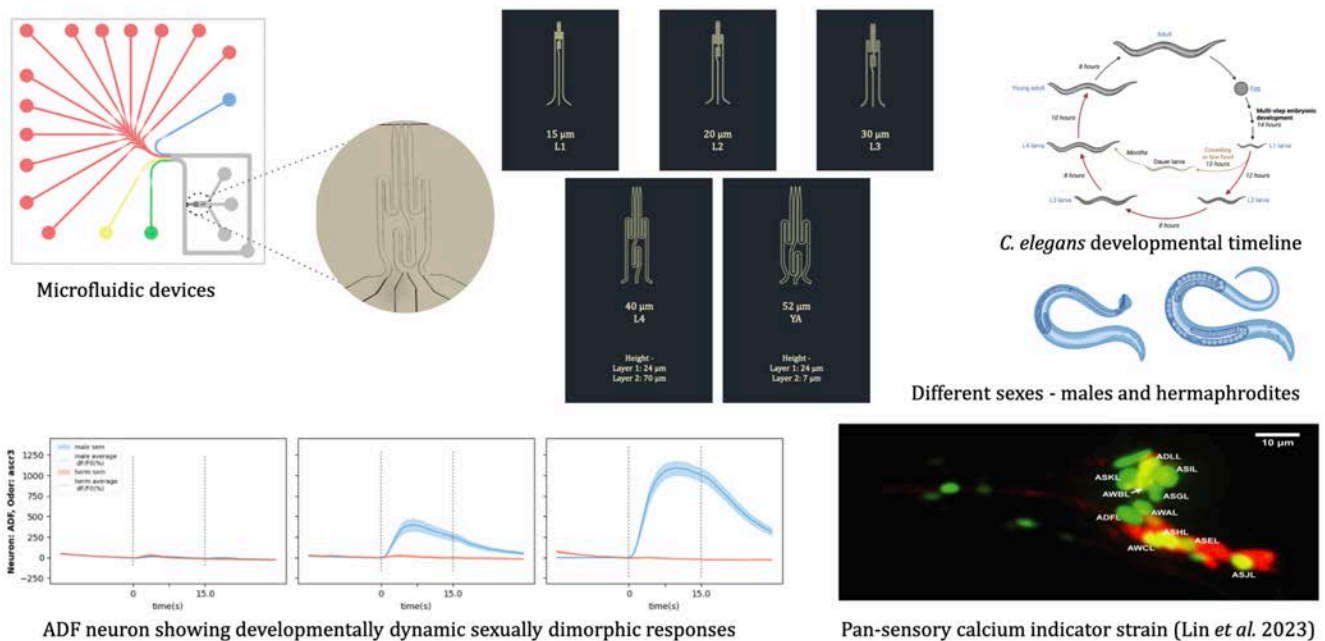
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Throughout its life, an animal engages with its environment, prioritizing tasks and adapting to the demands of its surroundings, in an age- and sex-dependent manner. These behavioral differences are often complemented by nervous system changes, across a variety of species. Previous connectomic studies revealed structural changes in the hermaphrodite nervous system across development (Witvliet *et al.* 2021) while Haque *et al.* (2024) highlighted how sexual dimorphism arises through distinct developmental gene expression patterns. To understand the functional neural dynamics underlying these changes, we developed 5 microfluidic devices optimized for immobilizing lower larval stage *C. elegans* (L1-YA) of both sexes to record pan-sensory calcium dynamics (Lin *et al.* 2023) during exposure to 12 ethologically relevant cues.

We observed many distinct patterns of developmentally dynamic responses. (1) Some neurons gradually change sensitivity throughout development in an odor-independent manner (e.g., ASK). (2) Other neurons exhibit odor-specific developmental dynamics (e.g., ASJ gradually increases in sensitivity to isoamyl alcohol, but peaks in responsivity to OP50 at L3). Additional complex responses arose when we compared male and hermaphrodite worms beginning at L3. (1) As animals develop, sensory cells typically show increasing sexual dimorphism in response to odors, though the dimorphism appears at different times for different cells (e.g., ADF responds monomorphically at L3 but dimorphically at L4). (2) Some neurons develop sexually dimorphic responses to different odors at different developmental times (e.g., AWA first responds dimorphically to copper at L3 and to OP50 at L4). To contextualize these sex-, stage- and odor- specific developmentally dynamic responses behaviorally, we have built microfluidic chips to study chemotaxis towards the stimuli through development.

Keywords: whole-brain calcium imaging, development, sexual dimorphism, chemosensation



Improving Tomographic fNIRS Contrast and Depth Sensitivity Using Activation-Prior-Guided Reconstruction

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Tomographic functional near-infrared spectroscopy (fNIRS), which relies on high-density optode configurations, remains limited by low spatial resolution and signal contrast, particularly in the context of complex cortical folding. We present an enhanced tomographic fNIRS reconstruction framework that improves spatial localization and activation contrast by incorporating a hypothetical activation prior into the inverse problem.

Our method iteratively evaluates a spatially localized activation prior, modeled as a Gaussian sphere, across a region-of-interest. When the prior aligns with a true activation, the resulting reconstruction exhibits significantly improved contrast and spatial precision. Notably, when the prior is misaligned, the reconstruction remains unaffected, indicating robustness to false priors. This property enables a systematic search across the cortical surface to recover true activations [1].

We demonstrate the utility of this approach using both simulated and empirical fNIRS datasets, reporting a threefold increase in activation contrast and a twofold reduction in spatial spread relative to conventional, unguided reconstructions. This method enables resolution of activations in deeper cortical structures, such as sulci, which are typically inaccessible with standard tomographic techniques. Simulations confirm successful recovery of sulcal activations.

Additionally, the framework shows improved performance under varying optode densities, including sparse and ultra-high-density configurations. This suggests its potential applicability across a range of hardware setups, including those constrained by limited optode availability.

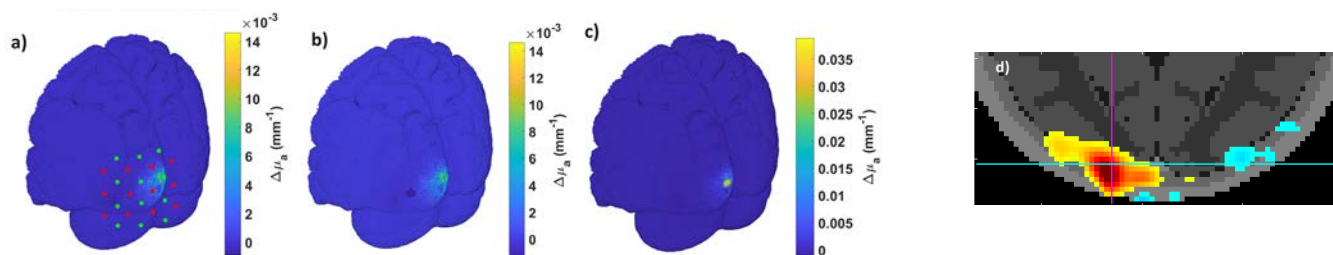


Fig. 1. Reconstructed absorption coefficients (1/mm) using *in-vivo* data with a cortically constrained reconstruction: a) with no activation prior, and those using b) discordant and c) concordant activation priors, compared with a volumetric reconstruction from NeuroDOT [2].

Keywords: optics, imaging, neuroscience, fNIRS

References:

- [1] Fang Q, "Method to localize small and high contrast inclusions in ill-posed model-based imaging modalities," US Patent US11246529B2. 2022 Feb 15
- [2] Eggebrecht et al. "NeuroDOT: An extensible Matlab toolbox for streamlined optical functional mapping," presented at the European Conference on Biomedical Optics, Optica Publishing Group, Jun. 2019, p. 11074_26. <https://github.com/WUSTL-ORL/NeuroDOT>

Labeling Macrophages with Lipid Microbubbles for Multi-Modal Imaging and Immunomodulation

Krunal Patel¹, Poulami Mondal¹, Tao Sun²

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Background: Macrophages are mobile, phenotypically plastic immune cells capable of shifting between pro-inflammatory (M1) and anti-inflammatory (M2) states. Tracking their location and modulating behaviors are essential for therapeutic strategies targeting inflammation and tissue regeneration. We hypothesize lipid microbubbles can effectively label macrophages and modulate their phenotypes.

Methods: RAW 264.7 macrophages (n=4) were incubated with lipid microbubbles (ratios 1:4–1:16) for 3 hours to achieve bubble labelling. To test the echogenicity of bubble-labelled macrophages, ultrasound imaging was performed using the VisualSonics F2 (B-mode, contrast mode) and a programmable ultrasound system (Verasonics Vantage) for passive cavitation mapping. Flow cytometry was conducted to assess macrophage phenotype. Brightfield, fluorescent, and live-cell confocal microscopy were utilized to monitor bubble uptake and lysosomal activation. Data were analyzed using via one-way ANOVA with significance set at $p < 0.05$.

Results: Confocal microscopy confirmed intracellular bubble accumulation of lipid microbubbles, co-localizing with lysosomal activity. Ultrasound imaging showed significantly enhanced contrast in bubble-labeled macrophages compared to unlabeled controls ($p < 0.05$). At 24 hours post-incubation, CD86 expression increased significantly at the 1:16 bubble ratio ($p < 0.05$). Anti-inflammatory markers (CD206, IL-10, VEGF) were markedly upregulated at higher bubble ratios. Additional immunomodulation, reflected in increased expression of CD80, HIF1 α , and Arg1, was observed following focused ultrasound (FUS) stimulation (1 MPa, 120 pulses, pulse repetition frequency 4 Hz, 500 kHz).

Conclusions: Lipid microbubbles effectively label macrophages for ultrasound and fluorescent imaging and promote a shift toward an anti-inflammatory phenotype. FUS further enhances this immunomodulatory response in a pressure- and bubble dose-dependent manner, suggesting significant potential for therapeutic and imaging applications.

Acknowledgments: Supported by Northeastern University and the Institute for Chemical Imaging of Living Systems (CILS).



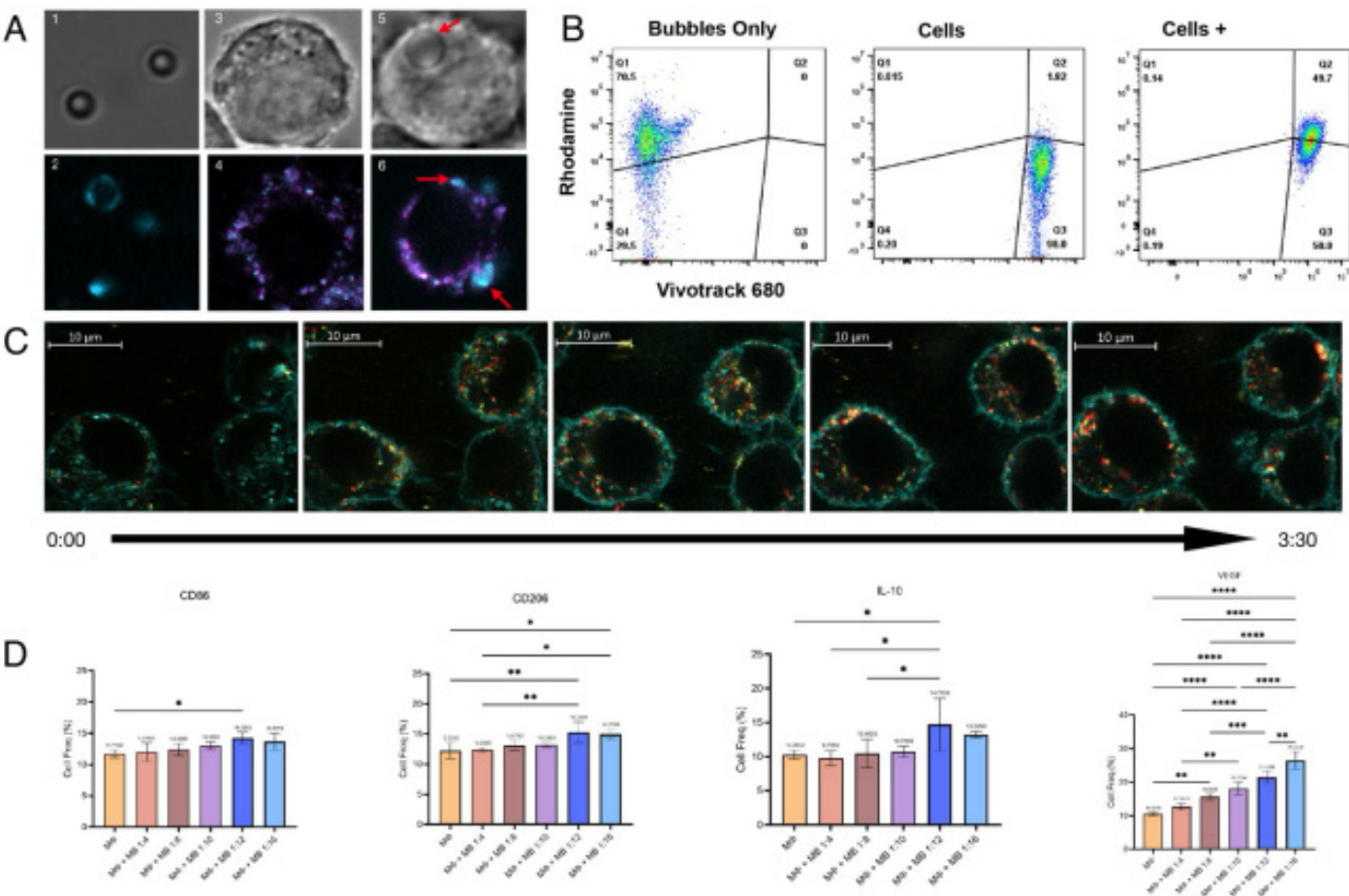


Figure 1. (A) Brightfield and fluorescence imaging reveal macrophage uptake of fluorescently labeled microbubbles (1, 2), membrane-stained cells (3, 4), and macrophages carrying microbubbles (5, 6) (red arrows). **(B)** Flow cytometry confirms bubble internalization by macrophages. **(C)** Time-lapse confocal microscopy (0–3.5 hrs) captures progressive microbubble uptake (red/orange) and increasing lysosomal activity (cyan) within macrophages. **(D)** Macrophage phenotype assessments at varying cell-to-microbubble ratios.

<i>Presenters</i>	<i>Lab</i>
Mahsa Karamzadeh	Loth
Vasuretha Chandar	Parameswaran
Victoria Williams	Bellini
Poulami Mondal	Sun
Christina Velez	Dai

5-Minute Thesis Abstracts

5-MINUTE THESES

Assessment of Cerebrospinal Fluid Dynamics During Normal Breathing and Valsalva Maneuver using Real-Time MRI

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The Valsalva maneuver, which involves forcefully exhaling while holding one's breath, increases pressure inside the head and spine. This pressure affects how cerebrospinal fluid (CSF) flows through the spinal canal. Previous research shows that CSF stroke volume (SV)—the amount of fluid moving with each heartbeat—drops during Valsalva but returns to normal soon after. However, standard MRI techniques can't capture these fast changes in flow.

In this study, we used a real-time imaging method called pencil beam (PB) MRI to measure CSF flow changes during the Valsalva maneuver in seven healthy individuals and nine people with Chiari Malformation Type I (CMI), a condition where brain tissue presses into the spinal canal. Participants performed the Valsalva maneuver while PB imaging recorded CSF motion.

We found that SV decreased by about 33% in healthy people and 40% in those with CMI during Valsalva. After the maneuver, SV quickly returned to normal levels. Heart rate also increased, but this alone didn't explain the drop in SV.

These results show that CSF flow is sensitive to pressure changes but can recover quickly. This rapid recovery may help explain why Valsalva-induced headaches in CMI are usually brief, even though the underlying cause of these headaches remains unclear. Key words: Magnetic resonance imaging, Chiari Malformation, Cerebrospinal fluid, Stroke Volume



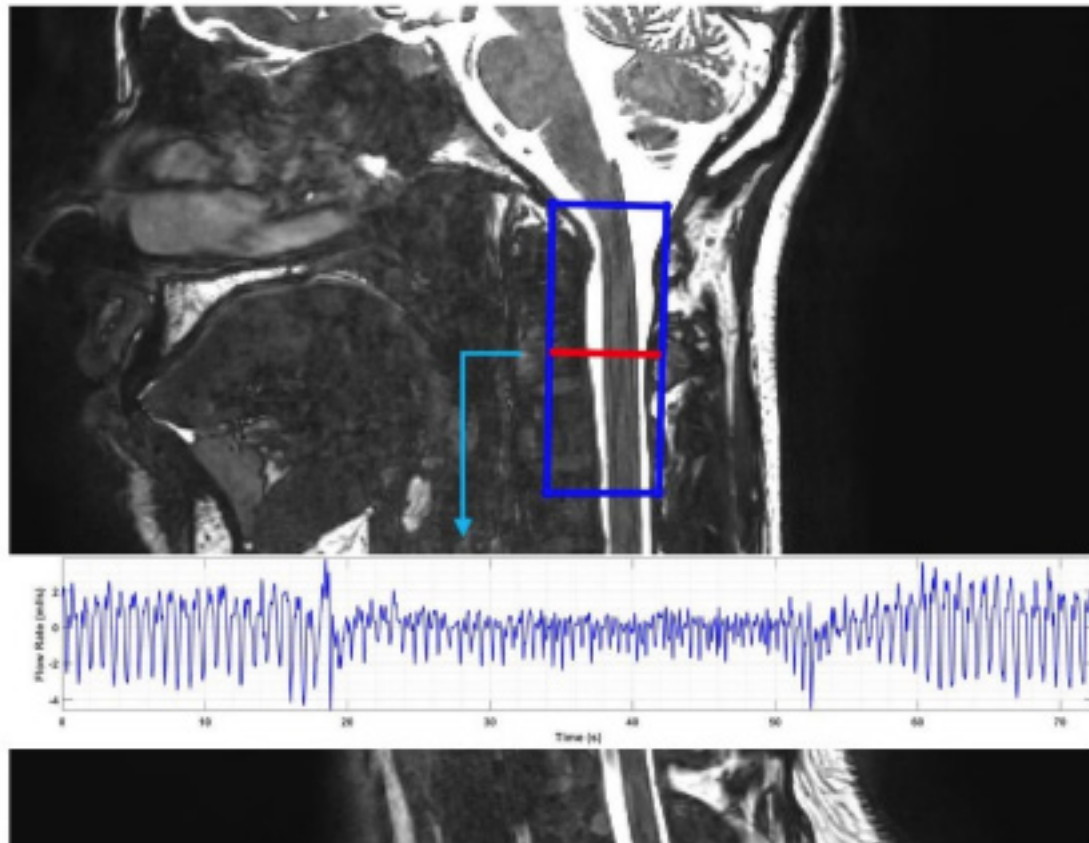


Figure 1: Real time assessment of cerebrospinal fluid during Valsalva maneuver

Intercellular contact is sufficient to drive fibroblast-to-myofibroblast transitions

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Fibroblast cells play a key role in maintaining the extracellular matrix. During wound healing, fibroblasts differentiate into highly contractile myofibroblasts, which secrete extracellular matrix proteins like collagen to facilitate tissue repair. Under normal conditions, myofibroblasts undergo programmed cell death after healing to prevent excessive scar formation. However, in diseases like fibrosis, the myofibroblasts remain active even after the wound is closed, resulting in excessive collagen buildup and a stiff, fibrotic matrix. The reasons for the persistence of myofibroblasts in fibrosis are not well understood. Here, we show the existence of a mechanism where direct physical contact between a fibroblast and a myofibroblast is sufficient for fibroblasts to transition into myofibroblasts (Fig 1). We demonstrate that the fibroblast-myofibroblast transition can occur even in the absence of known biochemical cues, such as growth factor activation or mechanical cues from a stiff, fibrotic matrix. Furthermore, we demonstrate that contact-based fibroblast-myofibroblast activation can be inhibited by the Gαq/11/14 inhibitor FR900359, which prevents the formation of myofibroblasts. These findings provide new insights into the persistence of fibrosis despite therapeutic interventions, suggesting a potential strategy for targeting the fibroblast-to-myofibroblast transition in fibrotic conditions.

Keywords: Mechanotransduction. Mechanobiology. Idiopathic Pulmonary Fibrosis. Intercellular contact. Cytoskeletal tension. PLC activation. Fibroblast-Myofibroblast transitions.

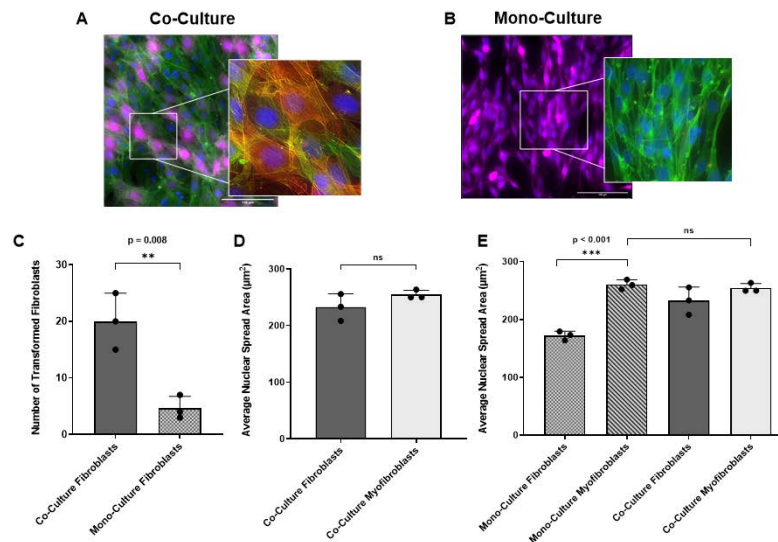


Fig 1: Fibroblasts when co-cultured with myofibroblasts on a soft ($E=300\text{Pa}$) PDMS substrate, undergo a phenotypic shift to myofibroblasts despite the absence of external biochemical stimuli (like growth factors) or mechanical stimuli from stiff substrate. Cells were stained for myofibroblast markers f-actin (green) and α -SMA (red) and nuclear stain DAPI (blue) after 3 days in culture. **(A)** Fibroblasts (magenta) in co-culture with myofibroblasts showed sequestration of α -SMA in their stress fibers when cultured on a 300Pa soft substrate, as in the inset (indicated by reddish-yellow stress fibers). **(B)** Fibroblasts in monoculture on 300Pa soft substrate retained their phenotype when not in contact with myofibroblasts. Scale bars 100 μm . **(C)** A significant number of fibroblasts (20 ± 5 cells) transformed into myofibroblasts as compared to fibroblasts in monoculture (4.66 ± 2.08) ($p = 0.008$, unpaired t-test, $n=3$, data represented as mean $\pm$ SD). **(D)** There is no significant difference in the nuclear spread area of fibroblasts in a co-culture compared to myofibroblasts in the co-culture (unpaired t-test, $n=3$). **(E)** There is no significant difference in the nuclear spread area of fibroblasts and myofibroblasts in the co-culture when compared to that of myofibroblasts in a monoculture (One-way ANOVA, $n=3$).

Qualifying Cardiovascular Health Using Biomarkers of Inflammation and Oxidative Stress in Response to Acute Wildfire Smoke Inhalation: A Path to Assessing Occupational Risk

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Wildfires account for over \$130 billion in health-related expenses across the U.S. and are a leading source of air pollution. Epidemiological evidence has shown increased rates of cardiovascular morbidities and mortalities immediately after a wildfire event, triggered by the inhalation of particulate matter, carbon monoxide, and other toxic chemicals in wildfire smoke (WFS). However, epidemiological studies underestimate the health risks faced by occupational workers by failing to collect data on the effects of repeated or chronic WFS inhalation. Wildland firefighters (WLFFs) are most at-risk for mortality due to cardiac events and are almost three times more likely to develop prehypertension and hypertension compared to the general population. The significant increased risk for CVD in WLFFs highlights the need to detect early indicators of the health effects of wildfire smoke inhalation, so we can improve exposure thresholds and guide public health policies to reduce WFS-related health risks. To do so, we will recruit northeastern WLFFs and measure their functional and biological indicators of health before and after a prescribed wildfire event. We will relate changes in health to biomarkers of inflammation and oxidative stress to identify early warning signs of cardiovascular damage.

Keywords: wildfire, inflammation, oxidative stress, occupational workers

Macrophage-Guided Phase-Change Nanoparticles for Precision Ultrasound-Triggered Drug Release

Poulami Mondal (1), Krunal Sanjay Patel (1), Tao Sun (1),

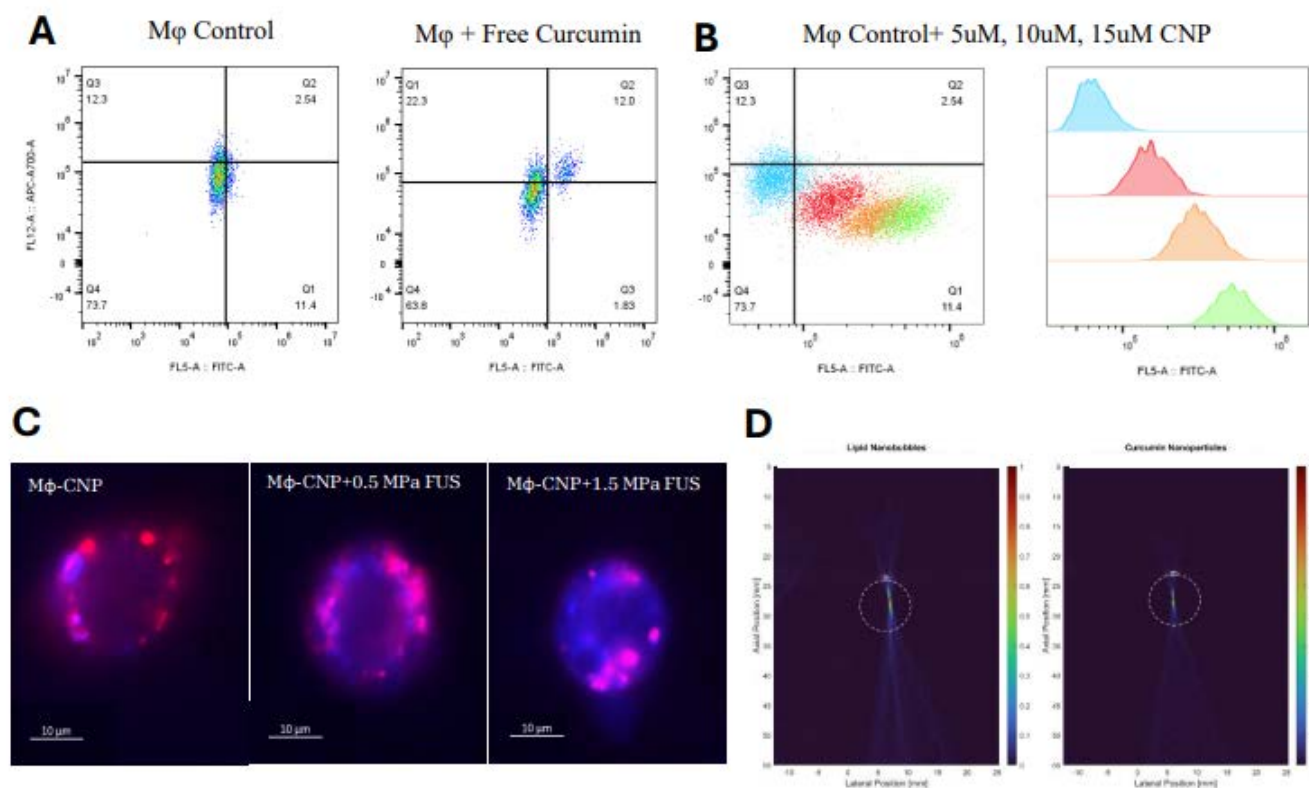
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We developed an ultrasound-activatable, phase-changing nanoparticle system for precision cancer therapy, with macrophage carriers enabling targeted, spatiotemporally controlled drug release. We engineered PLGA-PEG phase-changing nanoparticles encapsulating curcumin and perfluorooctylbromide using nanoprecipitation. These nanoparticles were rapidly internalized by macrophages, serving as carriers for targeted delivery. We characterized their physiochemical properties, encapsulation efficiency, and focused ultrasound (FUS)-triggered vaporization. Nanoparticles ranging from 280 ± 40 nm were successfully formulated. Curcumin-loaded nanoparticles (CNP) remained stable at 4°C for 30 days, with an encapsulation efficiency of 16–25%. Ultrasound induced phase transitions at 1.0 MPa ($p < 0.05$). Flow cytometry confirmed macrophage uptake, with 99.8% internalization compared to 12.4% for free curcumin (Fig. A-B), while macrophage viability remained high (93–95%) over 24 hours. Flow cytometry and confocal microscopy confirmed cellular uptake, while ultrasound exposure at varying pressures (0.5–1.5 MPa) facilitated drug release. Confocal microscopy verified ultrasound-triggered vaporization and intracellular drug release (Fig. C). Passive cavitation imaging (Fig. D) further supported the release mechanism. Cytotoxicity was evaluated in a GL261 glioma co-culture model, showing enhanced therapeutic efficacy with cytotoxicity increasing from 58% to 71% at 1.5 MPa sonication. Therefore, our macrophage-based nanoparticle system enables targeted, ultrasound-triggered drug release, offering a promising immune cell-mediated strategy to enhance therapeutic efficacy in cancer treatment.

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Key Words: Ultrasound-triggered drug release, Phase-changing nanoparticles, Macrophage-mediated delivery, PLGA-PEG nanoparticles, Cancer precision therapy





(A) Flow cytometry revealed two distinct populations compared to control macrophages, and the naked/free drug was inefficiently taken up by cells. (B) demonstrated efficient macrophage uptake of curcumin-loaded nanoparticles (CNPs), where increasing the amount of CNPs added, significantly increased the internalization in macrophages (blue: no treatment control, red: 5uM, orange: 10uM, green: 15uM curcumin respectively). (C) CNP internalization and subsequent vaporization into nanobubbles upon exposure to FUS (0.5–1.5 MPa), facilitating intracellular curcumin release. (D) PAM data validates cavitation in CNPs at 1.0MPa as compared to lipid nanobubbles control.

BIOENGINEERING A LONG-LASTING HUMAN NEUROVASCULAR NICHE WITH PERFUSED VASCULAR NETWORKS

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Recent research has suggested that prolonged exposure to microgravity due to space travel impacts the functionality of the blood-brain-barrier (BBB) and may also be linked to axonal damage in neurons caused by a pro-inflammatory phenotype. Bioengineered microfluidic models of the human neurovascular niche (hNVN)- a specialized region found in the adult brain that contains brain microvascular networks and neural stem cells (NSCs) capable of differentiating into new neurons- are particularly promising to bridge this gap in knowledge and uncover the underlying mechanisms causing BBB dysfunction and neuroinflammation. The vascular niche is known to orchestrate NSC fate; however, this dynamic interaction is not well defined in humans, especially under the effect of microgravity. Current microfluidic models of the hNVN can recapitulate this dynamic interaction, yet only maintain an intact BBB for less than a month which limits our ability to explore the effect of prolonged microgravity exposure.

Our research aims to improve the current microfluidic hNVN models to be long-lasting by upregulating key genes associated with vascular lumen durability, optimizing the hydrogel material to be more brain-specific and therefore better suited for NSC co-culture, and the addition of constant luminal flow to trigger mechanical pathways that will further strengthen the lumen.

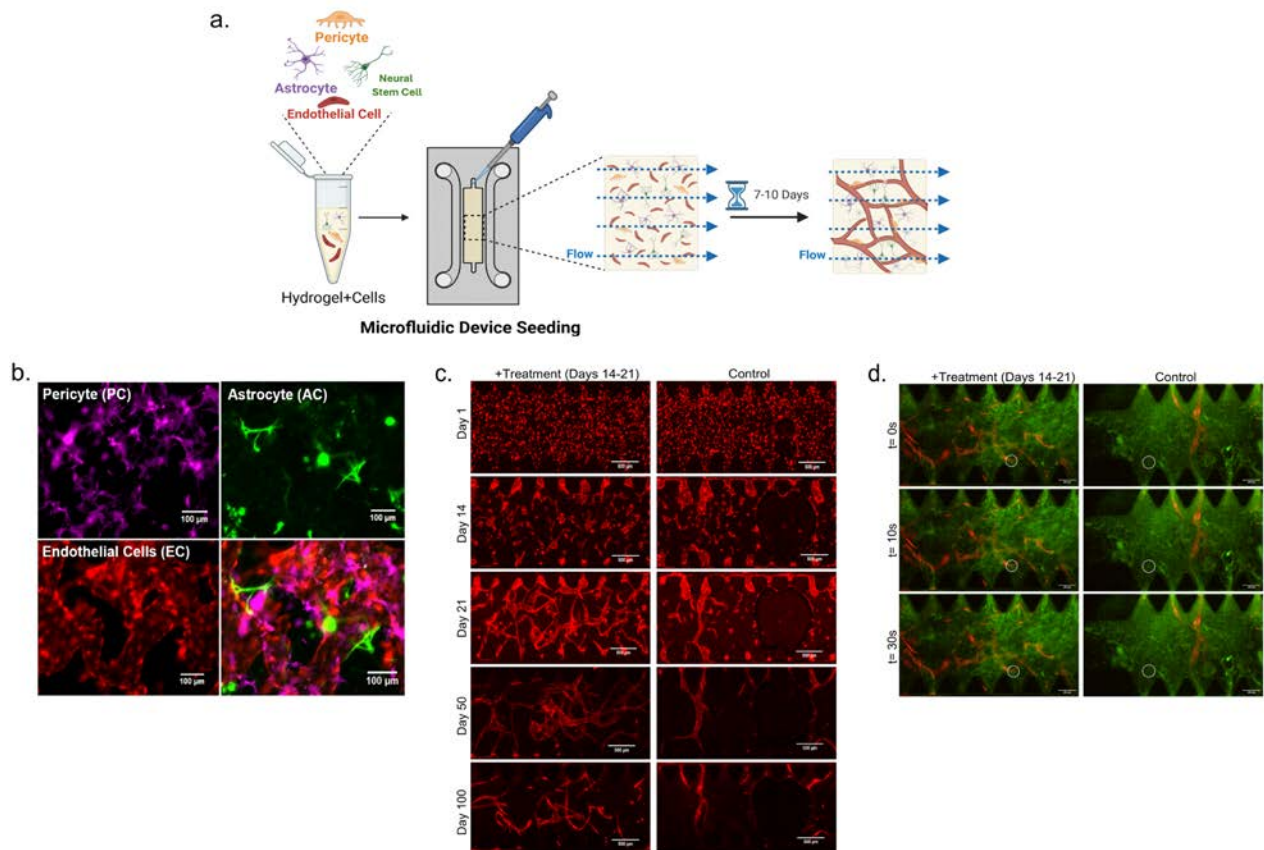


Figure 1: **A.** Schematic of hNVN model generation. Single cells are resuspended in a fibrin hydrogel and seeded into a microfluidic device under the presence of interstitial fluid flow across the bulk hydrogel. Within 7-10 days, the quad co-culture of cells will self-organize into microvascular networks recapitulating BBB morphology. **B.** Representative images of supporting cells interaction recreating BBB morphology. **C.** Images of vascular network formation and stability in quad culture samples over time. Red fluorescence is tdTomato brain endothelial cells transduced prior to co-culture. **D.** Representative images from time-lapse microscopy capturing neuronal firing based on the calcium reporter jRCaMP1a transduced into the NSCs prior to co-culture.

A photograph of two women standing on a modern wooden staircase. The woman in the foreground is wearing a yellow top and a black skirt, holding a yellow phone. The woman behind her is wearing a black top. The staircase has a light-colored wooden wall and a metal handrail.

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