Vanderbilt University Biomaterials Day
March 28, 2025

The Vanderbilt Student Chapter of the Society for Biomaterials was proud to host Biomaterials Day in the Student Life Center on Vanderbilt's campus on March 28, 2025. This one-day event consisted of several research sessions featuring early-career faculty from various regional institutions, rapid-fire trainee presentations, a poster session, two special session presentations, and a keynote address.

Participants: Vanderbilt's Biomaterials day consisted of 130 registered participants. Undergraduates, graduate students, postdocs, and faculty came from 13 distinct regional universities including Vanderbilt, University of Alabama in Huntsville, University of Cincinnati, Georgia Institute of Technology, University of Kentucky, University of Memphis, University of Mississippi, Purdue University, University of Tennessee, University of Alabama, Mercer University, University of Arkansas, and Washington University in St. Louis. A full list of registered participants can be found in the Appendix of this report.

Sponsors and Funding: Funding sources and funding amounts are outlined as follows:
- Society for Biomaterials: $2,500 (Taxed at 9% in TN taxes, $2268.75)
- Vanderbilt Student Organization Funds: $2,900
- Vanderbilt College of Engineering: $5,000
- Vanderbilt Institute of Nanoscale Science and Engineering: $3,000

Total Funding: **$13,400.00 ($13,168.75 after tax)**

Expenses: Major expenses for Biomaterials Day are outlined below:
- Breakfast: $842.85
- Lunch: $2,090.56
- Reception: $4,094.93
- Shirts: $1,453.34
- Transportation (flights, hotels, parking): $1,306.16
- Awards and honorariums: $1,925.00
- Other: $1,410.31

Total Expenses: $**13,123.15**

Speakers: Vanderbilt's Biomaterials Day was proud to have 8 faculty research presentations, 4 trainee plenary talks, 9 trainee rapid-fire talks, and 2 special session speakers. Speaker names and presentation titles are outlined below:

Keynote Presentation:
- **Dr. Andrés García**, Duke University - "Synthetic Hydrogels for Regenerative Medicine "

Faculty Presentations:

- **Dr. Leopold Green**, Purdue University - “Design Principles of DNA Origami for Cell Membranes Interfacing”
- **Dr. Eden Tanner**, University of Mississippi - “Bioinspired Ionic Liquids: Leveraging Chemistry to Achieve Targeted Drug Delivery”
- **Dr. Brittany Givens**, University of Kentucky - “Recent Advances In Endometrial Cancer Treatment Using Drug Delivery Systems”
- **Dr. Daniel Gonzales**, Vanderbilt University - “Polymer neural interfaces for simultaneous electrophysiology and imaging in vivo”
- **Dr. Xiaowei Li**, Washington University in St. Louis - “Angiogenic Biomaterials to Support Tissue Regeneration”
- **Dr. Briana Simms**, University of Cincinnati - “Design and synthesis of next generation lipid nanoparticles for therapeutic delivery”
- **Dr. Jonathan Brunger**, Vanderbilt University - “Engineered cell-material interactions to instruct tissue regeneration and repair”
- **Dr. Olga Liaudunskaya**, University of Cincinnati - “Cell-specific mitochondria dysfunction after moderate injury in human 3D in vitro brain model”

Trainee Plenary Talks:

- **Dr. Anastasia Varanko**, Vanderbilt University (Post Doc) - “A Regulatory T-cell Targeted Protein Nanocarrier for Cancer Immunotherapy”
- **Dr. Ryan Cree Miller**, Georgia Institute of Technology (Post Doc) - “Microfluidic Assembly of Mitochondria-Loaded Microparticles for On-Demand Delivery and Boosted Bioenergetics”
- **Mariah Bezold**, Vanderbilt University (Graduate Student) - “Hybrid Shear-thinning Hydrogels as an Injectable Delivery Platform for Repair of Diabetic Skin Wounds”
- **Karina Bruce**, University of Cincinnati (Graduate Student) - “Engineering of degradable linkers to improve oxidative sensitivity of thioketal-based biomaterials for regenerative medicine applications”
- A prize was awarded to the best plenary talk as voted on by attendees

Trainee Rapid Fire Presentations:

- **Jordan Berezowitz**, University of Kentucky - “Genotoxic Effects of Copper Oxide Nanoparticles in Endometrial Cancer Cell Models”
- **Sydney Bone**, University of Alabama Huntsville - “Liposomes as a Platform for CD40 Ligand Presentation for the Purpose of Feeder-Free B Cell Activation”
- **Sydney Henriques**, Vanderbilt University - “Bait and Switch: Exploiting Macrophage Behavior as a Cancer Treatment”
- **Daniel Hinrichsen**, University of Cincinnati - “3D In-Vitro Model of Human Neurovascular Unit to Study Traumatic Brain Injury”
- **Hayden Pagendarm**, Vanderbilt University - “STING-Agonist Conjugated Bispecific Albumin/PD-L1 Targeted Nanobody Antigen Fusions Demonstrate Potent Antitumoral Effects”

- **Sirjana Pun**, University of Cincinnati - “Leveraging a 3D bioprinted Microphysiological system of glioblastoma to investigate novel therapeutic delivery approaches: focused ultrasound and adeno-associated virus”
- **Keshav Shah**, Georgia Institute of Technology & Emory University - “Enzymatically-Degradable Hydrogel Microcarriers and Pro-Inflammatory Cytokine Licensing Modulate Mesenchymal Stromal Cell Secretome”
- **Amelia Soltes**, Vanderbilt University - “Optimization of siRNA Nanoparticles with Custom Surfactants for Osteoarthritis Treatment”
- **Priyavrat Vashisth**, University of Mississippi - “Ionic liquid-coated gold core polymeric nanoparticles for selective neutrophil hitchhiking and targeted endometriosis treatment”
- A prize was awarded to the best rapid fire talk as voted on by attendees

Special Session Presenters:

- **Dr. Brittany Givens**, University of Kentucky - “Biomaterials for Public Health Transformation: Endometrial Cancer”
- **Dr. Briana Simms**, University of Cincinnati - “Research and Entrepreneurship: Translating science from the benchtop and into the community”

Poster Session: Vanderbilt’s Biomaterials Day poster session consisted of 63 posters. Poster presenters represented all the regional universities that were present and consisted of undergraduate, graduate, and postdoctoral trainees. Six prizes were awarded including 1 third place graduate student prize, 1 second place graduate student prize, 1 first place graduate student prize, 1 first place post doctoral fellow prize, 1 first place undergraduate student prize, and 1 fan favorite poster prize. A list of poster abstracts can be found in the appendix.

Feedback: After the event, a survey was sent out to all the participants asking for their feedback about the event. The survey responses were overwhelmingly positive, especially in regard to the representation of the diversity of researchers in our region, communication of details, and the special topics sessions. Some of the criticisms included the busy schedule (especially in the evening), the lateness of the second poster session, and lateness of the award ceremony. Notably, the original schedule for this day was designed to address these issues, but the travel restrictions of the keynote speaker required us to push the events around. In the future, we can reduce the number of speakers to give more break time, in addition to ending the event earlier and being more prepared for the awards ceremony. Other feedback received that we will apply include encouraging more mingling and having the event more regularly.

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Poster Abstracts:

Adam Abduhlraman | *Vanderbilt University* | *Targeted RNA Therapeutics for Border-Associated Macrophages in Neurodegeneration*

Neuroinflammation is a key driver of Alzheimer's disease (AD), with border-associated macrophages (BAMs) playing a central role in modulating inflammatory pathways. This study develops targeted RNA therapeutic approaches to silence inflammatory mediators in BAMs while minimizing off-target effects. Our goals are: (1) to determine the lowest effective dose of divalent lipid-siRNA (L2-siRNA) that silences genes in BAMs while minimizing off-target accumulation in other CNS cell types and peripheral organs, (2) to characterize the BAM-targeting capacity of a trimannose-siRNA conjugate, and (3) to apply this platform to silence key pro-inflammatory genes implicated in AD.

L2-siRNA exhibits robust perivascular biodistribution after a single intracerebroventricular (ICV) injection, with BAMs—residing in the perivascular space—receiving high levels of delivery. Confocal microscopy and flow cytometry after fluorophore labeled L2-siRNA demonstrated greater BAM uptake compared to parenchymal microglia, with retention even at lower doses. This suggests that lowering L2-siRNA dosing may selectively enhance BAM targeting while mitigating silencing in other CNS compartments. In murine models, 2 nmol L2-siRNA induced gene silencing across BAMs, microglia, and astrocytes, as assessed by single-cell RNA sequencing. Future studies will optimize lower doses to improve BAM specificity.

BAMs uniquely express the mannose receptor (CD206), making them an ideal target for mannose-conjugated therapeutics. In vitro, CD206^hi macrophages exhibited increased mannose-siRNA uptake, and exhibit efficacious, carrier-free knockdown (IC50=12.09nM). In vivo RNAScope biodistribution analysis following ICV delivery in mice showed trimmanose-siRNA accumulation in perivascular cells, including those distant from the injection site 24 hours after ICV administration. This study establishes a framework for macrophage-targeted RNA therapeutics in neurodegeneration, optimizing BAM-specific silencing to enable precise investigation and therapeutic modulation of neuroinflammation in AD and other neurodegenerative diseases.

Kimia Abedi | *University of Cincinnati* | Bioprinting a Model of breast cancer-on-chip for assessment of drug efficacy in vitro

Breast cancer accounts for one in six cancer-related deaths in women, largely due to its metastatic nature and complex tumor microenvironment. Understanding the dynamic interactions between tumor cells, stromal components, and vascular networks is critical to developing effective therapeutic strategies for breast cancer. Conventional models often lack the physiological complexity needed to accurately predict drug responses. To address this challenge, we have developed a microfluidic 3D bioprinted Breast Cancer-on-Chip platform, integrating an endothelialized microfluidic channel capable of perfusion with cell culture medium and chemotherapeutic agents, effectively mimicking systemic drug administration. Our model incorporates patient-derived tumoral masses, generated via 3D bioprinting of self-assembled organoids composed of primary breast cancer epithelial cells, cancer-associated fibroblasts, and endothelial cells. We demonstrate that breast cancer cells actively drive the self-organization of a microvascular network and invade the surrounding matrix within the engineered tumor microenvironment. Additionally, our findings reveal that the tumoral assembloids develop a dense collagen capsule, which may shield tumor cells from circulating therapeutics, potentially contributing to drug resistance. This model will serve as a versatile and physiologically relevant platform to investigate mechanisms of drug resistance, testing immunotherapies in a controlled microfluidic environment.

Grace Adams | *Vanderbilt University* | Transparent polymer-based electrodes for combined electrophysiology and two-photon imaging

In mouse models, electrophysiology-based neural interfaces can measure neural activity on the millisecond scale, on par with the timing of neuronal firing. However, electrophysiology sparsely samples the surrounding tissue and does not provide structural information about neural circuits. Alternatively, two-photon (2P) imaging can provide dense, cell-specific structural mapping on long time scales. Combining these two modalities would create a strategic platform for investigating neural circuits with incredible detail. However, optical imaging is largely incompatible with traditional, silicon-based electrophysiology arrays. To address this gap, we are developing a flexible, transparent electrode array that can be integrated with 2P imaging. The transparency of this probe enables imaging of the specific cells providing electrophysiology signals, while its flexibility allows for the necessary hardware to be packaged outside of the optical window. The probes, fabricated using standard microfabrication techniques, consist of 32 channels of thin-film platinum and indium-tin oxide (ITO) encased in a thin layer of the transparent polymer Parylene C. ITO is a transparent and conductive alternative to conventional metals, but has high impedance and therefore is primarily implemented in millimeter-scale electrodes. By balancing electrode geometries, ITO electrodes can be scaled down to near the cellular scale with a sufficiently low electrochemical impedance and high transparency. The probes can be temporarily stiffened against a driving microwire with polyethylene glycol for implantation. Initial in vivo implantations of full platinum probes achieved preliminary acute, anesthetized recordings with detectable spike activity. The combined use of transparent electrodes and 2P imaging introduces significant technological advances for investigating fundamental neural circuit mechanisms.

Ella Adjei-Sowah | *Vanderbilt University* | Engineering lipid nanoparticles for targeted activation of RIG-I to potentiate antitumor immunity in renal cell carcinoma

Renal cell carcinoma (RCC) poses a substantial public health challenge. ccRCC represents approximately 2.4% of all adult cancers, with over 400,000 new cases diagnosed and 180,000 deaths globally each year. The gold standard for treating localized ccRCC is surgical resection via radical or partial nephrectomy. However, 30– 40% of RCC patients are diagnosed with metastatic disease at the time of diagnosis, and 20-30% experience metastatic recurrence following surgery, hence, there is a clear need for immunotherapies to improve patient outcomes. We engineered a lipid nanoparticle platform for 3pRNA delivery that maximizes RIG-I activation in the TME, and evaluated the efficacy and safety of this new class of immunotherapy for ccRCC. Our first generation 3pRNA/LNP platform reprogrammed the RenCa TME to enhance infiltration of activated and proliferating CD8+ and CD4+ T cells, NK cells, and cross-presenting dendritic cells, while reducing the frequency of M2 like macrophages after intratumoral administration. Furthermore, RIG-I activation in the RenCa TME induced vascular normalization as evidenced by reduced hypoxia, reduced vascular permeability, and increased pericyte coverage. This work demonstrates the immunotherapeutic benefit of targeting the RIG-I pathway in mouse models of ccRCC, and current efforts are focused on optimizing the LNP platform for more targeted 3pRNA delivery to open a new approach for improving immunotherapy outcomes.

Adekunle Akinmola | *University of Alabama in Huntsville* | Synthesis and self-assembly of folate-conjugated di- and triblock copolymers of poly(ethylene glycol), poly(Allyl-L-Gly) and poly(L-Leu) for delivery vehicles

It's essential that materials designed for biomedical applications are biocompatible and biodegradable. Ring-opening polymerization of amino acid N-carboxyanhydrides was investigated with α-hydroxy-ω-amino polyethylene glycol (PEG) as macroinitiator to synthesize amphiphilic block copolymers with varied chain lengths. The block copolymers were processed into micelles. The amphiphilic properties of the copolymer were investigated for physical entrapment of hydrophobic drugs and imaging agents. Further conjugation of folic acid to the block copolymer will enhance the binding efficacy of the micelles to targeted cells, increasing drug concentration on targeted site. Folate-conjugated and unconjugated PEGylated amino acids (poly(Allyl-L-Glycine and poly(L-Leucine)) with varying leucine monomer ratios were processed into micelles to deliver iron oxide nanoparticles. The resulting micelles were spherical with sizes range of 50 to 200 nm. The monomer ratio of the amino acid block was observed to determine the micelles' properties. Iron oxide encapsulated micelles show size variation because of an increase in the hydrophobicity of the micelle core.

Charitha Anamala | *University of Cincinnati* | Metabolic Regulation of Epigenetic Response Post Traumatic Brain Injury

Charitha Anamala, Sahan Kansakar, Sydney Sterben, Volha Liaudanskaya

Traumatic brain injury (TBI) disrupts mitochondrial metabolism, leading to bioenergetic failure and neurodegeneration. However, cell-specific metabolic responses and their long-term impact remain poorly understood. Here, we investigate the metabolic consequences of TBI in a 3D in vitro brain model, utilizing bioengineered silk scaffolds seeded with a triculture of mature human neurons, astrocytes and microglia to mimic the brain microenvironment. The AKG/2HG ratio

influences cellular bioenergetics by modulating mitochondrial metabolism, with AKG supporting ATP production and 2HG potentially disrupting oxidative phosphorylation and redox balance. Our study focuses on correlating α-ketoglutarate (AKG) and 2-hydroxyglutarate (2HG) levels with mitochondrial function and neurodegenerative progression post-injury. We hypothesize that TBI-induced (tricarboxylic acid cycle) TCA cycle disruptions—marked by decreased AKG and increased 2HG—drive mitochondrial dysfunction and epigenetic alterations, impairing neuronal recovery. Media samples were collected at 1h, 4h, 8h, 24h, 48h, and 72h post-injury to track AKG/2HG ratio dynamics to compare with neurodegeneration markers. Preliminary analysis of these samples revealed a lower AKG/2HG ratio compared to sham starting at 1h post injury, with a significant downward trend until 72 hours. Furthermore, the mitochondrial function of all three cell types (in terms of oxidative phosphorylation) showed a downward trend at these time points. Suggesting that the cellular ability to initiate the recovery required gene expression changes are compromised. We will next determine if this trend continues to chronic time points, followed by DNA methylation analysis of recovery and survival genes post- injury of 3D brain-like tissues to isolate genes of interest.

Jordan Berezowitz | *University of Kentucky* | Genotoxic Effects of Copper Oxide Nanoparticles in Endometrial Cancer Cell Models

In the U.S., endometrial cancer (EC) has the largest number of new cases and deaths compared to all other gynecological cancers. EC, the fourth most common cancer in women, is primarily treated with a hysterectomy—an invasive surgical procedure. Chemotherapeutic resistance to existing drugs is one of the greatest complications to cancer therapy. With few treatment options available, there is demand for research of anti-cancerous materials to more effectively treat EC. Research has shown that copper(II) oxide nanoparticles (CuO-NPs) possess anti-neoplastic potential, as Cu^{2+} ions trigger the generation of reactive oxygen species (ROS), leading to cytotoxicity. By harnessing this toxicity, we hypothesize that CuO-NPs will lead to apoptosis of EC cells. Our study utilized two EC cell lines (HEC-1A and AN3CA) and an endothelial cell line of a non-diseased state (HEK293). Viability assays and IC50 values quantified the cell's toxicity to CuO-NPs and provided a range of concentrations where cells experienced sensitivity to CuO-NPs. Apoptosis assay results confirmed toxicity profiles and quantified the cells' degree of apoptosis when exposed to CuO-NPs. All cell lines showed a concentration-dependent increase in gamma-H2AX expression, a marker for double-strand DNA breaks (DSBs). Investigation of GSH/GSSG ratios will explore the oxidizing power of CuO-NPs at different concentrations and exposure times. These in vitro toxicity studies demonstrate the anti-cancer properties of commercially available CuO-NPs and provide a foundation for the development of engineered nanoparticle drug delivery systems for targeted EC therapy, offering more effective treatment options and outcomes for patients.

Sydney Bone | *University of Alabama in Huntsville* | Biomaterials-based Synthetic Niches for in Vitro Expansion and Differentiation of Human Naïve B-cells

Nano- and Micro-scale Biomaterial Alternatives to Feeder Cells for Primary Human B-Cell Culture

Pearlson Prashanth, Sydney Bone, Anna Bell, Kyung-Ho Roh
University of Alabama in Huntsville

Improvement of in-vitro culture of immune cells is critical for improving our understanding of the immune system and advancing the treatment of diseases, whether pathogenic, cancerous, or autoimmune, via cellular immunotherapies. We are developing several platforms for the presentation of signaling molecules for the activation of human B cells without the use of feeder cells. Our recently published work reports success using commercially available iron oxide microbeads for CD40 ligand presentation. Additionally, we have promising early results using liposomes, which are small (50-200 nm) spherical assemblies of phospholipids and cholesterol that form a bilayer surrounding an aqueous solution. Both CD40L presentation mechanisms, when coupled with appropriate soluble signaling, can induce activation, proliferation, and differentiation of naïve B cells in-vitro without feeder cells.

Andrew Bryan | *University of Cincinnati* | Development of a piezoelectric scaffold with integrated bioactivity for traumatic injury

To address current limitations in biomaterials for trauma-associated tissue repair regarding a lack of physiologically relevant signaling and immune response following implantation, this study evaluated the development and feasibility of a multi-cue biomaterial with physiologically relevant chemical, electrical, and physical signaling. To accomplish this, a bulk-functionalized poly(vinylidene fluoride-trifluoroethylene) (PVDF-TrFE) scaffold was fabricated using blend electrospinning methods to incorporate decellularized extracellular matrix (dECM) primarily composed of fibronectin to mediate the foreign body response in vivo and promote enhanced wound healing. A known facilitator in the cell-ECM dynamic reciprocal relationship, the use of fibronectin as the primary component can advantageously regulate cell phenotype in a wound environment. Blended scaffolds were evaluated for physical, chemical, and electrical properties in vitro and further analysis of cell-material interactions in the context of peripheral nerve injury (PNI) repair and in vivo for immune response was performed. In vitro characterization demonstrated increased cell adhesion and repair-like morphologies in Schwann cells on blended scaffolds. Subcutaneous implantation of blended scaffolds in rats at 7 days revealed limited foreign body giant cell formation. Following 28 days in vivo, regenerative characteristics of the proliferative phase of wound healing were present with enhanced nerve growth and vasculature accumulating near the area of tissue inflammation. Overall, blend-electrospun scaffolds with incorporated, physiologically relevant dECM showed promise in mitigating pro-inflammatory responses and promoted advanced regenerative phenotypes in Schwan cells, thereby demonstrating the potential for an implantable biomaterial with the necessary physical, chemical, and electrical characteristics desired for clinically relevant tissue engineered applications.

Nina Cassidy | *Vanderbilt University* | Albumin-binding siRNA conjugates targeting Mcl-1 for the treatment of Triple-Negative Breast Cancer

Triple-Negative Breast Cancer (TNBC) comprises 15-20% of breast cancers. Because TNBC lacks targetable surface receptors, patients are relegated to more toxic and less effective chemotherapies. In addition to limited treatment options, outcomes for these patients are also worse than in patients with other breast cancer subtypes because TNBCs are highly invasive, prone to relapse, and have high metastatic potential. Short interfering RNA (siRNA)-based

therapies are a promising class of therapeutics capable of silencing traditionally ‘undruggable’ oncogenic targets. However, effective tumor delivery of siRNA faces many challenges including nuclease degradation and short circulation times. The recent clinical advancement of enhanced stabilization chemistries such as 2' ribose modifications and phosphorothioate linkages has enabled carrier-free delivery of metabolically-stabilized siRNA therapies. Beyond these chemical modifications, we have developed an siRNA lipid end-modification platform that promotes in situ albumin binding, a strategy that enhances circulation time and tumor delivery/silencing. In the current work, we showcase this platform for its therapeutic efficacy in treating both solid tumors and metastatic disease by targeting the anti-apoptotic protein Mcl-1. Furthermore, using a transgenic mouse model harboring the human Mcl-1 gene, we show that this siRNA conjugate avoids the dose-limiting on target toxicities of an Mcl-1 small molecule inhibitor that has reached clinical trials. In ongoing studies, we have appended the albumin-binding conjugate with a ligand for receptor-mediated targeting, an approach that further potentiates siRNA delivery in the spontaneous tumors MMTV-PyMT model. These promising results highlight the immense potential of molecularly defined siRNA therapeutics as a treatment option for TNBC.

Neil Chada | *Vanderbilt University* | Albumin-hitchiking nanobody STING agonist conjugates to improve adoptive cell therapy

Adoptive cell therapy (ACT) has demonstrated promise in treating solid tumors but remains hindered by the immunosuppressive tumor microenvironment (TME), which limits T cell infiltration and persistence. Stimulator of interferon genes (STING) agonists have emerged as a strategy to remodel the TME, but systemic delivery remains a challenge due to poor tumor accumulation and toxicity. To address this, we developed albumin-hitchhiking nanobody-STING agonists (AHNSA), which leverage albumin transport for enhanced tumor localization and controlled immune activation.

Our study evaluates AHNSA as an adjuvant to ACT in a TCR-transgenic T cell adoptive transfer model. First, we determine the optimal regimen for combining AHNSA with ACT to maximize antitumor efficacy. Next, we assess the effects of AHNSA on the phenotype of adoptively transferred cells, focusing on activation, proliferation, exhaustion, and cytotoxic markers using flow cytometry and immunohistochemistry. Finally, we examine immune cell composition within the TME to identify changes induced by AHNSA treatment.

Preliminary data indicate that AHNSA-mediated STING activation enhances T cell infiltration into solid tumors and reduces tumor burden. We hypothesize that systemic AHNSA administration will promote the infiltration of more activated, proliferative, and less exhausted adoptively transferred cells, leading to improved tumor control. This work establishes a novel immunotherapeutic strategy for overcoming the barriers of ACT in solid tumors by harnessing systemic STING agonism with targeted nanobody delivery.

Desirée Denman | *University of Tennessee, Knoxville* | PEGylated and PEOylated nanoparticles (NPs) interact with macrophages by directly binding scavenger receptor receptors

Poly-ethylene-glycol (PEG)-based nanoparticles (NPs) - including cylindrical micelles (CNPs), spherical
micelles (SNPs), and PEGylated liposomes (PLs) - are hypothesized to be cleared in vivo by opsonization
followed by liver macrophage phagocytosis. This hypothesis has been used to explain the rapid and significant localization of NPs to the liver after administration into the mammalian vasculature. Here, we show that the opsonization-phagocytosis nexus is not the major factor driving PEG-NP–macrophage interactions. First, mouse and human blood proteins had insignificant affinity for PEG-NPs. Second, PEG-NPs bound macrophages in the absence of serum proteins. Third, lipoproteins blocked PEG-NP binding to macrophages. Because of these findings, we tested the postulate that PEG-NPs bind (apo)lipoprotein receptors. Indeed, PEG-NPs triggered an in vitro macrophage transcription program that was similar to that triggered by lipoproteins and different from that triggered by lipopolysaccharide (LPS) and group A Streptococcus. Unlike LPS and pathogens, PLs did not increase transcripts involved in phagocytosis or inflammation. High-density lipoprotein (HDL) and SNPs triggered remarkably similar mouse bone-marrow-derived macrophage transcription programs. Unlike opsonized pathogens, CNPs, SNPs, and PLs lowered macrophage autophagosome levels and either reduced or did not increase the secretion of key macrophage pro-inflammatory cytokines and chemokines. Thus, the sequential opsonization and phagocytosis process is likely a minor aspect of PEG-NP – macrophage interactions. Instead, PEG-NP interactions with (apo)lipoprotein and scavenger receptors appear to be a strong driving force for PEG-NP–macrophage binding, entry, and downstream effects. We hypothesize that the high presence of these receptors on liver macrophages and on liver sinusoidal endothelial cells is the reason PEG-NPs localize rapidly and strongly to the liver.

Madisen Domayer | *Vanderbilt University* | Creation of Hyaluronic Acid Hydrogel with Bioadhesive Properties for the Delivery of High Drug-loading Nanoparticles for the Treatment of Osteoarthritis

Osteoarthritis is a chronic condition that leads to the degradation of cartilage, causing joint pain and disability. Current treatment options include corticosteroid and hyaluronic acid (HA) injections, but these treatments only provide temporary relief and do not slow the progression of disease. As a result, patients are often ultimately relegated to total joint replacement. HA consistently reduces joint pain associated with OA, but the half-life of HA is relatively short. Here, we are modifying HA to create bioadhesive, shear-thinning, hydrogels that are a composite of HA and nanoparticles. This design seeks to afford prolonged patient relief and also an opportunity for combining the benefits of HA with sustained local drug release from the nanoparticle component of the hydrogel. Furthermore, the NPs will be formed from polysulfides, an ROS responsive class of polymers that have inherent antioxidant, therapeutic function in the context of OA.

Sara Edgecomb | *University of Mississippi* | Enhancing enzyme replacement therapy for GM1 Gangliosidosis using ionic liquids

GM1 gangliosidosis is a neuropathic lysosomal storage disease (LSD) caused by a genetic mutation that leads to limited production of the enzyme β-galactosidase (β-gal) and early

childhood mortality. Unfortunately, the blood-brain barrier (BBB) limits treatment of this brain-centered disease. In previous studies, polymersomes, nanoparticles (NPs) made with di-block co-polymers, have been used as potential drug delivery vehicles for enzyme replacement therapy (ERT) as a treatment option. However, in LSD-affected patients, there is a lack of specificity of delivery since there is a widespread presence of low-density lipoproteins in areas of inflammation in various organs. In recent years, the efficiency and effectiveness of various types of NPs have been shown to be improved by the addition of biocompatible ionic liquids (ILs). ILs are low-melting salts (< 100oC) consisting of bulky asymmetric cations and anions. Their integration in polymeric NP systems broadly enhances their biomedical applications, not only improving the delivery of sparingly soluble drugs and in antimicrobial applications, but also enabling enhanced interactions between new biological membranes to improve efficiency via intravenous drug delivery. Due to the vast structure-to-function physicochemical tunability that is possible, their cationic and anionic components can be engineered to self-assemble onto interfaces through electrostatic interactions and hydrogen bonding. This coating then confers protective properties, such as protein-repulsion and cellular biocompatibility, for enhanced circulation half-life. By combining ILs and polymersomes, we develop NPs that can target the brain via “hitchhiking” on to red blood cells (RBCs) for ERT.

Gabriella Faircloth | *Vanderbilt University* | Optimizing Hydrogel Microsphere Formation for Suspension Cell Culture

The use of hydrogel microspheres has been recognized as a potential way to scale up the culture of traditionally adherent cell lines. Gelatin methacryloyl (GelMA) is a hydrogel that is UV crosslinkable upon exposure to the photoinitiator Lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP). Its crosslinked form exhibits robust mechanical properties, providing a 3D structure that supports higher cell seeding densities and extended culture durations, making it a strong candidate for microsphere medium. One proposed method of encapsulating cells consists of a flow-focusing microfluidic chip that facilitates formation of GelMA microspheres through the union of an aqueous phase and an oil phase. Previous optimization efforts studied the ratio of the oil phase to the aqueous and determined that the ratio of the oil phase and gel phase remaining the same but at higher velocities does consistently produce the same-sized spheres but at an increased rate, but spheres are not stable in culture conditions. In an attempt to continue this work and gain further understanding of how to manufacture consistently sized, fully crosslinked, spherical microspheres using this method, parameters such as the concentration of hydrogel used and the crosslinking time were optimized. Microspheres formed under each condition were then characterized by their size and production using fluorescent imaging coupled with image quantification. Additionally, rheological analysis on bulk GelMA samples was performed to better understand the structural integrity of the material under varying crosslinking times. Moving forward, additional microsphere optimization will be completed after the addition of cells.

Eva Gbur | *Vanderbilt University* | Endosomolytic albumin-binding siRNA conjugates for cholangiocarcinoma therapy

Cholangiocarcinoma (CCA) is an aggressive malignancy with poor overall survival and limited treatment options. Small interfering RNA (siRNA) therapeutics offer an avenue for silencing

oncogenic drivers of CCA that currently lack approved therapeutics, such as non-G12C KRAS activating mutations, yet face many challenges in delivery, including nuclease degradation, short half-life, and barriers to cytosolic delivery. In recently published work, we established siRNA conjugates modified with twin fatty acids (siRNA-L2), which promote non-covalent complexation with albumin after systemic delivery. The use of albumin as a carrier extends circulation time and promotes tumor uptake while avoiding toxicity associated with traditional cationic nucleic acid delivery vehicles, but the potency of these conjugates is limited by endosomal retention. Here, we designed an siRNA-L2 conjugate with chloroquine (CQ) incorporated into the branching tail structure (siRNA-CQ-L2), which maintains the ability to non-covalently bind albumin, and with multiple copies of CQ, mediates improved endosomal escape, cellular uptake/retention, and reporter gene knockdown in cancer cells. In addition, we have identified that co-targeting KRAS and anti-apoptotic MCL-1L with siRNA-L2 results in formally synergistic killing of KRAS-mutant CCA cells. We confirm synergistic effects on cell viability in vitro in multiple cell lines, with both siRNA-L2 treatment alone and siRNA-L2 in combination with standard chemotherapy. Finally, in immunocompetent transgenic mouse models of CCA, we demonstrate robust siRNA-L2 delivery to tumor and stromal cells, in contrast to surrounding healthy hepatocytes. In sum, this project provides new technological advantages for siRNA conjugates and addresses a pressing need for new targeted therapies for CCA.

Willem Graham | *University of Tennessee Space Institute* | Iron Nanoparticles for Magnetic Particle Imaging Applications

Magnetic nanoparticles have a history of being utilized as tracers for medical imaging techniques. One such imaging modality is Magnetic Particle Imaging (MPI), an imaging technique that is currently in development and demonstrates promising applications for cancer treatment due to its high sensitivity. MPI operates by directly measuring the magnetic response from the tracer particles, which are commonly iron-oxide nanoparticles. It is hypothesized that the signal received from the tracer could be improved with higher pure-iron content in the nanoparticles. The main challenge associated with producing pure-iron nanoparticles (FeNPs) is controlling the particle size without compromising the crystallinity of the nanoparticles. Therefore, we are investigating synthesis techniques to produce FeNPs that are ~20 nm in diameter and highly crystalline to provide the best tracer properties. To accomplish this, iron-oxide nanoparticles will be synthesized at the previously specified size and reduced to pure iron via a calcium hydride reduction reaction. To prevent agglomeration during the reduction reaction, the iron-oxide nanoparticles must be coated with silica before the reaction takes place. Initial reduction reactions have confirmed an increase in pure iron and crystallinity, as well as agglomeration. Currently, experiments are ongoing to reliably coat the ~20 nm iron-oxide nanoparticles synthesized from a solvent-surfactant reaction using oleic acid, oleylamine, and iron (III) acetylacetonate. The coating method currently being explored involves the functionalization of the nanoparticle surface with amines followed by a silica coating procedure tailored for amine-functionalized nanoparticles.

Martin Guerrero | *University of Alabama* | Glycosidic Effects of Natural Flavonoids on Nanoparticle Formation

Quercetin and rutin are plant-derived flavonoids widely used in the pharmaceutical and food industries due to their antioxidant, anti-inflammatory, and neuroprotective properties. However, their low aqueous solubility and stability limit their bioavailability for pharmaceutical applications. Previous studies suggest that flavonoid-metal complexation can enhance solubility and stability, yet glycosylation may influence the efficiency of nanoparticle formation and biological interactions. Understanding these structural differences is crucial for optimizing their biomedical applications. Rutin consists of quercetin with a disaccharide rutinose, while quercetin is an aglycone with no sugar moiety. The sugar group in rutin increases hydrophilicity but may introduce steric hindrance, affecting metal coordination and nanoparticle formation. To investigate these effects, UV-Vis spectroscopy was used to confirm nanoparticle formation, while FTIR verified Fe–O chelation, highlighting structural differences in metal binding. Antioxidant properties were evaluated using the DPPH assay, demonstrating that glycosylation impacts radical scavenging efficiency. A P-glycoprotein (P-gp) blocking assay assessed the ability of these flavonoid-based nanoparticles to inhibit efflux pumps, which play a crucial role in drug resistance. Furthermore, the Persian Blue assay was used to determine blood-brain barrier (BBB) penetration, revealing how glycosylation affects transport across biological membranes

Alexandra Teresa Gutierrez Vega and Maryam Afkhami | *University of Arkansas* | **Liquid Crystalline Collagen Substrates as a Model Platform for Investigating Tumor-Bone Interactions in Metastatic Cancer**

Tumor-induced bone disease (TIBD) is a significant clinical challenge that arises when tumors metastasize to the bone. Due to its complex pathophysiology, the biological factors that drive TIBD are poorly understood, and treatment options are limited. The prevailing theory of TIBD is known as the “vicious cycle” model, which posits that tumor-secreted factors, such as parathyroid hormone-related protein (PTHrP), activated by interactions between tumor cells and the rigid bone surface, drive osteoclast-mediated bone resorption. This process releases growth factors from the bone matrix, such as TGF-β, which further stimulate tumor growth and bone destruction. Therapeutic agents like bisphosphonates and RANK ligand (RANKL) inhibitors mitigate bone destruction, but do not hinder tumor growth nor extend life expectancy. There is a great need, therefore, to develop a deeper understanding of TIBD to guide future therapeutic approaches.

In this work, we investigate the expression levels of three key proteins involved in TIBD—integrin β3 (ITGB3), PTHrP, and Gli2—in breast cancer cells cultured on liquid crystalline (LC) collagen substrates, which mimic the bone marrow microenvironment. Collagen alignment, a proxy for the structural organization of the extracellular matrix in the bone, is hypothesized to influence tumor cell signaling and mechanotransduction. Thus, we use both aligned and random-ordered LC substrates to investigate the impact of collagen alignment on gene expression. Overall, we aim to elucidate how substrate alignment impacts tumor-bone crosstalk, including PTHrP-driven osteoclastogenesis and downstream signaling. Furthermore, our experimental work is complemented by a mechanistic computational model to explore the biochemical dynamics of TIBD-associated intracellular pathways, including TGF-β and Wnt/β-catenin. By integrating experimental and computational approaches, we seek to enhance the understanding of the tumor-bone interactions and molecular pathways that drive TIBD.

Insights gained from this work may ultimately aid in developing targeted therapies for metastatic bone disease, improving patient outcomes.

Sarah Hall | *Vanderbilt University* | Ultrahigh Paclitaxel-loaded Nanoparticles for the Treatment of Triple Negative Breast Cancer

Triple negative breast cancer (TNBC) accounts for over 15-20% of all breast cancers, and their treatment does not benefit from targeted therapies against estrogen, progesterone, and HER2 receptors. Motivated by the need for new therapeutics, we designed polymeric nanoparticles (NPs) to achieve 'ultrahigh' loading (LC ≥ 35%) of the common chemotherapeutic paclitaxel (PTX) to increase PTX maximum tolerated dose (MTD) and consequently anti-tumor efficacy. An ABA triblock copolymer library was synthesized with B blocks comprising a reactive oxygen species (ROS)-responsive polysulfide backbone and varied ratios of H-bonding (hydroxyl) and π-π interacting (Benzyl, %B) side groups. Polymers with higher benzyl composition (%B) displayed lower ROS responsivity and more sustained release profiles vs polymers with higher %hydroxyl. Nude mice were inoculated with 10^6 MDA-MB-231.Luc cells and treated at 150 mg/kg PTX (experimentally-determined NP formulation MTD) of either slow (80%B) or fast (40%B) release NPs or 20 mg/kg of Taxol as a gold standard treatment control. Mice received 5 treatments spaced every 10 days and the study ended on day 100 (n=8). The slow and fast release NP formulations demonstrated therapeutic efficacy superior to the clinical standard, Taxol. By day 100, the fast PTX release group showed 88% survival, while the slow release group maintained 100% survival. The slow release polymeric NP formulation eliminated tumors in 3 out of 8 mice and prolonged survival, exhibiting sustained, potent anti-tumor activity greater than the fast release NPs and Taxol. Thus, this delivery system shows great clinical potential for treatment of TNBC.

Sydney Henriques | *Vanderbilt University* | Injectable Cryogel to Repolarize Macrophage Behavior toward Anti-Cancer Immunity

The tumor microenvironment (TME) contains dysregulated signals that polarize macrophages toward M2 functions, suppressing anti-tumor immunity. Spatiotemporal drug release in the TME is critical for reprogramming tumor-associated macrophages (TAMs) to pro-inflammatory M1 functions, restoring immunosurveillance. To minimize systemic toxicity, localized delivery of immunomodulators like IFN-γ, IL-12, and CCL2 is essential. We developed an injectable cryogel (-20°C hydrogel) as a depot for these cytokines, designed to attract M2-like TAMs and repolarize them into M1-like cells, promoting anti-tumor immunity.

We developed a single layer cryogel and evaluated its impact on tumor growth. Peritumoral injection of the cryogel system into FVB female mice with PyMT-MMTV mammary tumors resulted in significantly suppressed tumor growth, an increase in T cell infiltration, and an increase in the M1:M2 ratio of TAMs.

To allow TAM attraction before their exposure to the inflammatory cytokines, we have developed a novel injectable multi-layered cryogel (MLC) composed of an inner layer and a peripheral layer. We loaded our inner layer with the inflammatory cytokines, while retaining the chemokine in the peripheral outer layer. This design ensures a burst release of CCL2 followed by sustained cytokine release. Our MLC is also tunable, in that the number of layers, layer thickness, and drug dose can be altered to release the biologics with fine control.

The injectability of our MLC allows for localized, non-invasive delivery of our depot to target the therapeutic site with a strong modulatory dose in the TME that has limited systemic toxicity. We believe our cytokine and chemokine loaded MLC will better modulate immune cell behavior to create an inflammatory tumor microenvironment and inhibit tumor progression.

Jordan Hill | *Vanderbilt University* | Modifying siRNA Delivery Using Blue Light PET-RAFT Polymer Conjugates

We evaluated the pharmacokinetic characteristics of a library of siRNA-polymer conjugates and elucidated a minimum polymer size of 40 kDa to reduce renal clearance as well as increase the bioavailability of the conjugates. We optimized reaction conditions for the polymerization of pDMA in a 384-well plate, created a siRNA macro-RAFT agent, and established a library of pDMA conjugates from 20 to 100 kDa. Conjugates over 40 kDa all showed increased half-life, decreased accumulation in the kidney, and increased accumulation in the liver. This size-dependent decrease in clearance rates translated into significant improvements in overall tissue distribution.

Daniel Hinrichsen | *University of Cincinnati* | 3D In-Vitro Model of Human Neurovascular Unit to Study Traumatic Brain Injury

Introduction: Traumatic brain injury (TBI) is characterized by an initial mechanical injury, which launches a complex cascade of poorly understood molecular and metabolic events which are amplified by interactions between the different cells in the brain [1]. Recent studies demonstrated a link between mild repetitive TBI (mrTBI) and the progression of a neurodegenerative tauopathy called chronic traumatic encephalopathy (CTE) [2]. The hallmark of CTE pathology is the accumulation of hyper-phosphorylated tau proteins around neuronal and vascular networks at the depths of sulci [3]. The human neurovascular component (NVU) plays a critical role in the onset and progression of this disease; however, the exact mechanisms remain unknown due to the lack of a physiologically relevant model that can replicate the CTE phenotype. Thus, here, we developed a human 3D in vitro model of the neurovascular unit to study the molecular mechanisms of mrTBI-induced neurodegeneration.

Methods: Our NVU model is comprised of 3 main cell types: green fluorescent protein-expressing human brain microvascular endothelial cells (GFP-hBMEC) (E), astrocytes (A), and pericytes (P) seeded into porous silk scaffolds at a ratio of 1:0.5:0.3 million cells and enveloped into collagen type I gel. Tested conditions (1) Extracellular matrix (ECM): laminin, fibronectin, collagen type 4, and Matrigel; (2) Cellular compositions: EAP, EP, and EA. All procedures were completed following the manufacturer's specifications.

Results: We optimized the 3D in-vitro neurovascular unit (NVU) model along 3 main factors: cell density, extracellular matrix (ECM) supplementation, and cell composition. First, to select suitable cell densities of BMECs, we used 2, 1, and 0.5 million cells. 1 and 2mln of green fluorescent protein-expressing human BMECs (GFP-hBMEC) showed vessel-like and vascular network-like structure formation, observed via confocal and epifluorescent microscopy at 14- and 28-days post-seeding. Second, we used the following ECM components: laminin, fibronectin, collagen type 4, and Matrigel. We discovered that each ECM component positively

affected the general cellular activity at 14 and 28 days; however, Matrigel had the strongest effect on vascular network formation. Third, various cellular compositions (EAP, EP, and EA) were used to assess how the multicellular interactions affect the formation of vessel-like and vascular network-like structures. Both co(di)-culture and tri-culture models showed improved vascular network-like structure and tight junction formation compared to cell density and ECM component experiment samples. Homeostatic growth of all cells was validated and confirmed by analyzing ZO1 and claudin-5 (tight junction markers), CD31 (BMEC functional marker), Aquaporin-4 (astrocyte marker), and α-Smooth Muscle Actin (pericyte) markers through western blot. At last, mrTBI demonstrated a significant increase in hpTAU expression, altered mitochondria network integrity, driven by pDRP1-dependent fission, increased IL-1b signaling, and decreased tight junction markers short and long-term after injury.

Discussion: Different seeding densities of hBMECs were studied, and 1 million cells/scaffold was chosen as it showed optimal endothelial cell growth yet had the potential for improvement. Extracellular matrix proteins drastically affected the network formation at early time points (up to 4 weeks), while after this point, the differences between ECM conditions and controls began to diminish. Co(di)-culture and tri-culture models with the combination of different cell types, including hBMEC, astrocytes, and pericytes, significantly boosted network formation at early time points, and these samples remained distinguished beyond two months. Moving forward, this model will be used with neuronal cells to study the molecular mechanisms that drive CTE-like phenotype in response to mild repetitive injuries.

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Sunghyun Jun | *University of Cincinnati* | Mild Repetitive Injury-induced Chronic Traumatic Encephalopathy Phenotype in Human Triculture 3D in vitro Brain Tissue Model

Introduction Chronic traumatic encephalopathy (CTE) is a progressive, neurodegenerative disease associated with mild repetitive head trauma. CTE's hallmark is p-tau aggregates, demonstrated in post-mortem brains (the only model to study the final stage of this disease). Thus, developing a system that allows interrogation of the molecular mechanisms and biomarkers associated with CTE disease is of utmost importance. Using an earlier developed human 3D in vitro triculture model, we determined the criteria for a mild injury that ensures neuronal network stability and allows for repetitive impacts. Next, we narrowed down the required injury conditions that lead to CTE-like p-tau phenotype.

Method Human neurons, primary astrocytes, and a microglial cell line were seeded in silk scaffolds (d=6mm) and embedded in a collagen gel at 2:0.5:0.1 million cells, respectively. The injury was inflicted using a controlled cortical impactor (CCI) with different conditions – tip size, speed, number of hits, time interval, and time point.

Results Our results demonstrated that 1mm and 3mm tips with a 1m/s injury preserved neuronal network integrity, thus allowing us to perform repetitive injuries. Next, we demonstrated that 7D and 14D intervals between injuries resulted in a p-tau aggregates increase 7D, 14D, and 4W after the last injury. At last, we demonstrated significant changes to energy production mechanisms in response to injury.

Conclusion Our results demonstrate the feasibility of using our model to study the onset and progression of mild repetitive injury-induced neurodegenerative disease. Based on our preliminary data, we will evaluate the vascular contribution to the disease progression.

Megan Keech | *Vanderbilt University* | Delivery and efficacy of therapeutic albumin-hitchhiking siRNA conjugates in post-traumatic osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease associated with aging or injury. Current treatments focus on symptom management but lack disease modifying osteoarthritis drugs. We are innovating siRNA conjugates leveraging our recently established a novel, carrier-free diacyl lipid end-modified siRNA (siRNA-L2) platform that, delivered intravenously, achieves potent and long-lived target gene silencing in OA joints. Building on our successful work targeting Mmp13, we are investigating new targets of interest in order to further develop a more potent disease modifying OA drug capable of modulating disease progression and treating symptoms.

Anna Kittel | *Vanderbilt University* | Hybrid Shear-Thinning Hydrogels as an Injectable Delivery Platform for Regeneration of Diabetic Skin Wounds

Introduction: Diabetic skin wounds affect 10-20 million people worldwide and result in 130,000 lower extremity amputations annually. Current treatments focus on removing damaged tissue and preventing infection but ultimately fail to facilitate wound regeneration. While stem cells are promising in promoting wound repair, chronic wounds are characterized by uncontrolled inflammation and overproduction of reactive oxygen species (ROS), limiting viability and benefits of cell therapies. We tested a nanoparticle (NP) and hyaluronic acid (HA) shear-thinning

hydrogel for delivery and protection of stem cells or stem cell-derived extracellular vesicles (EVs) in diabetic skin wounds.

Materials and Methods: A nanoparticle-based, shear-thinning hydrogel was assembled by guest-host chemistry between hyaluronic acid (HA) and self-assembled ROS-responsive polymer nanoparticles. The nanoparticle component composed of adamantane (AD) functional triblock co-polymers containing core-forming hydrophobic ROS-responsive poly(propylene sulfide), hydrophilic polydimethylacrylamide (PDMA), and DMA-co-AD was synthesized as the guest macromer and mixed with the host macromer, β-cyclodextrin grafted hyaluronic acid (HA-CD), to assemble nanocomposite hydrogels. Human urine derived stem cells (USCs) and USC-derived extracellular vesicles (EVs) were encapsulated in our previously identified lead hydrogel candidate (PPS D300-Ad20% 2CD/1AD), and delivered in vivo to transgenic (db/db) diabetic mouse skin wounds. Wound closure and mean perfusion were measured over two weeks.

Results and Discussions: In vivo wound healing studies in diabetic mice demonstrate that nanocomposite hydrogels delivering USCs and USC-EVs promote greater wound closure and higher perfusion levels in the wound bed compared to controls (Figure 1). This work shows promise in nanocomposite shear-thinning hydrogels for delivering therapeutic stem cells and EVs to regenerate chronic diabetic skin wounds.

Alexander Ligocki | *Vanderbilt University* | Cerebrospinal Fluid Delivery of a siRNA-Conjugate for Therapeutic Targeting in the Aged Brain

Effective drug delivery to the aging brain remains a critical challenge in developing therapeutics for neurodegenerative diseases. The cerebrospinal fluid (CSF) serves as an alternative route for drug distribution, by passing the blood-brain barrier (BBB). However, age-related changes in CSF dynamics may significantly impact drug transport and efficacy. This study investigates the pharmacokinetics, distribution, and efficacy of CSF-delivered therapeutics in aged animals, providing critical insights into their potential for treating neurodegenerative disorders.

Gaining clinical interest, short-interfering RNA (siRNA) therapies allow for targeted and robust silencing of disease-driving genes, however current siRNA technology is hindered by poor uptake and inability to penetrate deep brain regions. To combat this, we previously engineered a lipid conjugate capable of penetrating deep into CNS parenchyma through perivascular spaces, displaying potent gene silencing. Building on prior studies we assessed biodistribution and efficacy in aged mice, establishing comparable delivery and knockdown to young animals. Biodistribution was assessed 48 hours post infusion of a Cy5 labeled L2-siRNA into the CSF of young and aged mice. Comparable distribution was identified, with prominent perivascular delivery to deep brain regions. Efficacy was evaluated two weeks and three months post infusion identifying sustained gene silencing in aged mice. In addition, distribution to canonical sites of CSF efflux (lymph nodes, dura) as well as peripheral nerves, a newfound site of CSF flow was evaluated, to comprehensively characterize L2-siRNA biodistribution. Overall, L2-siRNA overcomes major hurdles for delivery in the aged brain, providing a versatile and effective therapeutic platform for the treatment of CNS disorders.

Jack Loken | *Vanderbilt University* | Fluorescein based polymers for the activation of synthetic notch receptors to direct and regulate engineered T cells

Cancer is projected to cause over 600,000 deaths and 2 million new diagnoses in the United States in 2025 alone, underscoring the critical need for advancements in cancer treatment. Traditional therapies such as surgery, chemotherapy, and radiotherapy have recently been complemented by immunotherapy, which has shown significant promise in hematologic malignancies. However, only 20-40% of patients typically respond to immunotherapy, and it may induce severe side effects such as cytokine release syndrome and autoimmune reactivity. Solid tumors, versus hematologic cancer, present unique challenges for immunotherapy with an immunosuppressive tumor microenvironment and dysfunctional vasculature preventing therapeutic efficacy. To improve prognoses over multiple cancer types, we explore the application of engineered cells with synthetic Notch (synNotch) receptors which function as cellular sensors for surface molecules. Many current applications of synNotch rely on endogenous protein inputs for localization and activation, but this approach requires population homogeneity for clinical applications. Instead, we look to activate synNotch engineered immune cells with varying polymer constructs that display fluorescein (Flu), a fluorescent small molecule used in medical imaging. By assembling Flu polymer constructs that are either bioadhesive in nature or soluble and multivalent, we can efficiently drive synNotch signal induction. With these two approaches, engineered cell-based therapies can either be directed to a site of interest, such as a tumor, following Flu injection and retention or systemically activated based on polymer size and pharmacokinetics. This platform aims to provide a foundation for precise regulation of engineered cell behavior to mitigate the current shortcomings in cancer immunotherapy.

William Lowery | *Vanderbilt University* | Protein-Polymer Composite Nanoparticles through the Photopolymerization of Pyrrole by Photosystem I

Conductive polymers can be used to wire biomolecules with man-made materials to create hybrid composites that exhibit synergistic properties, particularly in regard to photoactivity. Photosystem I (PSI), a protein isolated from the photosynthetic chain in green plants, can be interfaced with such polymer networks to utilize the photoactivity of PSI to power light-driven reactions. The conductive polymers aid the protein's performance by providing pathways for the electrons to be shuttled to and from the active sites. One of the primary challenges in wiring PSI to conductive polymers lies in properly aligning the active sites with the conductive material. A variety of approaches have been used to solve this problem, such as vapor deposition and electropolymerization. Recently, we showed how PSI could be utilized to photopolymerize conductive polymers in direct contact with one of the active sites. Building upon this previous work, we show how a composite film can also be obtained through a similar photopolymerization process, while yielding a product much closer to the electropolymerization entrapment scheme where proteins are entrapped within the polymer network. These composite films demonstrate how the system's photoactivity can be greatly improved through the direct photopolymerization connections, bringing the field even closer to more practical and efficient PSI applications.

Sarah Lyons | *Vanderbilt University* | Intravenous Delivery of Lipid-siRNA conjugates to Modulate Brain Barriers

Brain barriers, including the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB), play critical roles in regulating the entry of molecules from the periphery into the central nervous system (CNS). In many neurodegenerative diseases, the integrity of these barriers is compromised, allowing pathogens and immune cells to infiltrate the CNS and exacerbate neuroinflammation. While drug delivery strategies targeting the BBB have been explored extensively, no existing approaches directly target brain endothelial cells (BECs) to specifically restrict immune cell extravasation. Current immunosuppressive treatments largely focus on systemic immune modulation, neglecting the CNS-specific mechanisms driving immune cell migration. To address this gap, we propose an alternative strategy aimed at targeting the BBB and BCSFB, which are comprised of BECs and choroid plexus epithelial cells, respectively. These cellular barriers harbor receptors critical to immune cell extravasation, highlighting the need for gene-silencing approaches to mitigate their activity. We have developed lipid-siRNA conjugates capable of accumulating in BECs and the choroid plexus epithelial cells following an intravenous administration. A single 20 mg/kg injection achieves 50% gene silencing in CD31+ BECs, sustained for several weeks, and significant knockdown in the choroid plexus for over a month. Building on this drug delivery platform, we are currently investigating whether these lipid-siRNA conjugates can limit immune cell extravasation in models of multiple sclerosis. This approach has the potential to address a critical unmet need in neuroinflammatory disease treatment by restoring barrier integrity and reducing pathological immune infiltration.

Lauren Mehanna | *University of Kentucky* | Isolation of Poly(caprolactone) Nanoparticles in an Effective Size Range for Systemic Drug Delivery

Polymeric nanoparticles are a promising drug delivery vehicle for localized drug release of therapeutic agents. Poly(caprolactone) (PCL) is a widely studied polymer for its use in implantable biomaterials and drug delivery systems using single emulsion solvent evaporation [1]. For systemic drug delivery, favorable nanoparticles are < 200 nm in diameter [2]. For subcutaneous and intra-tumor injections, microparticles 1 – 1000 μm in diameter are appropriate [3]. Spherical PCL particles can be loaded with Rhodamine B (RHO), a fluorescent dye, to aid in understanding drug loading and release. Single emulsion (O/W) solvent evaporation was successful in synthesizing unloaded and RHO-loaded PCL spherical micro- and nano- particles. Sequential centrifugation from 1000 rcf to 15,000 rcf isolated various ranges of particle sizes. Centrifugation of large volumes (50 mL) of the particle emulsion hindered the capture of particles < 500 nm; for example, at 10,000 rcf unloaded particles had an average diameter of 755 ± 262 nm. A technique using microcentrifugation of the particle emulsion isolated smaller particle size ranges and improved particle yield. Microcentrifugation at 10,000 rcf significantly decreased the particle size four-fold compared with initial results with an average diameter suitable for systemic delivery of 213 ± 75 nm. Particles had similar hydrodynamic diameters in solution for all sizes and significantly less negative zeta potentials for particles collected with microcentrifugation. Release studies of RHO-PCL particles in aqueous solution revealed effective RHO loading and release, with a burst release within the

first 12 h, followed by sustained release thereafter. These results are promising for developing a PCL particle formulation for systemic drug delivery applications.
References:
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Samirah Salidu | *Vanderbilt University* **| Optimizing GelMACad Synthesis: Investigation of Conjugation Efficiency and Scale-Up Potential for Biomedical Applications**

Hydrogels are three-dimensional, cross-linked polymer networks capable of retaining significant amounts of water, making them ideal for various biomedical applications such as tissue engineering, drug delivery, and regenerative medicine. Gelatin methacrylate (GelMA) is a hydrogel with versatile physical properties, including UV crosslinkability, which enables a wide range of modifications to enhance interactions between the material and cells. Functionalization with N-cadherin (Cad) extracellular peptides further enhances GelMA's utility, such as its ability to seed neurons; however, the details of GelMACad synthesis remain unclear. This study investigates the effects of peptide conjugation timing, peptide:GelMA feed ratio, and scalability on conjugation efficiency. Fluorescent peptides were used to quantify peptide conjugation across reaction times ranging from 2 to 36 hours, revealing that a 6-hour reaction time achieves optimal conjugation. Feed ratio experiments with 0.5, 1, 2, 5, and 10 mg peptide/mL GelMA demonstrated a consistent conjugation efficiency of ~60%, independent of peptide concentration. Additionally, scaled-up synthesis using 3-5 g GelMA was successful with extended dialysis time. These findings optimize GelMACad synthesis protocols for improved reproducibility and scalability, broadening its potential for biomedical applications.

Gagan Singh | ***University of Mississippi*** **|** **Bioinspired Room-temperature Ionic liquids and GUMBOS as enHanced emission Tools (BRIGHT) for Enhanced Quantum Yields of Organic Near-Infrared Dyes in Aqueous Medium**

The low molecular brightness (MB) of near-infrared (NIR) dyes in biological environments remains a challenge for bioimaging, despite their advantages in reducing background interference. In this study, we systematically screen organic salts, including group of uniform materials based on organic salts (GUMBOS) and room-temperature ionic liquids (RTILs), with NIR dyes in aqueous environments. The formulation yielding the highest fluorescence emission profile is termed BRIGHT—Bioinspired Room-temperature Ionic liquids and GUMBOS as enHanced emission Tools.

We observe significant fluorescence enhancement in eight dyes, including IR-1061, which is otherwise non-emissive in water. The indolizine squaraine dye (SO3SQ) exhibits the most pronounced improvement, with MB increasing from 365 to 34,900 in 200 mmol L^{-1} choline deoxycholate ([Ch][Doc]), a 96-fold enhancement. This brightness increase, along with a high fluorescence quantum yield (FQY), is attributed to extended excited-state stabilization and delayed decay lifetimes, as demonstrated by transient absorption spectroscopy, time-resolved anisotropy, steady-state fluorescence, and computational studies.

[Ch][Doc] exhibits a strong biosafety profile, showing low cytotoxicity (HEK293, MCF10A) and minimal hemolysis in human red blood cells. We further demonstrate its application in high-resolution cell imaging, where [Ch][Doc] enhances brightness, resolution, and photoprotection. This study highlights the potential of biocompatible ILs to modulate emissive decay pathways of organic NIR dyes, opening new avenues for their use in biological applications.

Daniel Woods | *Vanderbilt University* | Flexible, transparent electrodes for acute recording in non-human primates

State-of-the-art in vivo electrophysiology probes are often silicon-based, but their rigidity poses challenges for stable recordings in the brain's soft tissue. Flexible polymer-based probes offer improved mechanical compliance and biocompatibility, enabling more robust data collection. Their transparent design also enhances optogenetic applications, which use light to selectively stimulate neurons, a rapidly advancing technique in neuroscience. Manufactured using VINSE cleanroom facilities with contact impedance around 1 MΩ, these probes were developed for acute recordings in non-human primates (NHPs) using standard microfabrication techniques of photolithography, metal sputtering, and plasma etching. Transparent polymer Parylene-C was used to encapsulate a conductive layer of platinum, which enables both flexibility and transparency of the probes. A variety of novel implantation methods were developed for use in both rodents and NHP. These provide reliable, robust extracellular electrophysiology of cortical neurons in both animal models. Preliminary NHP recordings during working memory behavioral tasks capture both local field potential and spiking activity of neurons in the prefrontal cortex. Chronic implementation of such probes is expected to reduce inflammation and foreign body response from surrounding brain tissue, an issue silicon based probes have yet to overcome. The successful integration of these probes in NHPs outlines a breakthrough for in vivo electrophysiology, and a path towards flexible, transparent optoelectronics in behaving animals.

Anna Ruth Madera | *Mercer University and Vanderbilt University* | Hydrogel Microsphere Formation for Suspension Cell Culture

Hydrogel microspheres are created via microfluidic devices that utilize multiple flow channels and hydrophobic interactions to create droplets of a polar substance in a non-polar substance. A variety of substances such as lipids, metallic materials, and hydrogels can be used to make the microspheres. The microspheres have a variety of applications such as drug administration, biomedical imaging, tissue engineering, and suspension cell culture.

Microfluidic devices are becoming a prominent tool in biological science and research and helping to push forward breakthroughs in biological research. This project aims to characterize the flow rates of the polar and nonpolar liquids in the device which determine the sizes and production rates of spheres and to examine the viability of implanting cells into the spheres during production for particular sizes.

Microspheres were made from gelatin methacrylate (GelMA) with LAP and FITC. Microspheres were created using different flow rates in the microfluidic device. Induced pluripotent stem cells

(iPSCs) were incorporated into the GelMA solution before the spheres were created. After being imaged, the microspheres were analyzed using ImageJ software.

By increasing the flow rate of the mineral oil flowing through the device while keeping the aqueous phase at a continuous flow rate, we have seen a decrease in the size of the GelMA microspheres created. If the ratio of the aqueous phase and the oil phase remained consistent with the ratio of the slower rates, the same size spheres were created but with faster productivity. Cells were able to be implanted into spheres during production.

Nicole Marguerite | *Vanderbilt University* | Batch Fabrication of Functionalized Gelatin Methacryloyl Microparticles for Cellular Delivery to the Central Nervous System

Cell-based therapies offer a unique opportunity to treat central nervous system (CNS) diseases that lack disease-modifying or curative therapeutics. However, progress of clinical trials is significantly hindered by limited cell survival and engraftment driven by factors such as immunological stress, inadequate nutrient access, and/or mechanical forces. While hydrogels have been leveraged as delivery vehicles, studies still report viability in single digits, possibly attributed to insufficient support for cell-specific biochemical outcomes. To facilitate neuron-specific maturation, our lab previously developed a peptide-functionalized gelatin methacryloyl hydrogel, termed GelMA-Cad, that enhances neurite extension and synapse formation of stem-cell derived neurons in vitro. For clinical translation, GelMA-Cad has been redesigned as jammed hydrogel microparticles (HMPs) to enable injectability and disease-specific customizations, such as molecule encapsulation or peptide presentation. Traditionally, GelMA-Cad is crosslinked into a hydrogel via free radical polymerization with photoinitiation; however, to fabricate HMPs using a batch technique, which is high throughput and scalable, an alternative crosslinking mechanism must be developed to ensure homogeneous crosslinking of each HMP. Toward this goal, we developed a strategy to crosslink GelMA-Cad in batch emulsions with thiolated polyethylene glycol via a base-catalyzed Michael-type addition. To support clinical translation and crosslinking kinetics, quality control methods were established for quantification of functional group and peptide concentration using nuclear magnetic resonance and spectrophotometry. Following optimized synthesis and purification strategies, HMPs were assessed for size distribution, rheological properties, and cytocompatibility by evaluating viability of stem-cell derived neurons embedded in jammed HMPs. This shear-thinning formulation of GelMA-Cad provides a template for engineering tailorized scaffolds for CNS cell transplantation with peptide presentation, drug delivery, or synthetic biology to enhance functional engraftment and improve clinical outcome.

Lindsey Marquez | *University of Cincinnati* | Evaluating Immune-Mediated ECM Remodeling to Reduce Foreign Body Response of Implanted Biomaterials

Biomaterials provide a promising solution to the construction of engineered tissues and implants for applications in regenerative medicine. Although these materials may show promising results when tested in vitro, these materials commonly incite a foreign body response (FBR) when implanted in vivo. The inflammation occurring as a result of FBR in vivo impedes long term integration of the scaffold and reduces effective recovery of the native tissue. A comprehensive understanding of the changes in extracellular matrix (ECM) deposition due to mediators

produced during inflammation and regeneration hold the potential for improving the long-term outcomes of implantable biomaterials. This work seeks to gain fundamental knowledge on how the ECM is modulated in response to inflammatory and noninflammatory stimuli. This was conducted by culturing rat perineural fibroblasts (RPF) in conditioned media from M1 and M2 macrophages. The ECM secreted from these cells is decellularized and characterized using a series of comprehensive protein assays, imaging and evaluation of gene expression. Results showed that exposure to conditioned media changed the relative level of ECM proteins being expressed and secreted by the RPFs. This is attributed to the ratios of M1 cytokines (IL-6 and TNF-α) and M2 cytokines (IL-10 and TGF-β1) within the conditioned media. The results of this research will inform the development of biomaterials that positively interact with their surroundings. The long-term impact of this work will inform the design of biomaterials with the intention to be used in applications for the peripheral nervous system and improve functional recovery for large gap injuries.

Haley Masters | *Vanderbilt University* | Exploring the impact of cell communication and mechanical forces on the maturation and functions of human choroid plexus epithelial cells

As the interface between the blood and cerebrospinal fluid (CSF), the choroid plexus (ChP) mediates body-brain homeostasis and has broad potential for regenerative medicine in the central nervous system (CNS). The epithelial cells of the choroid plexus, (CPECs), the functional component of the ChP, express amyloid beta production and clearance equipment and are potential contributors to the onset and progression of age-related diseases such as Alzheimer disease (AD). Despite this importance, a few shallow studies are the bulk source of knowledge regarding the human ChP or CPECs, primarily due to the lack of a reliable and representative model system for the human ChP, leaving much to be understood. Recently, I devised a simple and efficient protocol for CPEC differentiation from human pluripotent stem cells (hPSCs) to address this gap in the field. These derived CPECs (dCPECs) exhibit immature CPEC properties and functions in the absence of vascular, mesenchymal, and mechanical elements seen in vivo. The maturation of the dCPECs, based on transcriptome and functional signature, is stunted in this non-physiologically representative static system. Therefore, elevating the in vitro system to a more physiological representative system with the addition of endothelial and stromal cells and fluid flow, is likely to promote CPEC maturation. These activities will leverage innovative genetic engineering techniques and microfabricated devices not previously applied to ChP models. Overall, this proposed research will inform mechanisms of human ChP development and provide an improved system to study the role of the ChP in aging and AD.

Elizabeth Matlock-Buchanan | *University of Memphis* | Tosh Farms Sow Lameness Prevention Project: Using Biomedical Approaches in an Agricultural Setting for Intervention in Culling of Sows due to Lameness

In the swine industry, sow lameness can be a major contributing cause of economic loss for pig producers. Lame sows are typically euthanized resulting in loss of sow and current/future progeny. A primary cause of lameness is infection in cracked and overgrown skin on the hooves, which can lead to osteoarthritis, lesions, and osteochondrosis, among other ailments.

Standard of care treatment of foot lesions depends on early recognition and aggressive antibiotic medication before deep-seated abscessation has occurred. Hydrogels and chitosan composites containing natural therapeutics have demonstrated efficacy in preventing infection-causing biofilm formation and can form a barrier on the skin, making these composites potentially beneficial as wound treatments and lameness prevention. The use of therapeutic loaded hydrogels has multiple advantages over the standard of care treatment in that gels do not require reapplication for several days, do not contain traditional antibiotics that promote antimicrobial resistance, and form a barrier to support moist wound healing and while preventing further contamination. This study's purpose is to obtain preliminary data on the utility of hydrogel materials with and without antimicrobials in the treatment of lesions in sows. We will follow a small cohort of sows and perform assessments of treated sows during this preliminary study.

Josh McCune | *Vanderbilt University* | Hydrophilic Reactive Oxygen Species-Degradable Scaffolds for Wound Healing

Synthetic biomaterials represent a promising class of materials used for wound dressings. They are relatively inexpensive to produce and allow for fine control over physiochemical properties compared to biologic dressings. However, current synthetic wound dressings fail to fully optimize physiochemical properties as they are predominantly polyester-based materials subject to poorly controlled hydrolytic degradation and material-associated inflammation. We have previously developed a synthetic polythioketal urethane (PTK-UR) wound dressing which degrades in response to reactive oxygen species (ROS) in the wound environment. In porcine wound models, it was observed that critical wound healing factors such as tissue infiltration, vascularization, re-epithelialization, and reduced inflammation correlated with increasing scaffold hydrophilicity.

Our previous work was limited in the range of achievable hydrophilicity due to the synthetic approach and use of ethylene glycol monomers (EG). Here we have innovated a novel class of PTK materials capable of achieving significantly more hydrophilic wound dressings. These super hydrophilic PTK-URs have been validated to maintain morphological features (pore structure, pore size, and overall porosity) and degradation mechanisms previously optimized with the EG-PTK-URs. Despite comparable physical characteristics, these super hydrophilic scaffolds are more efficient at scavenging ROS, ultimately providing cytoprotection against oxidative stress in vitro. These scaffolds also exhibit larger swell ratios and improved moisture retention, a critical design feature for wound dressings. Currently, we are exploring the tunability of this new class of PTK-URs to allow for control over tissue responses and optimizing these next-generation scaffolds for evaluation in chronic wound models.

Megan McDonald | *Vanderbilt University* | Development of Novel Crosslinking for Bulk Hydrogel Embeddings with Functionalized Gelatin Methacryloyl

Hydrogels have emerged as promising biomaterials for tissue engineering applications and regenerative medicine due to their ability to mimic the extracellular matrix and aid in the maturation and differentiation of induced pluripotent stem cells (iPSCs). Previously, our lab developed a functionalized gelatin methacryloyl based hydrogel, GelMA-Cad, which effectively supports in vitro functional maturation of iPSC-derived neurons. In the current study,

three-dimensional (3D) GelMA-Cad hydrogels were characterized and evaluated in vitro to assess their ability to replicate neural tissue behavior. The commonly used crosslinking mechanism for methacryloyl-based hydrogels involves photoinitiation; however, this method presents several challenges including the generation of cytotoxic free radicals, poor light penetration into 3D printed materials, uneven crosslinking across large volumes, and incompatibility for in situ applications. To address these limitations, GelMA-Cad was crosslinked using Michael-type addition with thiolated polyethylene glycol (PEG-thiol). By manipulating reaction conditions such as pH, concentration, molecular weight, and the addition or absence of reducing agents, we aimed to tailor the functional properties of hydrogels including stiffness, gelation time, crosslinking time, and porosity. Mechanical properties of these gel matrices, like viscosity and elasticity, were analyzed using rheological techniques during hydrogel gelation. To evaluate cytocompatibility, iPSC-derived neurons were embedded in bulk hydrogels and assessed for viability. The implementation of this novel PEG-thiol crosslinking mechanism to methacryloyl-based hydrogels not only enhances the suitability of GelMA-Cad hydrogels, but also offers potential for a wide range of stem-cell related research, such as disease modeling and therapeutic development.

Rachel Moen | *Vanderbilt University* | Scalable Stem Cell-Based Platform to Produce Tissue Specific Extracellular Vesicles

Extracellular vesicles (EVs) contain a heterogeneous cargo of proteins, nucleic acids, and metabolites derived from their cells of origin. Mesenchymal stem cells (MSCs) naturally produce therapeutically relevant EVs that have potential to treat many diseases such as graft versus host disease, rheumatoid arthritis, and Crohn's disease. Collecting sufficient EVs for a therapeutic treatment is therefore resource-, cost-, and labor-intensive. This study aims to develop a scalable platform to produce EVs from tissue-specific cell types, using iPSC-derived MSCs as a proof of concept. We are optimizing EV production by adapting MSCs into a pseudo-suspension culture using microspheres made from a custom hydrogel, GelMA-Cad. GelMA-Cad is a gelatin-based hydrogel with N-cadherin attached to it to support cell growth by mimic cell adhesion to the extracellular matrix. We have shown that GelMA-Cad supports cell growth in both 2D and 3D culture. By seeding MSCs into hydrogel layers, adherent cells can be grown in a pseudo-suspension culture allowing for increased cell density and EV production per volume of culture. EVs are collected from the culture media for characterization (NTA, western blot, and TEM) and functional assays. MSC growth and EV production has been confirmed in GelMA-Cad layers. Here, we found that MSCs seeded in GelMA-Cad produced a significantly more small EVs than MSCs grown in a 2D adherent culture. A key objective of this study is to determine if the production of EVs from MSCs embedded in GelMA-Cad leads to higher volumetric productivity and greater reproducibility than adherent cultures. Future work will expand this platform to other iPSC-derived cell types beyond MSCs.

Riyanka Narasimhan | *Vanderbilt University* | Shear-Thinning Norbornene-based Hydrogel Microparticles (HMPs) for Allogeneic Cell Delivery

Hydrogel microparticles (HMPs) have been widely explored for cell encapsulation, as they can potentially promote the diffusion of therapeutic agents while protecting encapsulated cells from the host environment. However, it can be challenging to fabricate injectable, cell-laden HMPs

while maintaining cell viability. Natural alginate and hyaluronic acid-based HMPs have previously been developed as platforms for cell encapsulation, but synthetic hydrogels must be examined as they have high reproducibility and can extend cell viability. We grafted norbornene (NB) and adamantane (Ad) to RAFT polymerized poly(N, N-dimethylacrylamide-co-vinyl azlactone) p(DMA-co-VDMA) polymers to prepare fully synthetic photocrosslinkable hydrogel precursor macromers. This macromer solution is processed into HMPs via batch emulsion and UV light exposure, which will be tested for their mechanics, shear-thinning behavior, injectability, and biocompatibility. The new norbornene-based HMPs presented here are potential candidates for cell encapsulation and further development as an injectable platform for allogeneic cell therapies.

Olusola Olatona | *University of Cincinnati* | Silk-Collagen 3D Neuronal Model Uncover Injury Severity-Dependent Calpain Dynamics and Axonal Destabilization in Traumatic Brain Injury

This study investigated calpain-mediated microtubule pathology in traumatic brain injury (TBI) using a 3D human brain-like tissue model engineered from silk-collagen biomaterial scaffolds. Tricultures of neurons, astrocytes, and microglia were embedded in porous silk matrices supporting long-term stability and subjected to two injury severities: moderate (3 mm tip, 6 m/s, single impact) and mild (1 mm tip, 1 m/s, three impacts at 2-week intervals). A calpain inhibitor (acetyl calpastatin) was administered 30 minutes before the injury, with samples analyzed at 1 hour (1H), 24H, 48H, 72H, and 7 days (7D) post-injury.

In moderate injuries, protein analyses revealed acute calpain-1 suppression in inhibitor-treated groups at early time points (1H–48H), rebounding to sham levels by 72H–7D. Non-inhibited moderate CCI sustained calpain-1 elevation across all stages. Mild injuries showed no calpain-1/calpastatin differences between treated and untreated groups, though both diverged from sham early (1H–24H). Microtubule biomarkers—Tuj1, phosphorylated Tau (AT8), and Synapsin-1—exhibited severity-specific dysregulation, with pronounced destabilization in moderate injuries. Immunofluorescence correlated axonal swellings with calpain activity and injury severity.

The study demonstrates that biomaterial-enabled 3D models identify therapeutic thresholds: single-dose inhibition suffices for moderate but not repetitive mild CCI. Scaffold mechanical integrity enables reproducible injury modeling that is unattainable in 2D systems. Future work will optimize consistent and sustained inhibitor introduction for multi-impact paradigm, advancing personalized neuroprotective strategies using human-relevant tissue analogs.

Iliana Ontiveros | *University of Mississippi* | Investigation of the intermolecular interactions occurring in the assembly of IL coated NPs and the impact on their thermodynamics and kinetics

Polymeric nanoparticles (NPs) are at the forefront of drug delivery due to their diverse physical properties, allowing for precise customization to achieve specific functionality. When NPs are combined with ionic liquids (ILs), their efficacy has been shown to improve. ILs are salts composed of bulky asymmetric cations and anions, which result in low melting points due to their inability to pack into ordered crystal structures. Studies have demonstrated that various ILs can by integrated onto the surface of NPs formed by di-block co-polymers. Despite their

potential, the fundament forces, such as hydrogen bonding and electrostatic interactions, driving the physiochemical behaviors of IL-polymer NP assembly remain poorly understood. To address this, Nuclear Magnetic Resonance Spectroscopy (NMR) and Fourier Transform Infrared Spectroscopy (FTIR) can be used to investigate the interactions between different ILs and NPs. Additionally, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) can offer insights into the progression of IL-NP assembly and degradation. These combined techniques will contribute to a deeper understanding of the driving forces behind IL-NP assembly and provide knowledge to further manipulate IL-NP interaction for targeted drug delivery.

Samanta Paul | *University of Cincinnati* | Modeling of Covid-19 Spike Protein Induced Inflammation in a Blood-Brain Barrier on Chip System

The SARS-CoV-2 virus, responsible for COVID-19, has been linked to neurological symptoms in more than 35% of patients [1], including ischemic stroke, in some patients [2]. Research suggests that the COVID-19 spike protein may contribute to cerebrovascular issues by promoting blood clot formation [3]; however, its precise effects on the blood-brain barrier (BBB) remain unclear. Traditional BBB models often fall short due to their limited physiological relevance, inability to replicate the three-dimensional complexity of the human BBB. Developing a human-relevant BBB model could provide a more efficient and effective platform for investigating the impact of the SARS-CoV-2 spike protein on barrier integrity. Advances in organ-on-chip and bioprinting technologies offer an opportunity to overcome the limitations of traditional models, enabling the development of a BBB model that closely mimics human physiology. This study employs an advanced in vitro BBB-on-chip model, [4][5] incorporating astrocytes, endothelial cells, and a fibrin-thrombin gel matrix, to closely replicate human physiology. Different concentrations of the spike protein (0, 10, and 100 ng/ml) are perfused through the chip, with and without blood, to determine the thresholds for inflammation and endothelial disruption. In addition, the BBB model is pre-treated with IL-1B cytokine at varying doses to induce inflammation, providing a baseline comparison. Imaging and quantitative analysis focus on endothelial coverage, platelet signal intensity, and thrombi formation. The results of this study will provide critical insights into how the SARS-CoV-2 spike protein influences BBB integrity and inflammatory responses, potentially providing information regarding COVID-19 associated neuroinflammation and blood clotting disorders.

Angela Rast | *Vanderbilt University* | Polymeric Design of Synergistic Agonists

Immune responses can be modified or directed through the addition of adjuvants. This direction is critical in the success of vaccinations, especially cancer vaccines that often target “cold” or immunosuppressive environments. To this end, pattern recognition receptors (PRRs) have recently gained recognition due to their ability to activate the immune system. STING (stimulator of interferon genes) is a component of the cytosolic DNA sensing pathway that effectively activates innate immunity through activation of IFN-α, while toll-like receptors (TLRs) stimulate inflammatory cytokines regulated by NF-κB. Both adjuvants alone are limited by inefficient delivery, rapid clearance, and breadth of cytokine response. However, recent studies have highlighted the synergistic effects of STING and TLR signaling pathways. Therefore, this project aims to first explore the synergistic effects of TLR7 and STING agonists in a controlled

environment. Using these results, a polymer system was then developed allowing for the addition of TLR7 and STING agonists at predetermined ratios. The effects of the polymer on the activity of the agonists were also studied.

Jacob Ryan | *University of Cincinnati* | Inflammation-responsive antibiotic coatings for prophylactic bacterial infection treatment in orthopedic reconstructions

Patients receiving reconstructions are at an increased risk for osteomyelitis, an inflammatory bone disease caused by bacterial infections. Here, responsive antibiotic coatings placed on the surface of orthopedic implants are being developed to selectively release antimicrobials when triggered by infection-mediated inflammation. This is accomplished by constructing films with polymers that are specifically degraded by reactive oxygen species (ROS). The increase concentration of ROS during infection can be leveraged for “on-demand” antibiotic delivery to prolong local drug delivery, eliminate infections, and prevent reoccurring bacterial infections surrounding orthopedic implants .

The ROS-degradable polycation poly(thioketal β-amino amide) (PTK-BAA) and the hydrolytically degradable control polycation poly(β-amino ester) (PBAE) were successfully synthesized and purified. These polycation were incorporated into layer-by-layer (LbL) films by alternating layers of cations (PTK-BAA or PBAE and vancomycin) and anions (polyacrylic acid). HPLC was used to evaluate vancomycin loading and release. LbL films with 60 tetralayers had a drug loading of roughly 4.5 μg/cm2 and 11.2 μg/cm2 when constructed with respective PBAE and PTK-BAA polycations. Both PBAE and PTK-BAA films were incubated in saline or 1 mM H2O2 to assess vancomycin release. In both cases, the PBAE films released the bulk of the drug payload within the first 48 h. The PTK-BAA films incubated in aqueous conditions had an initial release of vancomycin while the films incubated in oxidative conditions demonstrated significantly greater drug discharge. On-going studies seek to improve film stability to decrease the initial burst release of drug before validating the effectiveness of these LbL films at mitigating Staphylococcus aureus infections both in vitro and in vivo.

Jake Schulman | *Vanderbilt University* | Engineering A Polymeric STING Agonist Delivery Platform to Improve Cancer Immunotherapy Efficacy

While immunotherapy, particularly immune checkpoint blockade (ICB), has transformed cancer treatment paradigms, many patients are primarily non-responsive or develop resistance to treatment over time. Inadequate immunotherapy outcomes can largely be attributed to immunosuppressive mechanisms within the tumor microenvironment (TME), which ultimately impede the magnitude and functionality of tumor infiltrating lymphocytes (TILs). It has been demonstrated that agonizing the Stimulator of Interferon Genes (STING) pathway leads to a potent type I interferon (IFN-I)-driven inflammatory response which creates an immunogenic TME, bolsters antitumor T cell immunity, and improves ICB responses. Despite their promise, the translation of STING agonists to the clinic has been unsuccessful. Clinical trials have predominantly utilized cyclic dinucleotides (CDNs), which are limited to intratumoral administration due to their poor drug-like properties. This has prompted the development of systemically deliverable STING agonists. Recently described synthetic STING agonist, dimeric amidobenzimidazole (diABZI), has demonstrated promise due to its anti-tumor activity, but it has

a short half-life, exhibits poor tumor tropism, and can indiscriminately activate STING, leading to immunotoxicities. To address these challenges, we leveraged reversible addition-fragmentation chain transfer (RAFT) polymerization-compatible diABZI-functionalized chain transfer agents (CTAs) to engineer a highly versatile, polymeric STING agonist delivery platform, known as p-diABZI. While diABZI cannot be modified into a prodrug, we demonstrate that prodrug-like behavior can be achieved via incorporation into macromolecular polymeric constructs with a TME-responsive linker. We see that p-diABZI elicits potent STING activation in vivo and antitumor activity in multiple murine cancer models.

Keshav Shah | *Georgia Institute of Technology and Emory University* | Enzymatically-Degradable Hydrogel Microcarriers and Pro-Inflammatory Cytokine Licensing Modulate Mesenchymal Stromal Cell Secretome

Culture of mesenchymal stromal cells (MSCs) on enzymatically-degradable hydrogel microcarriers (μCs) would enable more efficient cell harvest in vitro and facilitate in vivo delivery. Furthermore, licensing with pro-inflammatory cytokines can augment MSCs' secretion of pro-regenerative factors [1]. However, potential synergistic effects of degradable μCs and licensing in modulating the secretome of MSCs, a main mechanism of action in vivo, remain underexplored.

Degradable hydrogel μCs were fabricated with acrylated poly(ethylene glycol) conjugated to the MMP-cleavable peptide GGVPMSMRGGGK (PEG-VPM). PEG-VPM μCs degraded completely upon incubation in collagenase (phase contrast microscopy). From microscale mechanical testing, PEG-VPM μCs exhibited a compressive modulus of 54.3 ± 15.8 kPa.

Human MSCs (RoosterBio) were then cultured for 4d on PEG-VPM μCs, tissue culture polystyrene (TCP)-based Synthemax μCs, and planar TCP with either 0 (basal) or 100 ng/mL of both IFN-γ and TNF-α (licensed). Via PicoGreen assay, MSC numbers on PEG-VPM were significantly lower versus TCP surfaces; MSC numbers on microcarriers did not vary between basal and licensed. Via multiplex ELISA and principal component analysis, MSC secretomes separated distinctly based on licensing and culture surface ($R^2 = 0.853$, $Q^2 = 0.695$). While not secreted by basal MSCs, the anti-inflammatory protein interleukin-1 receptor antagonist (IL-1Ra) was secreted at 10-fold higher levels by licensed MSCs on PEG-VPM versus both TCP substrates. Additionally, licensed MSCs on PEG-VPM secreted 7-fold higher levels of the monocyte chemoattractant fractalkine versus Synthemax.

These results suggest that hydrogel carrier properties and pro-inflammatory cytokine licensing can combinatorially influence the MSC secretome. Further development may advance MSC manufacturing and therapeutic delivery.

Reference: 1. Lipat AJ et al. Stem Cells Transl Med. 2022; 11(9):971-986.

Cheick Sissoko | *University of Cincinnati* | Microfluidic-based sensor-enabled Neurovascular unit for modeling Alzheimer's Disease impact on the brain vasculature

Alzheimer's disease (AD), the most common neurodegenerative disorder, is marked by a significantly compromised blood–brain barrier (BBB), however, the precise mechanisms driving

this vascular dysfunction remain poorly understood. To address this knowledge gap, we proposed a microfluidic-based, sensor-enabled neurovascular unit (NVU) platform that replicates key structures and functions of the human brain–BBB axis. The device combines fluid flow, a functional brain endothelial vasculature, and advanced three-dimensional (3D) brain models in an optimized hydrogel matrix, while embedded sensors enable real-time monitoring of barrier integrity and key biological processes. By bioengineering essential features of the brain and BBB, this high-resolution, high-throughput system offers a powerful tool for elucidating the vascular contributions to AD pathogenesis and for evaluating therapeutic interventions. Here, we report the optimization parameters for modeling the brain-BBB environment on a chip. The ability to observe cellular interactions and vascular responses under precisely controlled conditions will shed new light on how neurodegenerative processes converge on vascular pathways. Ultimately, this sensor-enhanced microfluidic NVU stands to significantly advance our understanding of AD progression and accelerate the discovery of more effective treatments.

Amelia Soltes | *Vanderbilt University* | Optimization of siRNA Nanoparticles with Custom Surfactants for Osteoarthritis Treatment

Osteoarthritis (OA) is a globally prevalent degenerative joint disease, and there are currently no disease modifying osteoarthritis drugs (DMOADs) available. Small interfering RNA (siRNA) is a promising therapy for the knockdown of specific genes, especially in the context of OA, but endolysosomal escape presents a barrier against intracellular bioavailability and silencing potency. Here, siRNA-loaded nanoparticles (si-NPs) are conceptualized, synthesized, and screened for siRNA delivery. The si-NPs are comprised of a poly(dimethylaminoethylmethacrylate-co-butyl methacrylate) (PDB) and poly(lactide-co-glycolide) (PLGA) core to complex with the siRNA and enable pH-dependent membrane disruptive activity for endosome escape. New custom polymeric surfactants have been developed to improve gene knockdown and cytocompatibility, as well as to provide bioadhesive capabilities. These surfactants are polymer-lipid conjugates that contain aldehyde groups to enable reversible adhesion to the extracellular matrix (ECM) in the joint.

A library of diblock polymeric surfactants was synthesized with controlled reversible addition fragmentation chain transfer (RAFT) polymerization and verified using 1H NMR and GPC. Polymeric si-NPs were formulated via flash nanoprecipitation using a confined impinging jets (CIJ) mixer using a range of concentrations and surfactants from the polymer library. Dynamic light scattering (DLS) was performed to evaluate the size, polydispersity index (PDI), and zeta potential of the si-NPs, which were found to be monodispersed and neutrally charged. The viability and gene silencing activity of the si-NPs loaded with luciferase targeting siRNA were then assessed in luciferase-expressing ATDC5 cells 48 hours after treatment. Luciferase knockdown was achieved while maintaining cell viability. The bioadhesive capabilities of the si-NPs were evaluated using ex vivo porcine cartilage explants. Further studies are ongoing in order to determine a lead si-NP formulation.

Alexander Sorets | *Vanderbilt University* | Lipid-siRNA conjugate achieves deep brain region delivery and durable gene silencing in the rodent CNS

A key objective in the quest of developing disease modifying therapies for neurodegenerative disorders is achieving efficacious delivery of therapeutics to specific anatomical sites in the

brain. Along these lines, Huntington's disease (HD) is caused by mutations in the huntingtin (Htt) gene and current therapeutic efforts seek to lower Htt expression in affected cells of the striatum. Short-interfering RNAs (siRNA), in particular, have generated substantial clinical interest for treating HD owing to their ability to mediate sustained Htt knockdown. Yet, siRNA delivery to deep brain structures such as the striatum remains a considerable challenge for therapeutics administered into the cerebrospinal fluid (CSF). Here, we developed a lipid-siRNA conjugate (termed L2-siRNA) that enhances transport through CSF compartments, leading to deep brain region delivery and potent cellular uptake. We provide a detailed examination of both cell-specific and regional bulk tissue gene silencing in mice, highlighting potent striatal knockdown five months after a single injection without detectable toxicity. Intrathecal delivery of L2-siRNA in rats further illustrates effective transport and knockdown in a clinical standard model, collectively supporting L2-siRNA as a promising new platform for HD-modifying therapies.

Larry Stokes | *Vanderbilt University* | Development of a Carrier-Free CRIPSR/Cas9 Technology for Systemic Gene Editing

More than 4000 monogenic mutations are responsible for at least 80% of all rare diseases. The monogenic nature of these mutations makes many rare diseases potential candidates for CRISPR/Cas9 gene therapies; however, many delivery barriers must be overcome to achieve therapeutic levels of gene correction. To date, most research has focused on designing and optimizing viral vectors or lipid nanoparticles to deliver Cas9 in various cargo forms, but these delivery systems have associated drawbacks such as AAV immunogenicity or charged lipid cytotoxicity that makes the leading carrier formulations ineffective for systemic diseases such as Duchenne's Muscular Dystrophy (DMD). This creates a need for designing a Cas9 system that can act as a carrier-free gene editing therapeutic. Cas9 carrier-free gene editing has been investigated and has shown promising results in vivo following local administration of the Cas9 RNPs. However, many of these therapeutics are not directly translatable to systemic administration for DMD. To this point, many carrier-free siRNA therapies have been developed that leverage albumin hitchhiking to preferentially accumulate into inflamed or diseased tissues. Researchers have shown that albumin accumulates into injured/inflamed muscle in mdx mice. By leveraging albumin hitchhiking, the Cas9 protein can be engineered to act as a carrier-free therapy with the potential to target systemic diseases such as DMD.

Avanelle Stoltz | *Vanderbilt University* | A Cationic, ROS-responsive polymer for siRNA Conjugation for Delivery in vivo

Small interfering RNA (siRNA) has been proven as a promising therapeutic for treatment of various disease states, but delivery of siRNA has been a challenge due to endosomal entrapment. Our group has synthesized a library of novel cationic, ROS-responsive polymers for complexing with siRNA to facilitate endosomal escape and delivery. The parent co-polymers were synthesized using a ring-opening polymerization of propylene sulfide (PS) or a 50/50 copolymerization of PS and PS-tert-butyldimethylsilyl. After polymerization, various ROS-responsive amine groups (di-methyl, di-ethyl, and di-propyl) were then grafted onto the parent polymer via Carbonyldiimidazole addition. Chemical structure and final weights of the polymers were confirmed by 1H-NMR and GPC. Polymers were determined to be in the ideal

range (pKa ~ 6.5) to promote endosomal escape within the cell to aid in delivery of siRNA. Further characterization will include endosomal escape characterization, ROS-responsiveness, and DLS to characterize the polymer-siRNA complexes.

Payton Stone | *Vanderbilt University* | Fabrication of RIG-I-Activating Nanoparticles for Intratumoral Immunotherapy via Flash Nanoprecipitation

Intratumoral immunotherapy is a promising strategy for stimulating local and systemic antitumor immunity while eliminating or reducing immune-related adverse events often attendant to systemic administration. Activation of the cytosolic pattern recognition receptor (PRR) retinoic acid-inducible gene I (RIG-I) at tumor sites stimulates innate immunity that can potentiate a T cell-dependent adaptive antitumor immune response. However, the activity and efficacy of 5'-triphosphate RNA (3pRNA) agonists of RIG-I are hindered by poor in vivo stability, rapid degradation, limited cellular uptake, and inefficient cytosolic delivery. To overcome these challenges, we developed RIG-I-activating nanoparticles (RANs) assembled using a flash nanoprecipitation (FNP) process to load a potent stem-loop 3pRNA (SLR) RIG-I agonist into endosome-destabilizing polymeric nanoparticles. We leveraged FNP to induce turbulent micro-mixing between a corona-forming poly(ethylene glycol)-block-(dimethylaminoethyl methacrylate-co-butyl methacrylate) (PEG-DB) diblock copolymer, a hydrophobic core-forming DB counterpart, and a SLR RIG-I agonist, resulting in the self-assembly of densely loaded nanoparticles that promoted endosomal escape and cytosolic delivery of 3pRNA cargo. Through optimization of polymer properties and inlet feed ratios, we developed RANs with high and improved loading efficiency and increased serum stability relative to a previously reported micelleplex formulation assembled via electrostatic complexation with PEG-DB polymers. We found that optimized RANs exhibited potent immunostimulatory activity in vitro and in vivo when delivered intratumorally. As a result, in preclinical models of MC38 colon cancer and B16.F10 melanoma, intratumoral administration of RANs suppressed tumor growth and increased survival time relative to vehicle controls. Collectively, this work demonstrates that FNP can be harnessed as a versatile and scalable process for efficient loading of nucleic acids into polymeric nanoparticles and highlights the potential of RANs as a translationally promising platform for intralesional cancer immunotherapy.

Trisha Sullivan | *Vanderbilt University* | Effect of Molecular Weight on Different Methods of Polycaprolactone Particle Preparation

Cancer incidence is rising with an estimated 2 million new cancer diagnoses in the United States this past year [1]. Chemotherapy is used to treat many cancers; however, chemotherapy causes adverse side effects. Nanoparticle drug delivery systems offer an alternative treatment to traditional chemotherapy because they enable controlled and sustained drug release, minimizing exposure to healthy cells. Polycaprolactone (PCL) nanoparticles are one polymeric drug delivery system in development because they are biocompatible and biodegradable [2]. Producing nanoparticles that are less than 200nm is desired to ensure efficient cellular uptake. This study investigated the impact of PCL molecular weight (MW), 80,000MW vs. 25,000MW, on nanoparticle size using single-emulsion solvent evaporation with sequential centrifugation. Differences in size were observed; 25,0000MW resulted in significantly smaller particles compared to 80,000MW. Particles created using 80,000MW PCL had an average diameter of

755nm ± 0.270nm, and 25,000MW PCL particles had an average diameter of 480nm ± 151nm. We further investigated the effect of the emulsion volume on the resultant particle size and discovered that the smallest particles were sized at 566nm ± 170nm when using 80,000MW PCL. Future directions may involve using PCL with an even lower MW to further isolate smaller particles more suitable for systemic delivery.

[1] Collins, S. ACS. 2024. [2] Bartnikowski, M., Dargaville. Prog. Polym. Sci. 2019 96, 1–20.

Victoria Sullivan | *University of Tennessee* | Short -Term Plasticity of Memristive Droplet Interface Bilayers Mediated by Hydrocarbon Oils

The growing demand and advancements in artificial intelligence are clear. However, achieving high-efficiency, low-power, and cost-effective neuromorphic computing systems with synaptic plasticity and memory elements remain a challenge. Techniques utilizing biomembranes' adaptive capabilities, specifically droplet interface bilayers (DIBs), offer a promising solution. By using 1,2-diphytanoyl-sn-glycero-3-
phosphocholine (DPhPC) lipids doped with gramicidin, droplets with a monolayer of
these components can be merged to form an electrical circuit (biomembrane) that
mirrors the behavior of pre- and post-synaptic terminals. The biomembrane exhibits switching characteristics similar to the depolarizing pulses in nerve cells. The membrane dynamics, known as electrowetting and electrocompression, are controlled by an external voltage stimulus. This stimulus aligns the gramicidin monomers within the lipid membrane, allowing them to conduction charge. The biomembrane features a lipid bilayer, which acts as a capacitor and serves as the memory element, while gramicidin functions as a conductor with finite resistance. This combination makes it a powerful tool for harnessing synaptic plasticity, exploring how biological memory is stored within a bilayer, and creating neuromorphic hardware. Additionally, it contributes to a deeper understanding of neural plasticity at the membrane level. By using three hydrocarbon oils of varying lengths, the membrane dynamics capable of intramembrane insertion, capacitance, and the effects of resistance on short-term plasticity (STP) were studied through paired-pulse facilitation (PPF) and paired-pulse depression (PPD) protocols, which induced current responses. It was found that, under these protocols, voltage follows a quadratic relationship with respect to current. Additionally, the system can be described by an equation that uses membrane area to determine specific capacitance and connects specific capacitance to current. This approach allows for the determination of parameters for each oil system. Early stages of research suggest that parameters such as membrane area, time, and electrical current can be modified by membrane dynamics in response to voltage stimuli. These findings demonstrate how different oil types can uniquely modulate the membrane area by adjusting capacitance (memory storage), and, as a result, affect the electrical current response of the membrane to various electrical stimulations.

Brennen Thomas | *Vanderbilt University* | Collagen II-Targeted Peptide-siRNA Conjugate for Improved Delivery and Treatment of Osteoarthritis

Osteoarthritis (OA), one of the leading causes of disability worldwide, is degenerative joint disease marked by cartilage degradation, synovial thickening, and osteophyte formation. However, currently no disease-modifying therapeutics exist for the treatment of this joint

disease. Short-inferring RNA (siRNA) serve as a promising candidate to fill this void. Seeking improvements in the delivery of therapeutic siRNA, previous work utilized MMP13 selective siRNA loaded into a nanoparticle decorated with mAbCII, a monoclonal antibody targeting collagen II, the most prevalent architecture within the joint space. This design improved delivery, retention, and therapeutic outcomes of the nucleic acid molecules. Nanoparticles, however, are met with other concerns, such as immunogenicity. As a carrier-free method of siRNA delivery, application of an albumin-hitchhiking MMP13-selective siRNA conjugate saw marked improvements in delivery to injured joints leading to greater therapeutic disease modulation when compared to unconjugated siRNA. The ability to specifically target unique features of the joint space would provide greater ability to finely tune siRNA treatments to cell types of interest and limit off-target effects. As such, a collagen II-targeting peptide-siRNA conjugate was developed to circumvent the challenges associated with nanoparticle delivery and improve upon the albumin-hitchhiking design. Relying on WYRGRL, a previously established peptide that binds collagen II, as the targeting ligand, the siRNA conjugate saw improved in vitro retention in collagen II coated well plates, sustained knockdown of the gene of interest, and uptake in chondrocyte-like ATDC5 cells. This proof-of-concept conjugate design and testing demonstrate the potential utility of these carrier-free and targeted siRNA conjugates as a therapeutic modality for OA and reveal key considerations for next generation designs of these therapeutic strategies.

Ruben Torres | *Vanderbilt University* | Optimizing Compact Quantum Dot Surface Chemistry to Track Endogenous Dopamine Transporter Proteins in Native Neuronal Networks

The catecholamine neurotransmitter dopamine (DA) is central to control important behavioral roles including reward, mood, and cognition. The presynaptic DA transporter (DAT) determines dopaminergic signaling amplitude by shuttling receptor-available DA from synaptic to intracellular space. Importantly, genetic polymorphisms of the human DAT gene (DAT1, SLC6A3) have been associated to cases of ADHD and bipolar disorder. An unexplored DA regulatory mode is the horizontal trafficking of DAT to presynaptic terminals by lateral membrane diffusion. Here we develop and optimize a custom DAT-specific quantum dot probe to characterize brain region specific differences and define diurnal temporal profiles of native DAT membrane dynamics.

MD Imam Uddin, PhD | *Vanderbilt University* | Targeted delivery of shRNA-lipids to inhibit neovascularization in proliferative retinopathy

Objective: Diabetic retinopathy is a vision threatening condition in diabetic patients. We observed, using single cell RNA sequencing data analysis, activation of bone marrow-derived cells in peripheral blood from patients with Type-1 diabetes, and also in animal models of proliferative retinopathy. These activated monocytes and progenitor cells migrate into the retina in response to inflammation and neovascularization. However, contribution of these cells to neovascularization is largely unknown. We describe here the synthesis of a new hybrid nanoparticle for targeted delivery and gene silencing in activated monocytes that are associated with pathological neovascularization.

Methods: Single cell RNA sequencing data analysis were performed to characterize mRNA expression in peripheral blood from patients with Type-1 diabetes, and in oxygen-induced retinopathy (OIR) model. Targeted AS-shRNA-lipids were synthesized by conjugating diacyl-lipids to anti-sense short hairpin RNA with an anti-sense sequence complimentary to endoglin mRNA.
Results: Endoglin mRNA expression was associated with CD14high and IL1B positive activated monocytes in peripheral blood from Type-1 diabetic patients, and in OIR model. In addition, endoglin mRNA expression was inhibited by AS-shRNA-lipid. In addition, significant reduction of neovascularization was achieved in OIR after intraperitoneal injection of AS-shRNA-lipids.
Conclusions: Diabetes may contribute to monocyte activation and inhibition of endoglin mRNA could regulate the activation. We have developed a novel method for targeted delivery and inhibition of mRNA targets in activated monocytes in the living tissues using AS-shRNA-lipids. These studies may provide a framework for a novel strategy to inhibit retinal neovascularization.

Priyavrat Vashisth | *University of Mississippi* | Ionic liquid-coated gold core polymeric nanoparticles for selective neutrophil hitchhiking and targeted endometriosis treatment
Endometriosis is a chronic inflammatory gynecological disease affecting millions of women and people with uteri worldwide during their reproductive age. The disease is difficult to treat, with current surgical and hormonal therapy methods having several limitations and side effects including risk of recurrence. In this work, we explore the use of novel ionic liquid (IL)-coated gold core polymeric nanoparticles (NPs) for selective neutrophil hitchhiking for the targeted treatment of endometriosis via photothermal therapy. Neutrophils are the major circulating leukocyte population that acts against microbial infection and inflammation in our body and are present in higher levels than normal in endometriosis patients. In particular, the neutrophils tend to “home” to endometrial tissue. Hence, in this study, gold core PLGA NPs were synthesized by solvent evaporation method, and then coated with a neutrophil-selective IL. The synthesized NPs were characterized using Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS) to measure size, and Vis-NIR spectroscopy to investigate their optical properties. The hemocompatibility of NPs was tested in human female whole-blood K2EDTA. Neutrophil hitchhiking was quantified using fluorescence-activated cell sorting (FACS) and confocal laser scanning microscopy (CLSM) was used to visualize the NP uptake. The photothermal efficacy of the prepared NPs was tested using an 808 nm NIR laser against an endothelial endometrial 12Z cell line. After 5 min of laser treatment at 1W/cm2 more than 95% of cellular death occurred via apoptosis. We hypothesize that the IL coating will allow the NPs to specifically hitchhike onto neutrophils, accumulate in the diseased tissue, and allow for photothermal ablation of the affected site. Future studies will focus on the use of disease models to understand the in vivo trafficking of the particles.
Yunqian Zhang | *University of Kentucky* | Cell Encapsulation in Microgels for Controlled Metabolite Reprogramming and Enhanced Bone Regeneration
Immune cells are the first responders to tissue injury and play an essential role in tissue regeneration. Metabolic reprogramming of macrophages from an inflammatory to a regenerative phenotype after injury offers a safe and effective way to promote regeneration. However, metabolites are mostly small molecules that diffuse quickly from the injury sites after bolus injection. To address this challenge, we have developed macroporous hydrogel scaffolds

composed of individual microgels that serve as versatile carriers for macrophages and metabolites. We hypothesize that functionalizing the microgels with metabolites will create a desirable metabolic environment and actively control macrophage phenotype toward pro-regenerative status. Furthermore, the microgels will act as protective carriers, shielding macrophages from inflammatory factors in the injury site and enabling sustained delivery of metabolites to the encapsulated cells. To achieve this, we developed a microfluidic device that produced uniformly sized, photo-crosslinkable gelatin microgels and demonstrated their ability to sustain BSA release for up to 7 days. Moreover, microgels maintained high cell viability for encapsulated cells, demonstrating their potential to serve as a platform for cell-based therapies. Our ongoing work focuses on optimizing metabolite release kinetics by tuning microgel crosslink efficiency and evaluating the effects of varying metabolites on macrophage responses. We envision the microgel scaffolds, designed to create an optimal metabolic environment, as a versatile platform applicable to various tissue regeneration therapies.