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2024 REGIONAL SYMPOSIA

September 19–20, 2024

FINAL PROGRAM

SOUTHEAST SYMPOSIUM:

Georgia Institute of Technology

ATLANTA, GA

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2024 Regional Symposia

Southeast Symposium: Georgia Institute of Technology

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

7:30 AM - 8:30 AM	Registration and Continental Breakfast
8:30 AM - 8:45 AM	Welcome by Site Chairs
8:45 AM - 10:00 AM	Session I: Nanomaterials ***Invited Speaker: Evan Scott, University of Virginia***
10:00 AM - 10:15 AM	Coffee Break
10:15 AM - 11:30 AM	Session II: Biomaterial-Tissue Interaction ***Invited Speaker:*** ***Andrés J. García, Georgia Institute of Technology***
11:30 AM - 12:45 PM	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University*** *(2024 Clemson Award for Basic Research Recipient)*
12:45 PM - 1:45 PM	Lunch and Poster Session (#1 - 48)
1:45 PM - 3:00 PM	Session III: Immune Engineering 1 ***Invited Speaker: Jennifer Elisseeff, Johns Hopkins Univeristy***

Program Agenda

Thursday, September 19, 2024 *(continued)*

3:00 PM - 4:15 PM	Session IV: Drug Delivery 1 ***Invited Speaker: Thomas Werfel, University of Mississippi***
4:15 PM - 5:30 PM	Plenary Session II: ***Sarah Stabenfeldt, Arizona State University*** ***Danielle Benoit, University of Oregon***
5:30 PM - 6:45 PM	Session V: Tissue Engineering 1 ***Invited Speaker: Cherie Stabler, University of Florida***
7:00 PM - 9:30 PM	Dinner Reception

Program Agenda

Friday, September 20, 2024

8:15 AM - 8:45 AM	Registration and Continental Breakfast
8:45 AM - 10:00 AM	Session VI: Engineering Cells and Their Microenvironments
10:00 AM - 10:15 AM	Coffee Break
10:15 AM - 11:30 AM	Session VII: Immune Engineering 2 ***Invited Speaker: Marian Ackun-Farmmer, University of Maryland/Georgia Institute of Technology***
11:30 AM - 12:45 PM	Plenary Session III: ***Elazer Edelman, Massachusettes Institute of Technology*** *(2024 Founders Award Recipient)* ***Cynthia Reinhart-King, Rice University***
12:45 PM - 1:45 PM	Lunch and Poster Session (#49 - 91)
1:45 PM - 3:00 PM	Session VIII: BioInterfaces ***Invited Speaker:*** ***Juhi Samal, University of Alabama at Birmingham***
3:00 PM - 4:15 PM	Session IX: Drug Delivery 2 ***Invited Speaker: Jarrod Call, University of Georgia***

Program Agenda

Friday, September 20, 2024 *(continued)*

4:15 PM - 5:30 PM	Session X: Tissue Engineering 2 ***Invited Speaker: Daniel Abebayehu, University of Virginia***
5:30 PM - 6:45 PM	Concurrent Session XI: "POSTAR" Award Rapid Fire Talks

2024 Regional Symposia Chair

Anita Shukla, PhD, Brown University

Southeast Regional Symposium Co-Chairs:

Edward A. Botchwey, PhD, Georgia Institute of Technology
Ankur Singh, PhD, Georgia Institute of Technology

Program Committee:

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Simone Douglas-Green, PhD, Georgia Institute of Technology
Cheryl Gomillion, PhD, University of Georgia
Ana Maria Porras, PhD, University of Florida
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Tatiana Segura, PhD, Duke University
Susan N. Thomas, PhD, Georgia Institute of Technology
John Wilson, PhD, Vanderbilt University

2024 Society for Biomaterials (SFB) Southeast Symposia, Georgia Tech, Atlanta, GA

Plenary Speaker

Dr. Joel Collier
Duke University
(Clemson Award for Basic Research)

Invited Speaker

Dr. Jennifer Elisseeff
Johns Hopkins University

Invited Speaker

Dr. Cherie Stabler
University of Florida

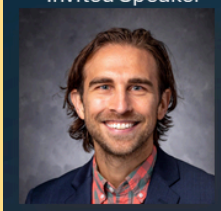
Invited Speaker

Dr. Andrés J. García
Georgia Institute of Technology

Invited Speaker

Dr. Evan Scott
University of Virginia

Invited Speaker



Dr. Jarrod Call
University of Georgia

Invited Speaker

Dr. Daniel Abebayehu
University of Virginia

Invited Speaker

Dr. Thomas Werfel
University of Mississippi

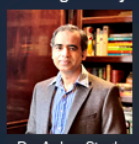
Invited Speaker

Dr. Juhi Samal
University of Alabama at Birmingham

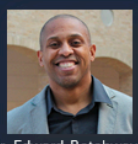
Invited Speaker

Dr. Marian Ackun-Farmmer
Georgia Institute of Technology

Regional Symposia Co-Chairs



Dr. Ankur Singh
Georgia Institute of Technology



Dr. Edward Botchwey

Program Committee:
Dr. John Wilson, Vanderbilt University
Dr. Daniel Abebayehu, University of Virginia
Dr. Simone Douglas-Green, Georgia Institute of Technology
Dr. Cheryl Gomillion, University of Georgia
Dr. Ana Maria Porras, University of Florida
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Registration Information

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ATLANTA

PLENARY SESSION I
Thursday, September 19

Dr. Shana Kelley
Northwestern University

PLENARY SESSION I
Thursday, September 19

Dr. Joel Collier
Duke University
(2024 Clemson Award for Basic Research)

PLENARY SESSION II
Thursday, September 19

Dr. Sarah Stabenfeldt
Arizona State University

PLENARY SESSION II
Thursday, September 19

Dr. Danielle Benoit
University of Oregon

PLENARY SESSION III
Friday, September 20

Dr. Elazer Edelman
Massachusetts Institute of Technology
(2024 Founders Award)

PLENARY SESSION III
Friday, September 20

Dr. Cynthia Reinhart-King
Rice University

Thank You to Our Speakers!

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2024 Regional Symposia

September 19th - 20th

- **Northeast: Northeastern University**
- **Midwest: Case Western Reserve University**
- **Southeast: Georgia Institute of Technology**
- **Southwest: University of Texas at Austin**
- **Western: University of Colorado, Denver | Anschutz Medical Campus**
- **Northwest: University of Washington**

SESSION I: NANOMATERIALS

8:45 AM - 9:10 AM **Invited Speaker: Evan Scott, UVA/NorthWestern**

9:10 AM - 9:22 AM

Ingestible Molecular Probes for Breath-Based Detection of Gastrointestinal Disease

Vishal Manickam, Georgia Institute of Technology

Vishal A. Manickam, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, vamanickam@gatech.edu

Carly E. Kimpling, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, ckimpling3@gatech.edu

Khoi N. Le, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, khoi30@gatech.edu

Amy Kang, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, akang60@gatech.edu

Leslie W. Chan, Ph.D., Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, 30332, USA, leslie.chan@gatech.edu

Endoscopic imaging is the gold standard for the detection and clinical assessment of many gastrointestinal (GI) diseases including GI cancers, ulcers, and inflammatory bowel disease. However, patient discomfort, potential complications with bowel perforation, cost, and length of the procedure deter individuals from recommended testing. The invasiveness of endoscopy also makes it an unsuitable tool for routine monitoring. Breath tests are non-invasive alternatives that are quick and can be completed with ease at great frequency and in a variety of settings for monitoring applications. Therefore, we have established a diagnostic platform that can induce exhalation of synthetic breath biomarkers that reflect disease activity in the GI tract.

Our platform is comprised of a new class of ingestible molecular probes that are metabolized by aberrant intestinal glycosidase activities into volatile (i.e gaseous) reporters that are exhaled in breath. Glycosidases are enzymes that hydrolyze glycosidic bonds in carbohydrates, and are essential for digestion, host immunity, and ECM remodeling. During GI disease, activities of certain host and microbiome glycosidases are altered. Therefore, we have developed ingestible sugar-based probes that transit the GI tract and release volatile reporters upon breakdown by specific intestinal glycosidases. Liberated reporters diffuse across the intestinal epithelium to enter systemic circulation and are detectable in breath after pulmonary gas exchange. Reporter trafficking to breath occurs in minutes, providing near real-time readout for probe activity.

Probes have been synthesized for glycosidases with altered activity in colorectal cancer (CRC). Probes were incubated with recombinantly-expressed glycosidases in cleavage assays, which confirmed that (1) probes are specifically cleaved by targeted glycosidases and (2) release volatile reporters, which are detectable via mass spectrometry. Cleavage assays using mouse tissue homogenates also confirmed

probe stability in the GI tract. In mouse studies, breath was collected 0-1.5h and 1.5-3h after oral probe delivery, the time intervals during which probes transit the small and large intestine, respectively. Exhaled reporter levels accurately reflected glycosidase activities in each GI segment. Preliminary studies demonstrate differential breath signals in healthy versus CRC mouse models. Altogether, we have developed ingestible probes that have the potential for broad utility in breath-based detection of GI diseases.

9:22 AM - 9:34 AM

Engineering protein self-assemblies through sequence-level tuning of thermal hysteresis

Kai Littlejohn, Georgia Institute of Technology-Emory University

Felipe Quiroz, Ph.D., Coulter Department of Biomedical Engineering, Georgia Institute of Technology-Emory University, felipe.quiroz@emory.edu

Intrinsically disordered proteins (IDPs) diverge from the traditional sequence-3D structure paradigm. Various IDPs self-assemble through stimulus-triggered phase separation, facilitating the formation of intracellular and extracellular structures. Recent advances in IDP engineering suggest the potential for fine-tuning IDP sequence and composition to control the dynamics and mechanical properties of IDP-assemblies. Recently, our group discovered that IDPs can be genetically engineered to access a range of nonequilibrium phase behaviors, such as thermal hysteresis, demonstrating its utility to program the stability of IDP nano-assemblies. However, the tunability of these hysteretic behaviors remains incipient. Here, we report the first observations of a sequence-level molecular dial of thermal hysteresis. To study this phenomenon, we exploit the repetitive architecture of IDP polymers (IDPPs). Previously, we developed thermoresponsive elastin-like IDPPs characterized by their degree of thermal hysteresis. Surprisingly, adding a polar residue (threonine) to a hydrophobic elastin-like motif led to IDPPs with the greatest hysteresis. Informed by a nanoscopic view of rehydration dynamics in hysteretic IDPPs, we posited that varying hydrogen bonding capability, by substituting threonine in the repeat unit with other polar residues (serine, asparagine and glutamine), would modulate hysteresis. To determine the length dependence of this behavior, we engineered, purified and characterized 40- and 80-mer IDPPs for each motif. UV–visible spectrophotometry revealed that increasingly polar IDPPs showed upward shifts in the phase transition temperature upon heating, enlarging the observable range of thermal hysteresis upon cooling. For large IDPPs, overall length was a weak modulator of hysteresis. Unexpectedly, near the phase transition temperature, controlled cooling of hysteretic IDPPs triggered the rapid growth of IDPP assemblies that also exhibited thermal hysteresis, exposing a novel self-assembly route that was inaccessible for conventional elastin-like IDPPs. Next, we applied our hysteretic dial to program the stability of IDP nano-assemblies. We engineered di-block IDPPs fusing a corona-forming IDPP encoding negligible hysteresis and hysteretic core IDPPs encoding large hysteresis. Our preliminary data suggest that tuning hydrogen bonding in hysteretic IDPPs enables potent stabilization of the cores of monodisperse IDPP nano-assemblies. Our findings demonstrate sequence heuristics to program thermal hysteresis in IDPPs, priming their use in bottom-up design of out-of-equilibrium IDP assemblies.

9:34 AM - 9:46 AM

Hysteretic phase separation dynamics as new variable for highly stable protein nanoparticle design

Alexa Regina Avecilla, Wallace H. Coulter Department of Biomedical Engineering at Emory University and Georgia Tech

Alexa Regina Chua Avecilla (alexa.avecilla@emory.edu; Wallace H. Coulter Department of Biomedical Engineering at Emory University and Georgia Tech), Felipe Garcia Quiroz (felipe.quiroz@emory.edu; Wallace H. Coulter Department of Biomedical Engineering at Emory University and Georgia Tech)

Phase-separation-driven assembly has enabled the engineering of protein-based nanostructures for drug delivery. Driving this progress are intrinsically disordered protein polymers (IDPPs) that reversibly phase transition above tunable critical temperatures. By engineering hydrophobic IDPPs fused to hydrophilic IDPPs, in a core-corona diblock architecture, IDPP nano-assembly can be guaranteed at body temperature. However, because phase-separated nano-cores exist in equilibrium, IDPP nanoparticles favor disassembly upon dilution —unavoidable for injectables. The recent discovery of non-equilibrium hysteretic phase transitions suggests a new variable to stabilize IDPP nanoparticles. Hysteretic IDPPs undergo phase separation upon heating above a critical temperature but require significant cooling below this temperature to regain solubility. Here, by recombinantly fusing IDPPs with divergent hysteretic behaviors to a common hydrophilic (non-hysteretic) IDPP, we engineered novel self-assembling hysteretic IDPP nanoparticles and characterized their nanomorphology and stability against conventional IDPP nanoparticles under physiologically-relevant conditions. Following temperature-dependent IDPP self-assembly with cryo-electron microscopy (Cryo-TEM) and UV-vis absorbance measurements, we captured two distinct assembly regimes: (i) nanoparticles, upon heating past the critical transition temperature of the core-forming IDPP (Tc1) and (ii) bulk phase separation, upon heating above the critical point of the corona-forming IDPP (Tc2, where Tc2>Tc1). Hysteresis determined the extent of sustained nanoparticle assembly below the initial Tc1~45°C, with moderately-hysteretic and highly-hysteretic nanoparticles respectively disassembling at 20 and 30°C below Tc1. This enhanced stability was also evident as a function of time at temperatures well below Tc1, resulting in fully stable nanoparticles at 37°C over at least 15 hours. Notably, hysteresis sustained nanoparticle stability as samples were diluted 25-fold to 1 μM. In contrast, non-hysteretic IDPPs (Tc1=30 °C) that readily accessed nanoparticle regimes at body temperature showed expected progressive shifts in Tc1 upon dilution. After a 25-fold dilution at 37°C, these non-hysteretic nanoparticles bordered on complete disassembly. Cryo-TEM also captured differences in nanoparticle stability under cryogenic conditions, with hysteretic IDPP diblocks consistently yielding high-contrast nanoparticles. As expected for the relatively slow process of vitrification (~ 1 ms), non-hysteretic diblocks failed to sustain nanoparticle assembly. Overall, our new findings demonstrate thermal hysteresis as a new engineering variable, separate from hydrophobicity, for the engineering of highly stable protein-based nanomaterials.

9:46 AM - 9:58 AM

Neonatal Fibrin Based Nanoparticles for Wound Healing

Sanika Pandit, Joint Department of Biomedical Engineering at UNC Chapel Hill and NC State

Sanika Pandit, spandit2@ncsu.edu, Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina at Chapel-Hill, Raleigh, NC; Comparative Medicine Institute, NC State University, Raleigh, NC

Kimberly Nellenbach, kanellen@ncsu.edu, Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina at Chapel-Hill, Raleigh, NC; Comparative Medicine Institute, NC State University, Raleigh, NC

Nina Moiseiwitsch, nina_moiseiwitsch@med.unc.edu, Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina at Chapel-Hill, Raleigh, NC; Comparative Medicine Institute, NC State University, Raleigh, NC

Anastasia Sheridan, aesherid@ncsu.edu, Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina at Chapel-Hill, Raleigh, NC; Comparative Medicine Institute, NC State University, Raleigh, NC

Ashley Brown, aecarso2@ncsu.edu, Joint Department of Biomedical Engineering, North Carolina State University and the University of North Carolina at Chapel-Hill, Raleigh, NC; Comparative Medicine Institute, NC State University, Raleigh, NC

Chronic non-healing wounds pose a significant clinical problem due to clotting deficiency and persistent inflammation. This deficiency in clotting results in a poorly formed fibrin matrix, which can contribute to the formation of non-healing wounds. In such cases, it is important to speed up the wound healing process. We have previously developed colloidal fibrin-based nanoparticles constructed from adult fibrinogen (aFBNs) that are pre-polymerized and use physiologically relevant thrombin and fibrin concentrations, unlike traditional fibrin glues typically used to treat non-healing wounds. The working time of the FBNs can be tuned by particle density, and the particles can be lyophilized and stored at room temperature. Previous work has shown that bulk neonatal fibrin scaffolds enhance in vitro wound healing.

To enhance wound healing and promote cell activity, we developed and evaluated colloidal neonatal fibrin-based nanoparticles (nFBNs) for wound healing applications. nFBNs can successfully be synthesized to comparable sizes of aFBNs. As compared to aFBNs, nFBNs are more effective in increasing fiber density within a fibrin clot. When incorporated into fibrin clots, nFBNs significantly impacted overall clot structure, resulting in decreased pore area and increased number of overall pores. Incorporating nFBNs into fibrin clots did not significantly impact the stiffness of the clots. However, nFBNs did slow clotting time as compared to aFBNs. Overall, aFBNs clotted faster and showed a dose dependent increase in clotting speed; no such relationship was seen with the nFBNs. nFBNs also promote cell attachment in a fibrin clot. Additionally, nFBNs enhance cell spreading and migration within a fibrin clot, as compared to aFBNs. To assess the wound healing capabilities of the nFBNs in vivo, full-thickness dermal wounds were created on diabetic mice and assessed over nine days. nFBNs increased the rate of wound healing and led to better healing outcomes, which were confirmed by transcriptomic analysis. Overall, nFBNs have immense potential as a wound healing therapeutic.

SESSION II: BIOMATERIAL-TISSUE INTERACTION

10:15 AM – 10:40 AM Invited Speaker: Andrés J. García, Georgia Institute of Technology

10:40 AM – 10:52 AM

Invited Speaker: Juan Mendenhall, Morehouse College, *Using Additive Manufacturing to Engineer Synthetic Tissues with Functional Gradients at Various Length Scales*

10:52 AM - 11:04 AM

Comparative proteomics analysis of wounds treated with microporous annealed particle scaffolds

Alejandra Suarez Arnedo, Duke University

Alejandra Suarez-Arnedo* (Duke University -ss1178@duke.edu), Eleanor L.P Caston* (Duke Unversity-elc67@duke.edu), Yining Liu (Duke University-sarah.yiningliu@gmail.com), Hellena

Bai (North Carolina State University-hbai3@ncsu.edu), David C Muddiman (North Carolina State University-dcmuddim@ncsu.edu), and Tatiana Segura (Duke University-ts313@duke.edu)

Microporous annealed particles (MAP) scaffolds have been shown to promote a unique regenerative response in skin wound healing. Specifically, MAP comprised of D-chiral crosslinkers (D-MAP) impart a wound healing response mediated by an adaptive immune response. However, the mechanism of action is yet to be elucidated. Using proteomic analysis based on LC-mass spectrometry, we identified 5,000 proteins common across different wound treatment groups, namely L-chiral MAP (L-MAP), D-MAP, and WounDres (a commercial control). In subsequent gene ontology analysis. we identified genetic pathways that are upregulated in different treatment groups. At early stage (Day 4), D-MAP-treated wounds showed an enrichment of multiple immune response processes, such as the adaptive immune response, complement activation, and lymphocyte development. In contrast, L-MAP promoted processes linked to keratinization and skin development. At Day 14, muscle contraction associated processes and keratinization were highly enriched in D-MAP, while L-MAP showed over presentation of functions associated with collagen organization and other extracellular matrix components. As the wounds progressed toward resolution (Day 21), we observed high enriched in keratinization, muscle contraction and low enrichment in immune specific terms when compared both D-MAP and L-MAP against WounDres. When we looked at the actual wound healing outcomes, D-MAP-treated wounds presented more appendages like structures, while L-MAP-treated wounds have extracellular matrix than resembles the architecture of normal skin. These results further elucidated the differences in regenerative characteristics between L- and D-MAP, as we characterized genetic pathway regulation as one of the key factors that contribute to a divergent wound healing response. Multiple temporal specific processes (e.g., adaptive immune response) contribute to the resolution of the MAP-treated wound and that functions are context in material specific when we compared MAP based on crosslinkers of different chirality. This study also showcased the power of proteomic analysis, with which we were able to uncover the relevance of the timing and the strength of which biological functions are enriched.

11:04 AM - 11:16 AM

Effects of Heparin Hydrogel Sulfation Level on TSG-6 Release and Tissue Response After Supraspinatus Muscle Injury

Jiahui Mao, Georgia Institute of Technology

Joseph J. Pearson; Georgia Institute of Technology; jjp276@gmail.com

Johnna S. Temenoff; Georgia Institute of Technology; johnna.temenoff@bme.gatech.edu

Tumor necrosis factor stimulated gene 6 (TSG-6) is a broadly anti-inflammatory protein that can help polarize macrophages towards anti-inflammatory phenotypes (M2). Its actions can be altered by complexing with glycosaminoglycans. In particular, fully sulfated heparin (Hep) potentiates the anti-plasmin effects of TSG-6 compared to desulfated heparin (Hep-). Rotator cuff tears can cause chronic muscle inflammation and degeneration that increases over time. We hypothesized that Hep + TSG-6 would promote muscle regeneration spatiotemporally after rotator cuff tear.

First, poly(ethylene glycol) (PEG) was conjugated with an MMP-cleavable peptide. Heparin sodium salt (Hep) and desulfated heparin derivative (Hep-) were prepared . The conjugated PEG and heparin gels were formed by free radical polymerization, fragmented to form particles, then injected into the rat supraspinatus muscle after rotator cuff injury. In vivo imaging evaluated TSG-6 release from Hep and Hep- systems for 21 days (n=6). In vivo inflammatory cell response was assessed through flow cytometry (n=8, days 3 and 7) and myogenic response through immunohistochemistry (n=5, days 7 and 14) for three groups: Saline injection, Hep+TSG-6 (Hep) and Hep-+TSG-6 (Hep-).

In vivo, similar rates of controlled release of TSG-6 were found from Hep and Hep- for 14+ days. Hep showed higher overall and type 2a, anti-inflammatory macrophages on day 7, (Hep: 2 +/- 1.3% cells, Hep-: 0.65 +/- 0.34%, Saline 0.54 +/- 0.3%) and increased embryonic myosin heavy chain (eMHC) positive fibers on day 7 compared to saline (Hep: 125 +/- 14 cm-2, Hep-: 69 +/- 80 cm-2, Saline: 5 +/- 15 cm-2). On day 14, Hep demonstrated significantly increased centrally located nuclei (CLN) compared to all groups (Hep: 560 +/- 205 cm-2, Hep-: 210 +/- 40 cm-2, Saline: 120 +/- 80 cm-2). Additionally, asymmetrical muscle regeneration was observed: samples in the myotendinous junction or near intramuscular tendon showed more eMHC staining on day 7 and more CLN on day 14 compared to samples from the muscle belly or far from intramuscular tendon.

Together, these results demonstrated that TSG-6 release from fully sulfated heparin resulted in increased overall anti-inflammatory cellular response and enhanced early muscle regeneration particularly in particular regions of this muscle injury model.

11:16 AM - 11:28 AM

Aligned, 3D, and electrically conductive multicompartment collagen-glycosaminoglycan scaffolds for musculotendinous tissue engineering in volumetric muscle loss

Geshani Bandara, University of Virginia

Cooper Wyatt, pmq4zx@virginia.edu, Department of Biomedical Engineering, University of Virginia.

Ryann D. Boudreau, zns8mx@virginia.edu, Department of Biomedical Engineering, University of Virginia.

Steven R. Caliari, caliari@virginia.edu, Department of Chemical Engineering, University of Virginia, Department of Biomedical Engineering, University of Virginia.

Skeletal muscle, composed of 3D, highly aligned, and electrically excitable muscle fibers, possesses an endogenous regeneration ability that aids in minor injury repair. However, this inherent regenerative limit is exceeded in the case of a volumetric muscle loss (VML) injury, due to the traumatic loss and damage of large amounts of muscle tissue. Tissue engineered scaffolds with combination of cells and conductive polymer particles are emerging due to the lack of effective treatment methods for VML injuries. However, fabricating biomaterials combining 3D structural alignment and electrical conductivity found in native skeletal muscle is challenging. Additionally, VML injuries are often associated with polytraumatic and accompanied by damage to the musculotendinous junction, adding complexity to the necessary treatment. We developed collagen-glycosaminoglycan scaffolds with an aligned microporous structure using a directional freeze-drying approach. 'Muscle' and 'tendon' compartments with a smooth interface mimicking the native interface are made by layering a type I collagen suspension with and without conductive polymer particles respectively. Polypyrrole (PPy) particles were synthesized and incorporated in type I collagen suspension to make the 'muscle' compartment of the scaffold electrically conductive. Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) techniques showed the successful formation of scaffolds with longitudinally aligned micropores due to directional freeze-drying and uniform distribution of conductive PPy particles respectively. C2C12 myoblasts and 3T3 fibroblasts cultured on muscle and tendon compartments respectively showed significantly increased metabolic activity (measured non-destructively using an alamarBlue assay) over 11 days of culture in growth and differentiation media. Both the compartments demonstrated enhanced cell infiltration, with the 'muscle' compartment showing positive myosin heavy chain (MHC) expression, indicative of myogenic differentiation. Our current research focuses on synthesizing nanoscale polypyrrole particles to enhance conductivity and promote phagocytosis/clearance in vivo. Furthermore, we are investigating the mechanical properties of multicompartment scaffolds and their ability to support differentiation of multiple cell types.

SESSION III: IMMUNE ENGINEERING 1

1:45 PM – 2:10 PM Invited Speaker: Jennifer Elisseeff, JHU

2:10 PM - 2:22 PM

PEG-4MAL Synthetic Immune Tissues for In Vitro Recapitulation of Adaptive Immune Response to E. Coli Bacterial Lysate

Valeria Juarez, Georgia Institute of Technology

Valeria M. Juarez (valeria.juarez@gatech.edu) 1;

Ankur Singh (ankur.singh@me.gatech.edu) 1, 2

Organizations:

1 Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA

2 Woodruff School of Mechanical Engineering and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, 315 Ferst Drive, 2310 IBB Building, Atlanta, GA, USA

Along the intestine, secondary lymphoid organs named Peyer's Patches (PPs) house adaptive immune cells which play an important role in human health and disease. B and T cells specifically help to orchestrate immune responses in the PP that generate memory and responses to bacteria in the gut, which lead to secretion of IgA. Here, we report synthetic immune organoids for evaluation of immunological changes when exposed to Escherichia coli (E. Coli), a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium commonly found in the intestine. The lymphoid organoid is generated culturing isolated human tonsil mononuclear cells within PEG-4MAL hydrogels with tissue specific bio adhesive cues, like fibronectin and collagen peptide mimics, RGD and GFOGER. Using this platform, we have evaluated increases in inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70, IFN-α2, IFN-β, IFN-λ1, IFN-λ2/3, IFN-γ, TNF-α, IP-10, GM-CSF), secreted immunoglobulin isotypes (IgG1, IgG2, IgG3, IgE, IgG4, IgA, IgM, IgD) as well as flow cytometry markers of the germinal center response (CD45, CD19, CD38, CD27, CD138, BCL-6). From these outputs, we have observed that by Day 10 we are able to obtain a significantly higher expression of sIgA levels in collected media in comparison to naïve lymphoid organoids (One Way ANOVA, $p = 0.0416$) as well as E. Coli lysate treated lymphoid organoids at day 4 (One Way ANOVA, $p=0.0190$). Flow cytometry analysis of isolated lymphoid cells indicates that cells retain high cell viability and increase in mature germinal center and plasma cell phenotypes, CD19+ CD27+ CD38+ and CD19-CD138+. We also see significant increases in inflammatory cytokines related to infection, especially with IL-6, IL-8, and GM-CSF. Lymphoid organoids fixed and imaged for CD19 and AID, a marker of germinal center response, show increase in AID. These results preliminarily demonstrate the efficacy of our system in recreating immune responses to bacterial inflammatory processes in vitro and hold promise in screening the effects of bacterium on adaptive immunity.

2:22 PM - 2:34 PM

RIG-I Activating Nanoparticles for Treatment of Glioblastoma

Alexander Kwiatkowski, Vanderbilt University

Alexander J. Kwiatkowski Vanderbilt University alexander.kwiatkowski@vanderbilt.edu

Christian R. Palmer Vanderbilt University christian.r.palmer@vanderbilt.edu

Asa A. Brockman Vanderbilt University asa.a.brockman@vanderbilt.edu

Rebecca A. Ihrie Vanderbilt University rebecca.ihrie@vanderbilt.edu

John. T Wilson Vanderbilt University john.t.wilson@vanderbilt.edu

Introduction: Glioblastoma (GBM) is a rare form of brain cancer with a dismal outlook for patients who face a mean survival time of 12-18 months post-diagnosis. Higher levels of the pattern recognition receptor retinoic acid-inducible gene-I receptor (RIG-I) are associated with improved survival outcomes in GBM, but RIG-I immunotherapy has not yet been explored for GBM. The clinical utility of 3pRNA RIG-I agonists is currently limited by significant drug delivery barriers, which we will seek to overcome using RIG-I activating nanoparticles (RANs).

Methods and Materials: Di-block polymer was formed with reversible addition–fragmentation chain transfer (polymerization using a first block of 10K polyethylene glycol and a second block of poly[(DMAEMA-c-butyl methacrylate. The di-block copolymer was dissolved in ethanol, diluted with sterile-filtered citrate buffer (pH=4.2), and complexed with RNA for 45 minutes to form micelles. Mouse Glioma261 (GL261) or CT2A cells were treated with RANs, then 24 hours later, supernatants were collected for ELISA, and cells were stained for flow cytometry. Mice were inoculated with 1,000,000 GL261 or CT2A cells on the right flank and treated intratumorally with RANS at a tumor volume of ~50 mm3 and on days 3 and 6 post treatment initiation. Alternatively, mice were engrafted with 200,000 GL261 cells in the right striatum at the following coordinates: +1.00 mm anterior, 2.0 mm lateral, and 3.00 mm deep.

Results, Discussion, and Conclusions: RANs were ~90 nm in diameter. We show that GL261 and CT2A cells treated in vitro with RANs produced significantly more interferon beta (IFN-β) and had increased MHC I expression following RAN treatment compared to cells treated with control RNA-loaded RANs (cRANs). In addition to cancer cells, RANs also activate antigen presenting cells, with THP1-Dual (monocytes) and RAW-Dual (macrophages) producing more type one IFN in response to RAN treatment. Following intratumoral treatment of GL261 and CT2A flank tumors with RANs, mice had prolonged survival with cRANs providing no benefit compared to vehicle control. We have successfully engrafted GL261 tumors and immunophenotyped the tumor and surrounding brain tissue. We demonstrate that RANs can activate multiple cell types and show efficacy against flank tumors.

2:34 PM - 2:46 PM

Improved transplant survival of human stem cell derived beta-cells overexpressing PD-L1 in a human HLA mouse model of autoimmune diabetes.

Cameron Manson, University of Florida

Cameron T Manson (cameronmanson@ufl.edu): J Crayton Pruitt Department of Biomedical Engineering, Gainesville, FL, USA

Jessie M Barra (jessiebarra@ufl.edu): Diabetes Institute, University of Florida, Gainesville, FL, USA

Adrienne Widener (adriennejan25@ufl.edu): J Crayton Pruitt Department of Biomedical Engineering, Gainesville, FL, USA

Jorge Santini Gonzalez (jorge.santini@ufl.edu): J Crayton Pruitt Department of Biomedical Engineering, Gainesville, FL, USA

Holger A Russ (holger.russ@ufl.edu): Diabetes Institute, University of Florida, Gainesville, FL, USA

Edward A Phelps (ephelps@bme.ufl.edu): J Crayton Pruitt Department of Biomedical Engineering, Gainesville, FL, USA

Type 1 diabetes (T1D) is an autoimmune disease characterized by T-cell mediated destruction of pancreatic beta-cells. Human stem-cell derived beta cells (sBCs) provide an abundant and renewable cell source for the replacement of these destroyed beta-cells. However, there is a need for localized immune tolerance to counter autoimmunity without the complications involved with systemic immunosuppression. We investigated whether sBCs engineered to overexpress the immune checkpoint molecule PD-L1 or that are knockout for HLA class I molecules could achieve localized protection against autoimmunity. We tested these engineered sBCs in a human HLA mouse model of autoimmune diabetes. NOD-HDD mice develop spontaneous diabetes, but express human HLA class I A2 instead of mouse MHC class I, such that islet-reactive mouse CD8+ T cells can mount an autoimmune attack in vivo compatible with human class I HLA. We have further developed this model by isolating T cells from spleens of diabetic NOD-HHD mice and adoptively transferring them into immune deficient NSG-HHD mice that also harbor human HLA Class I, thus transferring diabetes and challenging sBCs grafted under the kidney capsule. In the adoptively transferred NSG-HHD mice, there was improved survival of the sBCs overexpressing PD-L1 but eventual rejection of all sBC grafts by 3 weeks. As our strategy was primarily designed to control CD8+ T cells, we attributed the lack of long-lasting tolerance to graft infiltration by both CD4+ and CD8+ T-cells, indicating a need for a synergistic tolerance strategy that can also regulate the CD4+ T-cells.

From here, we developed an approach to extend presentation of T cell modulators (e.g. TRAIL) into the graft microenvironment using biomaterials engineering. To this end, we developed a granular hydrogel based on 4-arm polyethylene glycol (PEG) maleimide microparticles. Briefly, PEG-4MAL microgels with PEG-dithiol crosslinker were generated using a single-emulsion approach resulting in microspheres with an average diameter of 10-20 µm. These particles were further surface functionalized with immunomodulators using click chemistry approaches. We are currently conducting studies to investigate the synergistic ability of the biomaterials combined with engineered sBCs to control graft autoimmune rejection in the human HLA diabetic mice.

2:46 PM - 2:58 PM

STING Activating Polymer Drug Conjugates for Cancer Immunotherapy

Taylor Sheehy, Vanderbilt University

Alexander Kwiatkowski (alexander.kwiatkowski@vanderbilt.edu)

Karan Arora (karan.arora@vanderbilt.edu)

John Wilson (john.t.wilson@vanderbilt.edu)

Cancer remains the second leading cause of death in the United States. Immune checkpoint blockade (ICB) is revolutionizing treatment of diverse cancer types; however, these treatments only benefit a minority of cancer patients. Low response rates are correlated to immunogenically “cold” tumors, meaning they lack sufficient tumor antigen-specific CD8+ T-cell infiltration and other proinflammatory cell types. Therefore, there is a need for therapeutic systems that will shift tumors towards an immunogenically “hot,” environment, increasing antitumor innate immunity and enhancing responses to immunotherapy. This may be acheived through targeting the stimulator of interferon genes (STING) pathway. STING activation triggers a type I interferon (IFN-I)-driven inflammatory response, which stimulates dendritic cell cross-presentation of tumor antigens, leading to mobilization of tumor-specific CD8+ T cells, which reduce tumor progression and can lead to immunological memory.

STING agonists are being explored as next generation cancer therapeutics but are limited due to poor drug-like properties such as low bioavailability, high renal clearance, and off target toxicities. A dimeric amidobenzimidazole (diABZI) STING agonist developed by GlaxoSmithKline demonstrates promising antitumor effects through systemic administration; however, diABZI lacks tumor specificity and activates STING indiscriminately. Therefore, we have developed SAPCon, a STING activating polymer-drug conjugate, for the delivery of a chemically-modified diABZI that allows for enhanced circulation, tumor-targeting and environmentally-responsive drug release. diABZI-V/C-DBCO was designed for chemical-conjugation to the platform and intracellular drug release through cathepsin cleavage. The polymeric carrier platforms were synthesized through reversible addition- fragmentation chain-transfer (RAFT) polymerization and were conjugated to the STING agonist through copper-free click chemistry. Conjugation of the drug to various molecular weight polymers significantly improves pharmacokinetics and biodistribution, resulting in diminished tumor burden and prolonged survival in murine breast cancer models. These treatments successful shift the tumor microenvironment to a proinflammatory phenotype and synergize with approved ICB, opening an exciting door for the next generation breast cancer treatment.

SESSION IV: DRUG DELIVERY 1

3:00 PM - 3:25 PM

Glycopolymers enrich less immunogenic protein coronas and improve tumor delivery compared to PEG

Thomas Werfel, University of Mississippi

Kenneth R. Hulugalla1,3, Oluwaseyi T. Shofolawe-Bakare2, Sk Arif Mohammad3, Railey Mayatt5, Kelsie Hand3, Claylee Chism4, Sandeep K. Misra1, Tanveer Shaikh5, Eden Tanner4, Joshua Sharp1, Nicholas Fitzkee5, Thomas A. Werfel1, 2, 3, 6

1Department of BioMolecular Sciences, University of Mississippi, University, MS, USA
2Department of Chemical Engineering, University of Mississippi, University, MS, USA
3Department of Biomedical Engineering, University of Mississippi, University, MS, USA
4Department of Chemistry and Biochemistry, University of Mississippi, University, MS, USA
5Department of Chemistry, Mississippi State University, Starkville, MS, USA
6Cancer Center and Research Institute, University of Mississippi Medical Center, Jackson, MS, USA
Thomas Werfel: tawerfel@olemiss.edu

PEGylated nanoparticles remain the most widely employed vehicles for delivering cargo such as chemotherapeutics, targeted therapies, or nucleic acids to tumors. However, it is being increasingly appreciated that PEG causes immunogenic reactions and is susceptible to rapid clearance via the binding of anti-PEG antibodies. Therefore, as an alternative to nanoparticle PEGylation, we engineered nanoparticles with a glycopolymer-based corona. The particles are composed of a diblock copolymer, poly(2-(diisopropylamino)ethyl methacrylate)-b-poly(methacrylamidoglucopyranose) [PDPA-b-PMAG], that is synthesized via reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. In this study, we compared the physicochemical characteristics, biocompatibility, cell uptake, protein adsorption, and protein corona composition of PDPA-b-PMAG and PDPA-b-PEG NPs. We found that PDPA-b-PMAG and PDPA-b-PEG NPs could be produced with similar physicochemical characteristics (e.g., size, surface charge, and morphology) and cellular biocompatibility. However, these NPs exhibited vastly different biological interactions which were dependent upon the presence of the NP protein corona. Compared to PDPA-b-PEG NPs, PDPA-b-PMAG NPs exhibited roughly 5-fold higher uptake in MDA-MB-231 breast cancer cells in cell culture. Moreover, the PDPA-b-PMAG NPs also exhibited significantly less uptake in RAW 264.7 macrophages than PDPA-b-PEG NPs. These differences were exacerbated further in the presence of human serum because pretreatment of the NPs with human serum further increased the uptake of PDPA-b-PEG NPs in RAW 264.7 macrophages while PDPA-b-PMAG NP uptake in the macrophages reduced further. Upon analysis of the protein coronas of each NP formulation, it became clear that PDPA-b-PMAG NPs adsorb less immunogenic proteins than PDPA-b-PEG. First, SDS-PAGE and BCA experiments showed that PDPA-b-PMAG NPs adsorb significantly less protein than PDPA-b-PEG NPs. Moreover, the abundance of IgG-class and complement proteins was reduced ~5-fold and ~10-fold, respectively, on the surface of PDPA-b-PMAG NPs compared to PDPA-b-PEG NPs. These early results suggest that glycopolymers such as PMAG have great promise as less-immunogenic alternatives to PEG in nanomedicine. Our future studies will assess the in vivo delivery of PMAG-based NPs to confirm their utility as improved delivery vehicles for cancer and other diseases.

3:25 PM - 3:37 PM

Controlled release of rhBMP2 from porous tissue engineering graft promote bone regeneration in a rat spinal fusion model

Naboneeta Sarkar, Johns Hopkins Medicine

Naboneeta Sarkar1,2, Mahnoor Shafi1,2, Ethan Cottril1,2, Yicheng Zhang3, Zhicheng Yao3, Hai-Quan Mao3, Timothy Witham1,2*

nsarkar2@jhmi.edu, mshafi5@jh.edu, yzhan403@jhu.edu, zhichengyao@jhu.edu, twitham2@jhmi.edu,

1Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

2Spinal Fusion Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

3Department of Biomedical Engineering, Translational Tissue Engineering Center, School of Medicine, Baltimore, MD 21287, USA.

With a notable 118% surge in the last two decades, incidences of lumbar spinal fusion procedures in the United States are experiencing a remarkable upward trend. However, currently available FDA-approved collagen-based Infuse™ spinal fusion bone graft has been associated with serious complications including heterotopic ossification, paralysis, bowel/bladder dysfunction and local inflammation due to the presence of higher doses of recombinant human bone morphogenetic protein (rhBMP2), a potent osteogenic growth factor. These challenges have heightened the urgent need for novel spinal fusion bone grafts that offer greater fusion rate and superior clinical outcomes without the risk of adverse side effects. To address this, we have developed a novel porous biomimetic bone graft combining a pro-regenerative porcine small intestinal submucosa derived extracellular matrix (SIS-ECM) with an osteoinductive biphasic mixture of calcium phosphates (20% hydroxyapatite and 80% beta-tricalcium phosphate) in a 1:2 organic and inorganic ratio, closely resembling the molecular composition of native bone. Furthermore, this graft incorporates rhBMP2 encapsulated in a biodegradable polyelectrolyte polymer nanoparticle for controlled release of the growth factor over an extended period. This porous graft exhibits enhanced cellular attachment, proliferation and upregulation of osteogenic differentiation markers compared to the conventional collagen sponges. When implanted in a clinically translatable rat posterolateral lumbar spinal fusion model, the spinal graft accelerates bone formation and remodeling leading to early bony union after 8 weeks as shown by microcomputed tomography (μCT), histological analysis, and mechanical testing. Notably, the graft exhibits higher total and percentage bone volume, trabecular number as well as smaller trabecular separation compared to the control collagen sponge infused with 10-fold higher concentration of rhBMP2. Histological evidence by H&E and Masson's trichrome further confirms continuous bridging of bone this biomimetic bone graft whereas fibrotic tissue with nonunion could be seen for collagen sponges with the same amount of rhBMP2. Immunohistochemical staining and flow cytometry analysis of the post-operative tissue did not reveal any significant adverse immune reaction against the novel biomimetic graft. These findings underscore the promising potential of our novel biomimetic spinal fusion scaffold in advancing spinal fusion surgeries by promoting faster fusion rates and accelerated bone regeneration.

3:37 PM - 3:49 PM

Enhanced Delivery and Gene-silencing in Brain Border-Associated Macrophages using Lipid-siRNA Conjugates

Adam Abdulrahman, Vanderbilt University

Adam Abdulrahman, adam.m.abdulrahman@vanderbilt.edu, Vanderbilt Department Biomedical Engineering

Alexander Sorets, Vanderbilt Department Biomedical Engineering, alexander.g.sorets@Vanderbilt.Edu

Alexander Ligocki, alexander.ligocki@vanderbilt.edu, Vanderbilt Department of Chemical and Biomolecular Engineering

Katrina Schwensen, Vanderbilt Department of Chemical and Biomolecular Engineering, katrina.r.schwensen@Vanderbilt.Edu

Nora Francini, Vanderbilt Department Biomedical Engineering, nora.francini@vanderbilt.edu

Alena Shostak, VUMC Department of Neurology, alena.shostak@vumc.org

Andrew Kjar, Vanderbilt Department Biomedical Engineering, andrew.kjar@vanderbilt.edu

Joshua Park, Vanderbilt Department Biomedical Engineering, joshua.c.park@Vanderbilt.Edu

Rebecca Cowell, Vanderbilt Department of Chemical and Biomolecular Engineering, rebecca.cowell.1@Vanderbilt.Edu

Matthew Schrag, VUMC Department of Neurology, matthew.schrag@vumc.org

Craig Duvall, Vanderbilt Department Biomedical Engineering, craig.duvall@Vanderbilt.Edu

Ethan Lippmann, Vanderbilt Department Biomedical Engineering, Vanderbilt Department of Chemical and Biomolecular Engineering, Vanderbilt Brain Institute, VUMC Department of Neurology, ethan.s.lippmann@vanderbilt.edu

Despite the worldwide increase in the incidence of Alzheimer's Disease (AD), robust therapeutics to treat its underlying causes are lacking. There is increasing evidence that neuro-immune crosstalk plays a pivotal role in the development of neurodegenerative diseases such as AD. In particular, brain border-associated macrophages (BAMs), which are situated in the meninges, choroid plexus, and perivascular space, are emerging as critical effectors in these diseases. However, current strategies to therapeutically target BAMs do not exist. Nucleic acid therapeutics, such as short interfering RNA (siRNA), can induce potent and durable knockdown in specific gene targets. These treatments are commonly administered via the cerebrospinal fluid (CSF). We have developed a lipid-siRNA conjugate (termed EG18), which exhibits improved transport through the CSF into the perivascular space, choroid plexus, and parenchyma, and induces potent gene silencing across the CNS, including BAMs. Following injection into the lateral ventricles, EG18 distributes along CSF flow pathways, including perivascular spaces. The perivascular space is a fluid filled space which surrounds arterioles and venules in the brain, is involved in CSF fluid flow, and contains disease-relevant cells such as perivascular macrophages. Through immunofluorescence staining, we identified the localization of dye-labeled EG18 in the perivascular space. Additionally, to

assess EG18's delivery into BAMs, including specific disease-relevant subsets, we performed immunofluorescence staining 48 hours after injection. Furthermore, we found that EG18 induces robust BAM delivery even at lower doses (0.5 nmol), while mitigating off-target delivery to the spleen and liver. The effectiveness of gene silencing by EG18 in specific cell types, including BAMs, was evaluated using single-cell RNA sequencing. Owing to its ability to access perivascular spaces, our conjugate achieves widespread distribution throughout the CNS. Notably, we observed particularly robust delivery into BAMs, including the canonical Lyve1pos and MHCIIpos subsets, as evidenced by immunofluorescence staining. We found that EG18 potentiates gene silencing in a wide range of cell types including BAMs, compared to a non-targeting control.

In sum, EG18 represents a promising advancement in siRNA delivery technology within the CNS, effectively targeting key immune cells. This approach holds potential for developing treatments in AD through inhibition of disease-driving genes.

3:49 PM - 4:01 PM

Microfluidic Assembly of Mitochondria-Loaded Microparticles for On-Demand Delivery

Ryan Miller, Georgia Institute of Technology

Johnna S. Temenoff

Carol Ann and David D. Flanagan Professor

Georgia Tech/Emory University

email: johnna.temenoff@bme.gatech.edu

With the discovery that mitochondria can be actively transferred from one cell type to another and promote wound healing, new therapeutic approaches have been developed to harness the benefits of this phenomena. Nevertheless, promoting sufficient levels of exogenous mitochondria transfer to observe tissue level changes can be both variable and difficult to predict. As such, the goal of this work is to (1) increase mitochondrial uptake through polymer-based mitochondrial surface modification and (2) enable targeted mitochondria release through the encapsulation in matrix metalloproteinase (MMP) cleavable hydrogel microparticles. Using this system, we hypothesized that increased mesenchymal stem cell (MSC) derived mitochondria transfer to smooth muscle cells and osteoprogenitor cells would improve in vitro myogenesis and in vitro osteogenesis respectively. To facilitate free-mitochondria uptake, TAT-peptides (QPRRRQRRKKKRG) and triphenol phosphate (TPP) were conjugated to dextran via carbodiimide chemistry (TAT-dextran-TPP). The isolated mitochondria were incubated with 2.5wt% TAT-dextran-TPP for 12 hours and subsequently encapsulated in MMP-cleavable hydrogels (10µg/mL total mitochondria protein) using droplet microfluidics. MSC-derived mitochondria encapsulated in ~90µm diameter MMP-cleavable hydrogel microparticles were actively released over 48 hours when in the presence of 1mg/mL collagenase. No release was observed in a collagenase-free solution, illustrating selective release in the presence of MMPs. Furthermore, we observed a >6-fold increase in mitochondria uptake by C2C12 and MC3T3-E1 cells following 24-hour incubation with mitochondria decorated with TAT-dextran-TPP polymer and released from hydrogels. After 5 days, we observed greater differentiation of myoblasts into myotubes for conditions with increased mitochondria uptake. Similarly, we observed increased

osteogenic differentiation of precursor osteoblasts post mitochondria delivery marked by an increase in calcium deposition. From this study, we are able to illustrate that mitochondria can be isolated from MSC culture, loaded into MMP-cleavable hydrogel microparticles and released, all while preserving their function in vitro. Moreover, mitochondria surface modification with TAT-dextran-TPP increases internalization by C2C12 and MC3T3-E1 cells, promoting a larger degree of myogenic and osteogenic differentiation respectively. We note that while these results are most directly applicable for muscle and bone regeneration, on-demand mitochondrial release for localized cellular uptake has applications in a wide range of regenerative medicine applications.

4:01 PM - 4:12 PM

Supramolecular Peptide Hydrogel for Delivery of Locally Sustained Enzyme Therapeutics

Madeline Fuchs, University of Florida

Madeline Fuchs, Bethsymarie Soto Morales, Dillon Seroski, Gianna Scibilio, Benjamin Keselowsky*, and Gregory Hudalla* * Co-senior authors
J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL
madelinejfuchs@ufl.edu, bethsymarie.soto@gmail.com, dillonseroski@yahoo.com, gianna.scibilio@ufl.edu, bkeselowsky@bme.ufl.edu, ghudalla@bme.ufl.edu

Functional biomaterials fabricated from supramolecular self-assembly are of interest for many biomedical applications. However, to be useful for applications such as catalysis, whole protein must be installed to provide the biomaterial with this functionality. This can be challenging because covalently grafting proteins onto post-assembled biomaterials can alter protein bioactivity and is often non-specific and highly variable. Instead, the protein can be installed via recombinant covalent fusion to an assembly peptide in the pre-assembled state. The CATCH (Co-Assembly Tags based on CHarge complementarity) biomaterial system consists of a pair of oppositely charged variants of the synthetic -sheet fibrillizing peptide, Q11. Separately, the CATCH(4K+) and CATCH(6E-) peptides do not self-assemble due to electrostatic repulsion, but once combined, they co-assemble into nanofiber-based hydrogels. CATCH peptides recombinantly fused to folded proteins can be incorporated into the nanofibers during assembly, endowing the hydrogels with functionality. Immobilizing therapeutic enzymes to CATCH nanofiber-based hydrogels can locally prolong availability and functional efficiency of enzyme therapeutics. Localized delivery can decrease the rate of renal clearance and improve therapeutic outcomes, while also reducing off-target effects and administered dose.

The overall goal of this work is to show the versatility of this biomaterial system to be utilized in localized and sustained delivery of enzyme-based therapeutics. We show the functional incorporation of four different CATCH-fusion proteins. Using NanoLuc as a reporter enzyme, we observed that the CATCH vehicle can prolong retention of active enzyme in vivo for 20 days. Uricase, a clinically used therapeutic enzyme, reduces inflammation in a mouse model of gout when delivered locally in the CATCH vehicle. And finally, Indolamine-2,3-dioxygenase and Adensosine synthase A provide localized immunomodulation in response to LPS-induced inflammation when delivered in the CATCH vehicle. These results, and the modular design of the CATCH system, suggest that CATCH(4K+/6E-) could be a broadly useful vehicle for improving the local pharmacokinetics of existing and emerging enzyme therapeutics.

Session V: Tissue Engineering 1

5:30 PM – 5:55 PM **Invited Speaker: Cherie Stabler, PhD, University of Florida**

5:55 PM - 6:07 PM

A 3D bioprinted perfusable model of neuroblastoma to study the impact of tumor microenvironment on therapy response

Mehdi Salar Amoli, Emory University

- Mehdi Salar Amoli: Mehdi.salar.amoli@emory.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States

- Jenny Shim (co-first), jenny.shim@emory.edu, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, United States, Aflac Cancer and Blood Disorders Center, The Children's Healthcare of Atlanta, Atlanta, GA, United States, Winship Cancer Institute, Emory University, Atlanta, GA, United States

- Hunter Jonus, hunter.jonus@emory.edu, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, United States, Winship Cancer Institute, Emory University, Atlanta, GA, United States

- Martin Tomov, martin.lyubomirov.tomov@emory.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States

- Sarah Rezapourdamanab, sarah.rezapourdamanab@emory.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States

- Yamini Singh, yamini.singh@emory.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States

- Emma Howard, ehoward38@gatech.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States

- Margaret Wade, mwade42@gatech.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States

- Kelly Goldsmith, kgoldsm@emory.edu, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, United States, Aflac Cancer and Blood Disorders Center, The Children's Healthcare of Atlanta, Atlanta, GA, United States, Children’s Healthcare of Atlanta, Atlanta, GA, United States

- Vahid Serpooshan, vahid.serpooshan@emory.edu, Wallace H. Coulter Department of Biomedical Engineering, Emory University School of Medicine and Georgia Institute of Technology, Atlanta, GA, United States, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, United States, Children’s Healthcare of Atlanta, Atlanta, GA, United States

Introduction

Despite multimodal therapies, survival rate for patients with high-risk neuroblastoma (NB) remains <50%. Combining current therapies with monoclonal antibodies targeting disialoganglioside (GD2) on NB cells has improved survival. However, primary tumors in the soft tissue often remain unaffected. The tumor microenvironment (TME) and extracellular matrix (ECM) parameters can promote cancer cell survival and prevent anti-tumor immune cell infiltration. Understanding of these mechanisms is limited due to lack of robust in vitro models. Based on our previous models, this study employs 3D bioprinting and perfusion bioreactor technologies to develop a tunable in vitro model of NB, paving the way for studying the impact of ECM and TME properties on NB growth, aggression, and therapy response.

Methods

Models were designed to include vascularization channels, and a chamber for NB spheroid. Methacrylated gelatin (GelMA) was synthesized and bioprinted using a BioX bioprinter, with gelatin microparticles as support bath. Print fidelity, chemical and mechanical properties of the constructs were evaluated. Human umbilical vein endothelial cells (HUVECs) were seeded into the bioprinted vascular channels. Human derived NB cell line, IMR-5, were grown as neurospheres in neurobasal media and seeded in the housing, along with peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, and cultured under static and dynamic perfusion conditions. The cells were then analyzed using brightfield microscopy, Live/Dead imaging, flow cytometry and immunohistochemistry (IHC).

Results and Discussion

The constructs were bioprinted at a high fidelity, accurately representing the 3D design for vascular channels, and the NB/PBMC chamber. Seeded HUVECs displayed a high viability and proliferation in three days, forming a uniform endothelial layer in both static and dynamic culture conditions. NB spheroids and PBMCs demonstrated adequate viability, as assessed via Live/Dead imaging. IHC and flow cytometry analyses demonstrated an optimal population of PBMC and NB cells following culture, confirming the ability of 3D engineered TME in maintaining viable and functional multicellular components. Compared to previous model, the novel perfused 3D bioprinted models developed and optimized here were shown to accurately replicate different cellular/ECM elements of TME, demonstrating potential for use as a research enabling platform for studying NB aggression and treatment strategies.

6:07 PM - 6:19 PM

Sialic-acid Modified Adult Fibrinogen to Promote Neonatal-like Wound Healing in Adults

Anastasia Sheridan, University of North Carolina at Chapel Hill and North Carolina State University

-Anastasia Sheridan, Joint Department of Biomedical Engineering of University of North Carolina, Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA, Comparative Medicine Institute, North Carolina State University, Raleigh, NC 27606, USA, aesherid@ncsu.edu

-Kimberly Nellenbach, Joint Department of Biomedical Engineering of University of North Carolina, Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA, Comparative Medicine Institute, North Carolina State University, Raleigh, NC 27606, USA,kanellen@ncsu.edu

-Grace Hardy, Joint Department of Biomedical Engineering of University of North Carolina, Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA, Comparative Medicine Institute, North Carolina State University, Raleigh, NC 27606, USA, grhardy@ncsu.edu

-Elizabeth Byrnes, Joint Department of Biomedical Engineering of University of North Carolina, Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA, embyrnes@ncsu.edu

-Ashley C. Brown, Joint Department of Biomedical Engineering of University of North Carolina, Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA, Comparative Medicine Institute, North Carolina State University, Raleigh, NC 27606, USA, Department of Material Science and Engineering, North Carolina State University, Raleigh, NC 27606, USA, aecarso2@ncsu.edu

Chronic wounds are a worldwide problem impacting 10.5 million people in the United States alone. Chronic wounds do not follow a timely repair which can cause infection leading to amputation and death. Fibrinogen plays a major role in wound healing as it undergoes proteolytic cleavage; thereby, turning into a crosslinked fibrin network that acts as a provisional matrix for platelets, growth factors, and other cell types to assist in hemostasis. However, if this matrix is insufficient, non-healing wounds can occur. Therefore, topical therapies that expedite the wound healing process and avoid infection should be explored. Neonates exhibit faster wound closure than adults. Fetal fibrinogen, a molecular fibrinogen variant, is integral to accelerated healing. Fetal fibrinogen has different subunit molecular weights and higher sialylation levels; it also exhibits greater cell attachment and accelerated healing. Given ethical concerns and lack of availability of fetal fibrinogen, we propose modifying adult fibrinogen to be more fetal-like to enhance wound healing. Because fetal fibrinogen has higher sialic acid levels than adult fibrinogen and sialic acid has been shown to alter clot properties, we hypothesize that increasing sialic acid on adult fibrinogen can achieve neonatal-like wound healing. To create adult fibrinogen with varying sialic acid concentrations, adult fibrinogen was mixed with α-2,3-sialyltransferase and cytidine-5'-monophospho-N-acetylneuraminic acid sodium, incubated for 24-48 hours, and quantified via NANA assay. Polymerization kinetics were evaluated by measuring clot turbidity over time. Clot structure was evaluated by adding 488-labeled fibrinogen to clots and imaging on confocal microscope. Cellular interactions were evaluated by seeding fibroblasts onto clots and incubating for 24 hours. Cell nuclei and membranes were stained to measure cell count and area. Results show adult fibrinogen with varying sialic acid levels can be created, and sialic acid level has an inverse relationship with clot turbidity. Furthermore, increased fibroblast attachment and spreading was seen on clots created from fetal fibrinogen and adult fibrinogen with high sialic acid. In conclusion, sialylation can be modified on adult fibrinogen to create neonatal-like clots that promote cell attachment and spreading. Future experiments will be conducted to evaluate cell activation, clot mechanics, and in vivo wound healing outcomes.

6:19 PM - 6:31 PM

Anisotropic granular fiber hydrogels for skeletal muscle tissue engineering

James Gentry, University of Virginia

James L. Gentry, University of Virginia, jg9zk@virginia.edu

Steven R. Caliari, University of Virginia, sc6au@virginia.edu

Volumetric muscle loss (VML) injury results in permanent deficit of skeletal muscle function and drastic reduction in quality of life. Granular materials promote de novo myogenesis by enabling cell infiltration from surrounding tissue, pro-regenerative immunomodulation, and promotion of angiogenesis. However, these materials composed of discrete spherical particles prevent functional recovery due to their tortuous, isotropic pore structure that inhibits myofiber alignment. Therefore, development of a macroporous granular scaffold with anisotropic structural cues would be useful for VML repair. To address this need, we have developed a microfiber-based granular scaffold comprised of both sacrificial (gelatin) and non-sacrificial (gelatin-methacryloyl or GelMA) fibers, providing space for myogenesis and aligned structural cues respectively. Fibers were fabricated using a bulk fragmentation approach. Warm solutions of either gelatin or GelMA were drawn into a tipless syringe and incubated at 4ºC until solid. The gel was extruded through a cell strainer, yielding microfibers of the same geometry as the cell strainer pores. Pore apertures of 20, 40, or 80 µm were used as they cover a wide range of myofiber diameters. The mean diameter of the 80 µm fibers was found to be 88 ± 20 µm based on confocal images of fluorophore-labeled fibers. To determine if the GelMA fibers exhibit properties necessary for 3D printing, photo-crosslinked fibers underwent a cyclic strain rheometric test. Similarly to other granular systems, this material exhibited recovery within 30 seconds of strain cessation. Extrusion-induced alignment of fibers was visualized with fluorescent confocal imaging. Quantitative analysis showed fiber orientation along 0° in the aligned group, while orientation was uniformly distributed in the unaligned control. Preliminary cell culture experiments suggest high cell motility and proliferation in the granular fiber hydrogel compared to a nanoporous group. In summary, we developed an anisotropic granular material for use as a skeletal muscle tissue scaffold, and we are working to characterize the effect of pore size and alignment on in vitro myogenesis.

6:31 PM - 6:43 PM

Application of Tissue Penetrating Hyaluronic Acid Hydrogel Augments The Chondrocyte Microenvironment Reducing Catabolic Activity

Tristan Pepper, Georgia Tech

Tristan Pepper - Georgia Tech - tpepper3@gatech.edu
Lauren Foster - Emory - lauren.foster2@emory.edu
Hicham Drissi - Atlanta VAMC
Thanh Doan - Emory
Jay Patel - Atlanta VAMC - jay.milan.patel@emory.edu

Joint injuries are increasingly common and initiate a degenerative cascade in the articular cartilage. Chondrocytes experience both intra- and extra-cellular changes during the initial phases of this process, starting a degenerative process that progresses towards osteoarthritis (OA). Thus, restoration of chondrocyte homeostasis and maintenance of its microenvironment may be critical towards slowing, or even preventing, OA. Previously, we developed a tissue-penetrating hyaluronic acid (tp-HA) system that reinforced damaged cartilage and prevented its breakdown; the objective of this study was to explore the micro-scale implications of tp-HA treatment. In particular, we 1) characterized the integration and localization of tp-HA, 2) established its ability to reinforce the chondrocyte microenvironment, and 3) determined how HA augmentation of the chondrocyte microenvironment influenced cellular morphology and catabolic activation.

To characterize the diffusion of tp-HA, bovine cartilage explants were harvested. tp-HA solution (4 wt%, fluorescently tagged) was applied to the cartilage superficial layer , allowed to diffuse for 5 minutes, and photocrosslinked for 5 min to gel within the cartilage. We demonstrated that tp-HA integrated into the tissue approximately 150μm. Furthermore, when stained for type VI collagen to visualize the PCM, we observed increased tp-HA (via fluorescence) in the PCM, indicating its integration into the chondrocyte microenvironment. To determine how the tp-HA system restored cartilage micromechanics, explants were digested in collagenase for 30 min (to mimic OA) and reinforced with tp-HA. Explant cryosections (5μm) were stained for type VI collagen, and nanoindentation was used to map non-reinforced and reinforced regions, with tp-HA demonstrating restoration of PCM mechanics to near-healthy values. Finally, to investigate how HA hydrogel impacts chondrocyte behavior, bovine chondrocytes were encapsulated within gelatin hydrogels (5% methacrylated gelatin; GelMA) or gelatin-HA hydrogels (4% GelMA, 1% MeHA), followed by IL-1β application (10ng/mL) for 2 hours. Gels were stained for NF-κB (catabolic activation) and phalloidin (F-Actin). Chondrocytes in GelMA demonstrated sharp increases in cell perimeter and NF-κB intensity, both of which were almost completely mitigated in GelMA-MeHA gels.

In summary, our tp-HA hydrogel integrated with the chondrocyte microenvironment and restored its mechanical properties; incorporation of HA into the microenvironment early in OA may help prevent its progression.

SESSION VI: ENGINEERING CELLS AND THEIR MICROENVIRONMENTS

8:45 AM - 8:57 AM

Guest-host interlinked microporous annealed particle hydrogels as an injectable microenvironment for islet transplantation

Adrienne Widener, University of Florida

Adrienne E. Widener, J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, adriennejan25@ufl.edu

Edward A. Phelps, J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, ephelps@bme.ufl.edu

Microporous annealed particle (MAP) hydrogels are composed of closely packed microgels that form interstitial spaces to create porosity. These microgels can slide past one another to form a shear-thinning and injectable material for cell and tissue engineering. We use guest-host interactions to interlink polyethylene glycol maleimide (PEG-MAL) microgels (MAP-PEG) for increased stability and self-healing of the material, which we characterized through rheology. Clinical islet transplantation has been used as a therapy for type 1 diabetes to replace insulin-producing beta cells in the islets of Langerhans. This therapy has been successful in controlling blood glucose, but due to alloimmunity and reoccurrence of autoimmunity, the transplants can be rejected. We investigated MAP-PEG as a tunable and injectable microenvironment for the protection of transplanted islets in vivo. Syngeneic mouse islets were co-delivered with MAP-PEG under the kidney capsule of C57BL/6 mouse to determine viability and engraftment. After 14 days, microgels remained well-compacted and did not cause an inflammatory foreign body response in vivo. We found that the addition of guest-host interactions did not negatively affect the viability and engraftment of the islets compared to unfunctionalized microgels and islet-only implants. Mason's trichrome straining showed little to no fibrotic encapsulation of MAP-PEG transplants but substantial fibrotic collagen deposition around degradable bulk hydrogels and islet only controls. The MAP-PEG gels were permissive to islet graft revascularization at 14 days without additional growth factors. Gels without islets had significantly lower vessel density, suggesting that islets within the MAP-PEG released sufficient angiogenic signals to vascularize the material. The advantage of MAP-PEG is that it provides the ability to control the cellular signaling environment near the islet graft. We have successfully conjugated immunomodulatory biomolecules to the surface of MAP-PEG hydrogels. Ongoing in vivo studies are characterizing a panel of immunomodulatory proteins and drugs presented on MAP-PEG co-transplanted with beta cells to prevent autoimmune rejection in a humanized mouse model of type 1 diabetes.

8:57 AM - 9:09 AM

Biomaterials-based human lung with integrated mucosal and humoral immune responses

Eshant Bhatia, Georgia institute of technology

Eshant Bhatia1,2, Rachel Ringquist1,2, Zhe Zhong1,2, Zhengying Wang2, Nikhil Kasaghatta,2, Sandip Das,2, Anirudh Sriram2, Krishnendu Roy1,2,3, Ankur Singh1,2

1. George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia

2. Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia

3. School of Engineering, Vanderbilt University, Nashville, Tennessee

The need for lung mucosal and lymph node systemic immunity is crucial for comprehensive protection against respiratory pathogens. The balance of mucosal and systemic immunity is critical in determining the outcome of severe, lethal influenza. An effective immune response can help to control the virus and prevent severe complications, while an overactive or dysregulated response can contribute to the severity of the illness. Influenza research often relies on animal models for pre-clinical studies, but these models do not accurately replicate human immune responses or the immunological diversity of the human system. To address this limitation, we have developed a fibrin-collagen-based immune-competent lung-on-chip system that mimics mucosal responses to influenza infection in a vascularized, perfusable lung epithelium. The immune competency here corresponds to the presence of all immune cells, and using single-cell RNA sequencing we demonstrate a strong immune-stroma crosstalk in infected lungs (manuscript in Review). While this model represents a step toward a more complete human-based system, it does not fully capture the physiological complexity needed to understand the impact of pulmonary infection on secondary lymphoid organs, where most humoral immune responses are generated. To overcome this critical gap, we have further developed a maleimide-functionalized polyethylene glycol hydrogel-based lymphoid organoids and integrated it with a fibrin-collagen-based immune-competent lung on the chip. The chip design allowed for molecular transport from lung to lymphoid by establishing interstitial flow promoting antigen transfer from lung to lymphoid organoid. We demonstrate the lung-lymphoid immune crosstalk and generation of adaptive immunity in response to influenza infection in the lung. We noted that infection of the lung epithelium with live virus triggered the activation of immune cells in the lung interstitial space, while also stimulating antibody-secreting cell maturation in lymphoid organoids.

9:09 AM - 9:21 AM

Matrix stiffness influences response to chemo and targeted therapy in brain metastatic breast cancer cells

Venu Yakati, The University of Alabama

Venu Yakati, Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL 35487, USA, vyakati@ua.edu

Lalita A. Shevde, Department of Pathology, O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35233, USA, lsamant@uab.edu

Shreyas S. Rao, Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL 35487, USA, srao3@eng.ua.edu

Breast cancer is the most common malignancy accounting for 12.5% of all newly diagnosed cancer cases across the globe. Breast cancer cells are known to metastasize to distant organs (i.e., the brain), wherein they can exhibit a dormant phenotype for extended time periods. These dormant cancer cells exhibit reduced proliferation and resistance to therapy. However, the mechanisms by which the dormant cancer cells exhibit resistance to therapy, in the context of brain metastatic breast cancer (BMBC), is not well understood. In this study, we employed hyaluronic acid (HA) hydrogels with varying stiffnesses (i.e., ~0.4 kPa vs. ~4.5 kPa) to study drug responsiveness in dormant vs. proliferative BMBC cells. We found that cells cultured on soft HA hydrogels (~0.4 kPa) that showed a non-proliferative (dormant) phenotype exhibited resistance to Paclitaxel or Lapatinib. In contrast, cells cultured on stiff HA hydrogels (~4.5 kPa) that showed a proliferative phenotype exhibited responsiveness to Paclitaxel or Lapatinib. Moreover, dormancy-associated resistance was found to be due to upregulation of serum/glucocorticoid regulated kinase 1 (SGK1) gene which was mediated, in part, by the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Finally, we inhibited the expression of SGK1 using a SGK inhibitor (GSK650394) in BMBC cells cultured on soft HA hydrogels, which resulted in a dormant-to-proliferative switch and response to therapy. Overall, our study demonstrates that matrix stiffness influences dormancy-associated therapy response and that the p38/SGK1 axis, in part, mediates therapy response in our HA hydrogel platform.

9:21 AM - 9:33 AM

Quantitative Effects of Extracellular Matrix Composition on Breast Cancer Phenotype

Kyndra Higgins, University of Georgia

Kyndra Higgins; ksh01779@uga.edu, Cheryl Gomillion, Ph.D.; ctgomillion@uga.edu
School of Chemical, Materials, & Biomedical Engineering, University of Georgia

Detection of metastatic breast cancer (BC) will not occur until after the cancer has spread, resulting in delayed treatment. Therefore, it is critical to understand the mechanisms underlying metastasis for better detection of metastatic cancer. The role that extracellular matrix (ECM) composition plays on BC aggressiveness, separate from stiffness, is not fully understood. In the present work, eight mammary cell

lines were cultured and evaluated using an impedance-based assay system to elucidate the relationship between ECM composition, cell phenotype, and 2D migration. The long-term goal of this work is to improve current cancer assessment methods and determine a correlation between quantifiable impedance-based characteristics, 2D cell morphology, and cancer subtype.

Selected cells included two non-cancerous cell lines and six BC cell lines, five of which are triple-negative (ER-/PR-/HER2-), a highly aggressive and often metastatic subtype. Cells were cultured using a Maestro Tray-Z (Axion Biosystems). The plates were thinly coated in ECM protein (collagen I, collagen IV, fibronectin, laminin, Matrigel) prior to cell seeding and cell proliferation was monitored by impedance. At confluency, cell mediums were switched to a nutrient-reduced formula. Wells were scratched to create a wound and cell migration was monitored for 36 hours. A distinct difference in impedance was observed across all cell lines that can be linked to single-cell morphology and growth patterns. Three of the triple-negative BC cells showed wound closure under multiple ECM conditions, confirming cancer aggressiveness based on reported cell line characterization. ECM was also shown to affect cell-cell adhesion for five cancer cell lines. ECM composition was not shown to affect wound closure for any cell line evaluated, suggesting that the specific processes underpinning breast cancer outgrowth and epithelial-to-mesenchymal-transition are found outside of the cell-ECM protein relationship.

Our hypothesis that ECM composition affects cell behavior was supported, although ECM proteins did not affect 2D migration specifically. These findings demonstrate the feasibility of using an impedance-based assay to quantitatively characterize cancer cell migration, proliferation, and response to microenvironment. Overall, this work creates a foundation for our long-term goal of improving current assessment methods to yield a more detailed and objective evaluation of cancer metastatic behavior.

9:33 AM - 9:45 AM

Epigenetic modulation of malignant B cells by T cells in lymphoid cancers

Lucy Britto, Georgia Institute of Technology

Lucy S. Britto, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA, lucy.britto@gatech.edu

Deepali Balasubramani, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA, dbalasub3@gatech.edu

Sona Desai, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA, sonadesai@gatech.edu

Phunterion Phillips, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA, pphillips43@gatech.edu

Neev Trehan, St Richards Hospital, University Hospitals Sussex NHS Foundation Trust, Chichester, West Sussex, UK, neevtrehan@hotmail.com

Ethel Cesarman, Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, ecesarm@med.cornell.edu

Jean Koff, Winship Cancer Center, Emory University School of Medicine, Atlanta, GA, USA, jkoff@emory.edu

Ankur Singh, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA, Winship Cancer Center, Emory University School of Medicine, Atlanta, GA, USA. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, Georgia 30332 USA, ankur.singh@me.gatech.edu

Activated B cell-like diffuse large B-cell lymphoma (ABC-DLBCL) is an aggressive subtype of non-Hodgkin's lymphoma associated with poor prognosis. Despite new pharmacological targets identified though molecular profiling of ABC-DLBCL, clinical trials using target therapy have not benefited these patients. To improve therapeutic strategies, immune-competent tissue models are needed to understand how DLBCL cells evade or resist treatment. This study employed synthetic hydrogel-based lymphoma organoids to illustrate how cues from the lymphoid tumor microenvironment (Ly-TME) can impact B cell receptor (BCR) signaling and tri-methylation of histone 3 at lysine 9 (H3K9me3) to dampen the effects of BCR inhibition. Using imaging techniques, we showed T cells directly increased DNA methyltransferase 3A expression and cytoskeleton formation in neighboring ABC-DLCBL cells that was regulated by H3K9me3 expression. Using expansion microscopy on lymphoma organoids captured T cell-mediated increase in the size and quantity of spatially segregated H3K9me3 clusters in proximal ABC-DLBCL cells, suggesting restructuring of high-order chromatin structures that may be associated with novel transcriptional states. Treating ABC-DLBCL cells with an inhibitor of G9α histone methyltransferase prior to inhibition of the BCR pathway protein MALT1 reversed T cell-induced H3K9me3 upregulation and mitigated T cell-mediated the dampened treatment response to BCR pathway inhibition. This study underscores the need for biologically relevant tissue models to understand how Ly-TME signals can alter DLBCL progression, spatially and temporally, and suggests targeting both aberrant signaling pathways and epigenetic cross-talk could enhance treatment efficacy for high-risk patients.

9:45 AM - 9:57 AM

Effect of 3D Viscoelasticity on Endothelial-to-Mesenchymal Transition in Atherosclerosis

Ishita Jain, Stanford University

Ovijit Chaudhuri, Stanford University, chaudhuri@stanford.edu

Ngan Huang, Stanford University, ngantina@stanford.edu

Endothelial-mesenchymal transition (EndoMT) is a biological process in which vascular endothelial cells (ECs) acquire a mesenchymal identity that give rise to lineages like fibroblasts and smooth muscle-like cells. Pathologically, EndoMT is involved in the progression of numerous cardiovascular diseases such as atherosclerosis, in which ECs give rise to smooth muscle-like cells within the plaque. However, there is essentially a dearth of knowledge of how the biochemical and biomechanical cues from the extracellular matrix (ECM) milieu directly influence the progression of EndoMT. To address the limited knowledge of ECM effects on EndoMT, we propose to develop a 3D tissue engineered model with independently tunable biochemical and biomechanical cues, including stiffness, stress relaxation rate and ECM composition. The direct effects of biomechanical properties on EndoMT are difficult to study in vivo, owing to complex

microenvironmental factors of soft tissues. In contrast, in vitro platforms allow the study of biomechanical and associated mechanosignaling pathways in a well-controlled environment. The main research objective was to encapsulate primary human aortic endothelial cells into tunable viscoelastic 3D alginate hydrogels and study EndoMT progression in 3D. To study this, we optimized alginate hydrogels properties to have modular mechanical and viscoelastic properties. Primary human coronary artery endothelial cells were co-cultured with human fibroblasts to get a blood vessel network in the 3D alginate hydrogels. Additionally, we observed viscoelasticity dependent migration of ECs into the alginate hydrogel. Lastly, we quantified higher SM22 expression (EndoMT) in ECs in fast relaxing hydrogels, pointing towards viscoelasticity dependent progression of EndoMT. These results underscore the importance of mechanical factors such viscoelasticity in addition to stiffness in progression of diseases like atherosclerosis. Future work involves performing transcriptomics analysis to uncover the mechanism behind viscoelasticity dependent EndoMT progression.

SESSION VII: IMMUNE ENGINEERING 2

10:15 AM - 10:40 AM

Incorporating covalent crosslinks into immune constructs to enable co-delivery of distinct immunomodulatory cues

Marian Ackun-Farmmer, Georgia Institute of Technology

Christopher M. Jewell1,2, cmjewell@umd.edu

1.Fischell Department of Bioengineering, University of Maryland, College Park, MD; 2.US Department of Veterans Affairs, VA Maryland Health Care System, Baltimore, MD.

Multiple sclerosis occurs when autoreactive lymphocytes attack the protective myelin sheath surrounding neurons in the central nervous system. Current MS treatments are non-curative and cannot distinguish between healthy and diseased immune cells, motivating the need for new strategies to treat the disease. Toll-like receptors (TLRs), which traditionally recognize pathogen-associated molecular patterns (PAMPs), are upregulated in MS patients. Thus, an emerging therapeutic strategy is to deliver TLR antagonists and self-antigens, to achieve immune tolerance without broad immunosuppression. Herein, we developed covalently cross-linked carriers built entirely from immune cues – termed immune polyelectrolyte multilayers (iPEMs) to co-deliver self-antigen - myelin oligodendrocyte glycoprotein (MOG) and TLR9 antagonist - GpG. We pursued the co-delivery of Rapamycin (Rapa), a small molecule drug well-documented to expand TREGS, simultaneously to target innate and adaptive immune cells involved in MS. Specifically, we hypothesized that Rapa loading could be achieved in iPEMs, and covalent cross-links could be used to stabilize MOG and GpG cargo without disrupting cargo loading and impeding Rapa loading.

iPEMs synthesized using 3-bilayer bilayer depositions of MOG and GpG were incubated with varying glutaraldehyde (GA) cross-linking solutions to achieve covalent cross-links prior to removal of the sacrificial template. We found that cross-linking density could be modulated by adjusting GA concentration and incubation times; without impacting the functional capabilities of iPEMs. We importantly show comparable loading of small molecule Rapamycin between non-cross-linked iPEMs. Using an adoptive transfer approach, we show that myelin-specific T cells respond to cross-linked and non-crosslinked designs comparatively. Altogether, this work shows a proof-of-concept design to simultaneously engage innate and adaptive immune cells to drive robust immune tolerance in the context of MS and can be generally adapted to the autoimmune diseases.

10:40 AM - 10:52 AM

Albumin hitchhiking nanobody-STING agonist conjugates to improve immunotherapy

Neil Chada, Vanderbilt University

Neil C. Chada1 2 (neil.chada@vanderbilt.edu), Jonah Finkelstein3 (jonah.e.finkelstein@vanderbilt.edu), Alex Kwiatkowski1 (alexander.kwiatkowski@vanderbilt.edu), Vijaya Bharti3 (vijaya.barti.lnu@vanderbilt.edu), Blaise R. Kimmel3 (blaise.kimmel@vanderbilt.edu) , John T. Wilson1 3 (john.t.wilson@vanderbilt.edu)

1 Department of Biomedical Engineering, Vanderbilt University School of Engineering

2 Medical Scientist Training Program, Vanderbilt University School of Medicine

3 Department of Chemical and Biomolecular Engineering, Vanderbilt University of School of Engineering

While immunotherapy has revolutionized cancer treatment, the tumor microenvironment (TME) limits the efficacy of therapies CAR-T cells especially in solid tumors. To combat the immunosuppressive nature of the TME, we focused on targeting the stimulator of interferon genes (STING) pathway. We developed an “albumin hitchhiking” strategy that leverages an anti-albumin nanobody to which drugs can be conjugated and delivered.

We first characterized the anti-albumin, anti-B7H3, and anti-albumin/anti-B7H3 fusion nanobody using mass spectrometry. Next, the ability to conjugate payloads to nanobodies was tested by conjugating Cy5, a fluorescent molecule, to each nanobody and confirmed using SDS-PAGE as well as mass spectrometry. For initial studies, we selected only the anti-albumin nanobody conjugated to Cy5 (a fluorescent dye) to assess pharmacokinetics and biodistribution. We observed an increased half-life of approximately twentyfold compared to a control nanobody against GFP administered alone. Further, the protein conjugate was highly localized to the tumor compared to other internal organs. The activity of the protein conjugates when linked to diABZI was tested in vitro on A549D and THP1D (IFN-ß reporter lines), which showed increased expression of interferon-beta (IFN-ß), an inflammatory marker. Furthermore, in an in vivo breast cancer model, bulks tumors were processed analyzed via PCR to show an increase in inflammatory markers that promote anti-tumor responses. Flow cytometry analysis in this same model showed an increase in CD8+ T cells and within that population an increase in CD69 expression. An in vivo survival study in a subcutaneous breast cancer mouse model showed a decrease in tumor burden. In a less immune responsive model of neuroblastoma, tumor growth was similarly attenuated when treated with anti-albumin nanobody drug conjugates as well as the fusion anti-albumin and ant-B7H3 conjugate compared to controls. Current studies are underway to determine the effect of combining our treatment with CAR-T cell therapy in an immunosuppressive neuroblastoma model. Further, we will perform flow cytometry to determine the extent of immune reprogramming in the tumor post-treatment and identify changes in CAR T phenotype when administered with a STING agonist. Taken together, this data indicates potential to improve efficacy of already existing therapies for immunosuppressive solid tumors.

10:52 AM - 11:04 AM

Controlling Naïve T Cell Fate with miRNAs using Cationic Polymer-Functionalized Nanowires

Shaylyn Grier, Georgia Institute of Technology

Zhonghao (Eric) Dai, Georgia Tech, zdai43@gatech.edu
Ankur Singh, Georiga Tech, ankur.singh@me.gatehc.edu

T cell-based therapies leverage the phenotypic plasticity of T cells and responsiveness to microenvironmental cues. Manufacturing T cells from naïve or stem-like memory T lymphocytes can enhance antitumor and anti-viral responses while reducing cytokine release syndrome. However, it is

extremely difficult to deliver genetic material to naïve T cells and direct differentiation towards beneficial phenotypes such as stem-like memory (TSCM) and effector (TEFF)) during ex vivo T cell engineering processes. Consequently, conventional processes hinder long-term therapeutic efficacy and can result in early-onset T cell exhaustion. Here, we present a cationic polymer-functionalized nanowire technology, with 20 nm tip size, for efficiently delivering T cell fate-determining miRNAs to naïve mouse and human T cells, therefore bypassing the need for prior activation of naïve T cells using T cell receptor (TCR)-stimulating antibodies or cytokines. Covalent conjugation of cationic polymer Polyethyleneimine (PEI) to a silicon nanowire surface enabled the adsorption of miR-23a, miR-28, and miR-130, which modulate T cell functions, and pre-program phenotypic and functional trajectory during ex vivo differentiation. After genetic pre-programming, primary naïve human or murine T cells were activated with TCR-stimulating antibodies. Flow cytometry revealed that the nanowire platform delivered miR-29-mimic to naive murine and human T cells with >90% efficiency and viability, surpassing lentiviral, nucleofection, and lipofectamine approaches. A single delivery of 6-FAM-miR-29-mimic downregulated transcriptional targets T-bet and Eomes while increasing CD62L expression, maintaining less-differentiated T cell phenotypes. Additionally, we have demonstrated codelivery of 6-FAM-miR-29-ASO and APC-miR-130-mimic to non-activated murine T cells, resulting in additional increases in CD45RA+CD62L-CCR7- TEFF cell yield and proinflammatory cytokine expression (i.e., perforin, granzyme A and B, IFNγ, and IL-2). Interestingly, delivery of a distinct modulator of T cell exhaustion, miR-23a, biased T cell fate towards a significantly higher % CD45RA+ CD62L+ TSCM and induced rapid ex-vivo expansion. Our findings demonstrate a unique ability of perturbing multiple regulatory pathways in naïve T cells using a biomaterials-based nanoscale technology to modulate T cell phenotype and function for therapeutic T cell production.

11:04 AM - 11:16 AM

Macromolecular Polymeric STING Agonists for Cancer Immunotherapy Applications

Jacob Schulman, Vanderbilt University

Karan Arora, Vanderbilt University, karan.arora@vanderbilt.edu
John Wilson, Vanderbilt University, john.t.wilson@vanderbilt.edu

Cancer immunotherapies have revolutionized the treatment of many cancer types, offering patients hope for complete tumor regression and life-long immunological memory to prevent disease recurrence. One promising cancer immunotherapy target is the Stimulator of Interferon Genes (STING) pathway, as it is a crucial link between innate and adaptive anti-tumor immunity. Activation of the STING protein triggers a type-I interferon (IFN-I) inflammatory response which provides the proper immunological context to activate antigen presenting cells (APCs), T cells, and natural killer cells, leading to potent anti-tumor responses. Unfortunately, local and systemic administration of endogenous STING agonists (e.g., 2’3’-cGAMP) are limited by poor stability and cytosolic delivery, which has prompted the development of non-nucleotide, synthetic STING agonists. Of the non-nucleotide STING agonists in use, recently described diamidobenzimidazole (diABZI) compounds have been shown to facilitate potent STING activation and anti-tumor activity but they pose significant formulation and administration challenges due to their hydrophobicity and short half-life, respectively. Consequently, we have developed reversible addition-fragmentation chain transfer (RAFT) polymerization-compatible diABZI-based chain transfer agents (CTAs)

and have leveraged these to create macromolecular polymeric STING agonists for systemic cancer immunotherapy and neoantigen peptide cancer vaccine applications. Herein, we have created a library of N,N-dimethylacrylamide (DMA)-based polymeric constructs using diABZI-CTAs with cleavable and non-cleavable linkers. We see that STING agonism from the polymeric constructs is independent of molecular weight, but dependent on linker cleavability, as polymers synthesized with cleavable diABZI-CTAs demonstrate robust STING activation and antitumor activity in murine cancer models. Additionally, we created a polymeric neoantigen peptide vaccine platform with the diABZI-CTA system through the copolymerization of DMA and pyridyl disulfide ethyl acrylamide (PDSMA), which enables peptides containing an N-terminal cysteine to be conjugated to the polymer via a disulfide exchange reaction. We synthesized vaccine constructs containing synthetic long peptide variants of MHC-I and MHC-II-restricted ovalbumin model neoantigens and found these constructs were able to significantly outperform soluble mix controls in antigen presentation and T cell priming both in vitro and in vivo.

11:16 AM - 11:28 AM

PEGylation of Indoleamine 2,3-Dioxygenase for Systemic Immune Regulation

Jennifer Simonovich, University of Florida

Jennifer A. Simonovich: University of Florida, jsimonovich@ufl.edu
Sabrina Macias: University of Florida, slfmacias@gmail.com
Dorina Avram: Moffitt Cancer Center, Dorina.Avram@moffitt.org
Benjamin G. Keselowsky: University of Florida, BKESELOWSKY@bme.ufl.edu

Indoleamine 2,3, dioxygenase (IDO), the rate-limiting enzyme of tryptophan catabolism, is anti-inflammatory in many inflammatory and autoimmune diseases. Exogenously delivered IDO is immunosuppressive, allowing dendritic cells to maintain an immature state. These properties make IDO an attractive protein therapy candidate. However, it faces quick clearance in vivo. Coupling poly(ethylene glycol) (PEG) with IDO creates a modified protein-polymer complex that persists in circulation while maintaining its enzymatic function, resulting in systemic immunosuppression. To conjugate the PEG polymer to the protein, PEG chains with a single maleimide reactive group were combined with IDO. Size of the PEG-IDO product was varied by increasing the length of the PEG chain. To determine circulation time, PEG-IDO was injected intraperitoneally; IDO in blood was analyzed via blood draws and quantified via Western blot, using an exponential decay curve to determine circulation half-life. A mouse model for psoriasis was used to determine therapeutic effects of systemically delivered PEG-IDO. Disease state was assessed via clinical scoring of erythema, scaling, and skin thickening. Mice were treated on day 3 and day 8 as needed. PEG-IDO had a greater half-life than IDO alone, and increasing the PEG chain length increased the half-life – 10kDa PEG-IDO persisted to 5 days in circulation and 30kDa PEG-IDO showed no drop in circulation levels out to 7 days, while WT IDO was barely detectable even 2 hours post injection. Mice treated with two doses of 10kDa PEG-IDO showed lower cumulative scores from day 5 onwards as compared to saline treated mice, while mice treated with 30kDa PEG-IDO only required one dose to achieve similar decrease in scores. This showed that treatment with longer persisting PEG-IDO formulation is more effective than the same treatment with a shorter persisting formulation

SESSION VIII: BIOINTERFACES

1:45 PM – 2:10 PM Invited Speaker: Juhi Samal, University of Alabama ant Birmingham

2:10 PM - 2:22 PM

Copper coated nanotextured stainless steel for antibacterial application

Anuja Tripathi, Georgia Institute of Technology

Julie A. Champion

julie.champion@chbe.gatech.edu

Georgia Institute of Technology

Bacterial adhesion to commonly shared surfaces can lead to severe infections, posing a risk of mortality and significant healthcare costs. Antibiotics have addressed this concern to a certain extent, but continuous usage of antibacterial agents can lead to development of drug-resistant bacteria which caused approximately 1.27 million deaths worldwide in 2021. Various studies have explored non-chemical methods of killing Gram-negative and Gram-positive bacteria. However, the outer membrane of Gram-negative bacteria makes them particularly resilient. Hence, there is a significant need to develop antibacterial surfaces effective for both Gram-positive and Gram-negative bacteria. Some of the conventional approaches include antifouling materials, which often contain toxic compounds as coating such as tributyltin and dichlofluanid. Additionally, nanotextured polymers, metals, and metal oxides have been explored for their potential in antibacterial surfaces. While these materials have shown efficacy against Gram-positive bacteria, they fall short against Gram-negative strains. Hence, there is a need to create an affordable antibacterial surface for shared environments which can kill Gram-negative and Gram-positive bacteria without promoting drug resistance.

Stainless steel 316L (SS316L) is widely used in public settings, including sinks, toilets, surgical tools, and cardiovascular and orthopedic implants, owing to its favorable mechanical strength, corrosion resistance, and biocompatibility. In this study, we have demonstrated nanotextured stainless steel (nSS) fabrication followed by Cu coating using an electrochemical technique and its potential as an antibiotic-free biocidal surface against Gram-positive and negative bacteria. The synergistic effects of nSS and Cu provide a promising avenue for combating bacterial infections without contributing to drug resistance. The dual antibacterial activity rises due to the small grooves and ridges on the nanotextured surface, which make it difficult for bacteria to attach and spread, and release of copper ions that kill or inhibit the growth of bacteria. Our method involves the application of a copper coating on nanotextured stainless steel, resulting in significant antibacterial activity within 30 minutes. Cu-coated nSS demonstrated a remarkable reduction of 97% in Gram-negative and 99% in Gram-positive bacteria. Overall, our material holds promise for developing effective, scalable, and sustainable solutions to mitigate bacterial infections stemming from surface contamination, all without exacerbating drug resistance issues.

2:22 PM - 2:34 PM

The Impact of Porous Scaffold Geometry on Host Responses In Vivo

Taylor Lansberry, University of Florida

TR Lansberry (taylorlansberry@ufl.edu), University of Florida;

RP Accolla (rpaccolla@gmail.com), University of Florida;

I Labrada-Miravet (ilabrada@ufl.edu), University of Florida;

J Walsh (justinwalsh@ufl.edu), University of Florida;

C Crouse (cameroncrouse@ufl.edu), University of Florida;

JP Liang (limeon.q@gmail.com), University of Florida;

CL Stabler (cstabler@bme.ufl.edu), University of Florida

Cell-based therapies, such as 3D porous scaffolds, are a promising treatment for many diseases, whereby the scaffold mechanically supports and distributes donor cells while facilitating host engraftment. The optimal scaffold geometry to fulfill these key objectives has yet to be identified. Published studies have found correlations between global pore size and foreign body responses, yet the results are contradictory and confounded by material degradation in vivo. To overcome this, we have created a porous 3D-printed, biostable polydimethylsiloxane (PDMS) scaffold, which can provide control over implant geometry to clearly interrogate the impact of geometrical features on host responses to the implant. These scaffolds are fabricated using a reverse cast method that involves integrating PDMS into a 3D-printed sacrificial mold, curing overnight, and removing the mold to leave a flexible scaffold. Three distinct prototypes were modeled with PDMS rung thicknesses of (300, 200, or 150 µm in x-y; all 100 µm in z) and uniform pore size (300x300x100 µm; x-y-z). Actual feature sizes were validated using brightfield imaging and ImageJ. These scaffolds were transplanted into the epididymal fat pad of C57BL/6 mice before explantation; intra-device vascularization was visualized with tomato lectin staining and host engraftment with trichrome staining. Prototype images showed significant differences in scaffold rung thickness ($p < 0.0001$) with uniform pore sizes ($p = 0.082$). In vivo, scaffolds with thicker rungs exhibited increased collagen deposition and fibrotic features than scaffolds comprised of thinner rungs. Promising scaffolds (150x150x100 µm rung thickness) were then loaded with allogeneic islets within a fibrin hydrogel and transplanted into the omentum of diabetic Lewis rats. Optimized 3D printed scaffolds were compared to PDMS scaffolds fabricated using traditional particulate leaching methods. In our diabetic model, both scaffold fabrication methods reversed diabetes; however, the time to normoglycemia ($p = 0.0189$) and risk of rejection ($p = 0.0351$) was significantly lower with the 3D-printed scaffold, demonstrating the impact of geometric features on transplant efficacy. In conclusion, geometric scales can shift host responses from favorable to deleterious and impact transplant outcomes. Future work will identify which cells are driving these variable responses using digital spatial profiling.

2:34 PM - 2:46 PM

Engineering Nitric Oxide-releasing Antimicrobial Dental Coating for Targeted Gingival Therapy

Natalie Crutchfield, University of Georgia

Manjyot Kaur Chug, University of Georgia, manjyot@nytricx.com

Mark Garren, University of Georgia, mgarren@uga.edu

Hitesh Handa, University of Georgia, hhanda@uga.edu

Elizabeth Brisbois, University of Georgia, ejbrisbois@uga.edu

Bacterial biofilms play a central role in the development and progression of periodontitis, a chronic inflammatory condition affecting the oral cavity. One solution to current treatment constraints is using nitric oxide (NO) – with inherent antimicrobial properties. In this study, an antimicrobial coating is developed from the NO donor S-nitroso-N-acetylpenicillamine (SNAP) embedded within polyethylene glycol (PEG) to prevent periodontitis. The SNAP-PEG coating design enabled controlled NO release, achieving tunable NO levels for more than 24 h. Testing the SNAP-PEG composite on dental floss showed its effectiveness as a uniform and bioactive coating. The coating exhibited antibacterial properties against Streptococcus mutans and Escherichia coli, with inhibition zones experiments. Furthermore, SNAP-PEG coating materials were found stable when stored at room temperature, with 93.65% of SNAP remaining after 28 d. The coatings were biocompatible against HGF and hFOB 1.19 cells through a 24 h controlled release study. This study presents a facile method to utilize controlled NO release with dental coatings comprising SNAP-PEG. This coating can be easily applied to various substrates, providing a user-friendly approach for targeted self-care in managing gingival infections associated with periodontitis.

2:46 PM - 2:58 PM

Shape-shifting protein nanostructures driven by molecular juxtaposition of opposing phase-separation grammars

Jeremy Hannon, Emory University

Jeremy Hannon, Maria Camila Giraldo Castano, Alexa Regina Chua Avecilla, Felipe Garcia Quiroz.

Intrinsically-disordered proteins (IDPs) exhibit environmentally-sensitive conformational dynamics and phase transitions that drive the formation and dynamics of protein assemblies across biological systems. Temperature-responsiveness distinguishes phase transitions above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST). IDP polymers (IDPs) with these opposing behaviors are known to have distinct sequence grammars. However, the interplay of these opposing grammars in multi-domain IDPs and IDPPs is poorly understood. We and others previously showed that UCST-LCST diblock IDPPs can be programmed as nanoparticles that undergo disassembly upon heating. Unexpectedly, the phase behavior of the UCST-IDPP domain was strongly influenced by molecular juxtaposition of the LCST-IDPP, as evidenced by dramatic shifts in the expected critical temperature for nano-assembly. This behavior contrasted with the highly predictable critical temperature for assembly of LCST-LCST diblock IDPPs. To understand how opposing phase-separation grammars interact to dictate the

overall phase behavior, here we recombinantly synthesized and characterized a library of novel UCST-LCST diblock IDPPs. These designs explored the molecular-level fusion of three UCST IDPPs of progressive hydrophobicity but fixed length, and two LCST IDPPs of increasing hydrophilicity and length. Excitingly, temperature-dependent UV-visible absorbance measurements showed that UCST-LCST diblocks in our library often exhibited a rarely-seen band-pass phase behavior marked by dual UCST and LCST transitions (LCST>UCST). Select diblock IDPPs transitioned from nanoparticles into micron-sized assemblies exclusively in a narrow temperature range (~10-15°C), before dispersing into single-chain IDPPs at higher temperatures. Across our designs, the hydrophobicity of UCST-IDPP domains tuned the LCST-type transition and set the width of the band-pass regime. Surprisingly, UCST-LCST IDPPs with short LCST blocks formed nanoparticles at low temperatures that rapidly reconfigured through an LCST-type transition within physiologically relevant conditions. At higher temperatures, these diblock IDPPs also showed band-pass behavior. The shape-shifting regime was highly reversible and Cryo-TEM images showed that the LCST-triggered shape-shifts involved monodisperse nanoparticles. Unlike folded proteins with well-insulated modules, our findings suggest that the phase behavior of multi-domain IDPs results from protein-level integration of the juxtaposed phase-separation grammars. The discovery of shape-shifting UCST-LCST nanoparticles will stimulate the integration of complex phase separation grammars in IDP engineering and nanotechnology.

SESSION IX: DRUG DELIVERY 2

3:00 PM - 3:25 PM

Advancing β-adrenoreceptor agonism for recovery after volumetric muscle loss through biomaterial delivery

Jarrod Call, University of Georgia

Jennifer McFaline-Figueroa(1), Christiana J. Raymond-Pope(2), Joseph J. Pearson(3), Albino G. Schifino(1), Junwon Heo(1), Thomas J. Lillquist(2), Emma E. Pritchard(2), Elizabeth A. Winders(1), Edward T. Hunda(1), Johnna S. Temenoff(3,4), Sarah M. Greising(2), Jarrod A. Call(1,5)

(1) Department of Physiology and Pharmacology, University of Georgia, Athens, GA 30602, USA

(2) School of Kinesiology, University of Minnesota, Minneapolis, MN 55455, USA

(3) W.H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30032, USA

(4)Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA

(5)Regenerative Bioscience Center, University of Georgia, Athens, GA 30602, USA

Volumetric muscle loss (VML) injury results in the unrecoverable loss of muscle mass and strength. The β2-adrenergic receptor agonist formoterol produces a modest recovery of muscle mass and strength when delivered orally in VML-injured mice. The objective of this study was to determine if a regenerative medicine paradigm could enhance the recovery of VML-injured muscle. Regenerative medicine involved direct delivery of formoterol to VML-injured muscle using a nonbiodegradable poly(ethylene glycol) biomaterial. Preliminary biomaterial characterization included batch-to-batch size optimization, in vitro formoterol release analysis, visual and histological evaluation of the biomaterial residence within the muscular compartment. Based on the preliminary findings, VML-injured mice were randomized to receive a single intramuscular biomaterial injection at 1-month post-injury with either empty or with formoterol. Muscle mass, contractile function, metabolic function, and histological evaluations were used to determine if the regenerative medicine approach was effective at 2-months post-injury. The regenerative medicine model produced greater permeabilized muscle fiber mitochondrial respiration and electron conductance through the electron transport system compared to untreated VML-injured mice; however, the non-biodegradable biomaterial was associated with lower muscle quality (i.e., lower muscle mass-normalized contractility) and fewer total muscle fibers. The conclusions reached from this study are: i) regenerative medicine strategies utilizing formoterol require further optimization (e.g., biodegradable optimization) but showed promising outcomes; and ii) in general, β-adrenergic receptor agonism continues to be a physiologically-supportive intervention to improve muscle metabolic function after VML injury.

3:25 PM - 3:37 PM

Sustained Release of siRNA from Antioxidant Polymer Microparticles for the Treatment of Osteoarthritis

Patricia Poley, Vanderbilt University

Carlisle R. Dejulius, University of Michigan, cdejuliu@med.umich.edu

Craig L. Duvall, Vanderbilt University, craig.duvall@vanderbilt.edu

When reactive oxygen species (ROS) levels exceed the body's natural antioxidant abilities, cells experience oxidative stress, which has been attributed to the progression of degenerative diseases, such as osteoarthritis (OA). One factor attributed to the cause of OA, age, triggers cells to become senescent, a state in which replication is ceased but the cell does not die. Senescent cells secrete the SASP (senescence-associated secretory phenotype) which includes inflammatory cytokines, proteases, and growth factors that influence the behavior of nearby cells, contributing to OA progression. A common senescence target, Cdkn2a, encodes for p16 and p19, two cell cycle inhibitor proteins. We hypothesized that knockdown of Cdkn2a could inhibit cells from becoming senescent, decreasing SASP factors, and reducing joint damage caused by post traumatic osteoarthritis (PTOA).

Previous delivery systems have utilized PLGA microparticles (MPs), however these have been shown to cause negative immune responses in vivo. Instead, poly(Propylene sulfide) (PPS) has been shown to be inherently antioxidant. Due to its amorphous nature, MP formation with PPS yielded particles of small size (~2um). To yield MPs of larger size, PPS was copolymerized with ethylene sulfide (PPSES), a more crystalline polymer. PPSES was synthesized via an anionic ring opening polymerization with molar ratios of the two monomers ranging from 0-50% ES. Polymers with greater than 40% ES exhibited enough crystallinity to be formulated into MPs. Specifically, 50:50 PPSES formed large, stable MPs, making it an ideal candidate for sustained intra-articular drug release in OA.

Chemically-stabilized siRNA lipid conjugates were loaded into 50:50 PPSES MPs via solid/oil/water emulsion. A cyclic repeat loading model was used to induce PTOA in mice via bilateral loading. On day zero, particles were injected intravenously into mice at 1 mg PPSES/5.5 ug siRNA. After four weeks, tissues of interest were harvested for qPCR. Free siRNA conjugates failed to have significant knockdown of Cdkn2a. In contrast, PPSES-siCdkn2a MPs achieved ~68% knockdown compared to a control sequence in joint tissues, indicating sustained efficacy with MP delivery. Future work will investigate phenotypic joint changes with sustained Cdkn2a knockdown to further characterize the effect of siRNA-MPs in PTOA.

3:37 PM - 3:49 PM

Mucosa-targeted Inflammatory Bowel Disease Therapeutics for Localized Intestinal Immunosuppression

Anna Davis, Georgia Institute of Technology

Anna M. Davis: adavis359@gatech.edu, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

Coauthors:

Ayowole Akinsola: aakinsola6@gatech.edu

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

Paul Dawson, Ph.D., FAASLD: paul.dawson@emory.edu

Division of Gastroenterology, Hepatology and Nutrition, Emory University School of Medicine

Leslie W. Chan, Ph.D.: leslie.chan@gatech.edu

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, 30332, USA

Background: Crohn's disease (CD) is an inflammatory bowel disease mainly affecting the small intestine. Immunosuppressive drugs, including TNF-α-neutralizing antibodies and small molecule immunomodulators, are the cornerstone of CD treatment and are taken for months to years. However, widespread drug biodistribution after intravenous (IV) administration leads to systemic immunosuppression and increased risk for severe infections. Targeted delivery of CD drugs to the inflamed intestinal mucosa is needed to improve drug efficacy and localize immunosuppression to minimize off-target effects. Additionally, sustained release formulations are needed to improve medication compliance. To address these needs, we have developed a new delivery platform for sustained and targeted CD drug delivery to the intestinal mucosa that couples two components: (1) mucosa-homing bile acid (BA)-drug conjugates and (2) biodegradable microspheres for drug depot formation after intramuscular (IM) injection.

Methods: BAs are produced in the liver and released into the small intestine for lipid absorption. BA transporters facilitate efficient BA recycling, enabling reabsorption from the small intestine into blood circulation and uptake of circulating BAs back into the liver. To determine if hepatobilliary BA transporters could be leveraged for intestinal delivery, a model cargo (fluorescein) was conjugated to cholic acid (CA), a primary BA. CA-fluorescein biodistribution was evaluated in mice via IVIS imaging after IV and IM injection. To assess utility of biodegradable microspheres for sustained intestinal delivery, oil-in-water emulsion evaporation methods were used to encapsulate CA-fluorescein in poly(lactic-co-glycolic) acid microspheres for biodistribution studies.

Results: CA-fluorescein conjugates homed to the small intestine within minutes after IV/IM injection. Fluorescence signal was strongest in the small intestine with signal also appearing in the gallbladder, the organ that stores BAs before their release in the small intestine. Altogether, this supports the proposed mechanism of circulating conjugate uptake via hepatobiliary BA transporters for subsequent intestinal delivery. Small intestine signal was absent by 4h. For prolonged delivery, CA-fluorescein was successfully encapsulated in 30-µm diameter microspheres. Biodistribution studies are underway and formulations will be optimized for up to 2-week long release. CA conjugates with the small molecule immunomodulator, methotrexate, have been synthesized more recently to assess this delivery system using a CD drug cargo.

3:49 PM - 4:01 PM

Emulsion-Induced Polymersomes Taming Tetrodotoxin for Prolonged Duration Local Anesthesia

Chao Zhao, The University of Alabama

Chao Zhao, The University of Alabama, czhao15@eng.ua.edu

Injectable local anesthetics that can provide a continuous nerve block approximating the duration of a pain state would be a life-changing solution for patients experiencing post-operative pain or chronic pain. Tetrodotoxin (TTX) is a site 1 sodium channel blocker that is extremely potent compared to clinically used local anesthetics. Challengingly, TTX doses are limited by its associated systemic toxicity, thus shortening the achievable duration of nerve blocks. Here, emulsion-induced polymersomes (EIP) are explored as a drug delivery system to safely use TTX for local anesthesia. By emulsifying hyperbranched polyglycerol-poly (propylene glycol)-hyperbranched polyglycerol (HPG-PPG-HPG) in TTX aqueous solution, HPG-PPG-HPG self-assembles into micrometer-sized polymersomes within seconds. The formed polymersomes have microscopically visible internal aqueous pockets that encapsulate TTX with an encapsulation efficiency of up to 94%. Moreover, the polymersomes are structurally stable, enabling sustained TTX release. In vivo, the freshly prepared EIP/TTX formulation can be directly injected and increase the tolerated dose of TTX in Sprague–Dawley rats to 11.5 μg without causing any TTX-related systemic toxicity. In the presence of the chemical penetration enhancer sodium octyl sulfate (SOS), a single perineural injection of EIP/TTX/SOS formulation produces a reliable sciatic nerve block for 22 days with minimal local toxicity.

4:01 PM - 4:12 PM

Albumin-binding nanobody-antigen fusions enhance antigen presentation and improve vaccine responses through pharmacokinetic modulation

Hayden Pagendarm, Vanderbilt University

Neil Chada (neil.chada@vanderbilt.edu) - Vanderbilt Unviersity

Alexander Kwiatkowski (alexander.kwiatkowski@vanderbilt.edu) - Vanderbilt University

Jonah Finkelstein (jonah.e.finkelstein@vanderbilt.edu) - Vanderbilt University

Taylor Patino (taylor.m.patino@vanderbilt.edu) - Vanderbilt University

Christopher Lofts (christopher.a.lofts@vanderbilt.edu) - Vanderbilt University

Blaise Kimmel (kimmel.85@osu.edu) - The Ohio State University

Karan Arora (karan.arora@vanderbilt.edu) - Vanderbilt University

John T. Wilson (john.t.wilson@vanderbilt.edu) - Vanderbilt University

Peptide vaccines hold the potential to treat many diseases. These therapies utilize the exact antigen epitopes (~2 kDa) that are presented by major histocompatibility complexes (MHCs). Unfortunately, the efficacy of peptide-based vaccines has been limited due to inefficient trafficking to the draining lymph

node (dLN), rapid renal clearance, susceptibility to proteolysis, and presentation in an inappropriate context without the spatiotemporal co-delivery of an adjuvant. To address this, we have developed a platform for peptide antigen delivery using a nanobody (Nb) targeting mouse serum albumin (MSA). Nbs are alpaca-derived single-domain antibodies of small size (~12-15 kDa) and high target specificity. About ten times smaller than conventional antibodies (Abs), Nbs exhibit efficient tissue penetration and clear rapidly when unbound to their target, making them effective delivery vehicles for conjugated therapeutic cargos. We hypothesized that the fusion of peptide epitopes with a Nb targeting MSA (69 kDa), termed nAlb, would enhance antigen draining to the lymphatics by surpassing the renal clearance cutoff (~40 kDa). In this work, we synthesized fusions of nAlb and relevant peptide antigens. For stimulatory vaccines, we adjuvanted using a conjugate of nAlb and the STING agonist diamidobenzimidazole (diABZI). For pharmacokinetic and biodistribution studies, an nAlb-Cy5 conjugate was generated. Conjugates were generated using a combination of sortase and SPAAC reactions. Serum half-life was determined by treating mice with 2 nmol equivalent Cy5, collecting blood at indicated timepoints, and quantifying the Cy5 concentration via fluorescence spectroscopy. Ex vivo biodistribution, demonstrating accumulation in the draining lymph node (dLN), was determined by sacrificing mice 24 h post Cy5 injection and imaging the dLNs and major clearance organs using IVIS. Cy5 uptake by cell type in the spleen and dLN was determined using flow cytometry. Treatment with nAlb-OVA251-270 and nAlb-diABZI generated robust antigen specific immunity, quantified using a tetramer stain, that improved therapeutic responses in both prophylactic and therapeutic vaccination models of B16.F10 melanoma. Additionally, as robust antigen presentation absent co-stimulation drives tolerance, treatment of mice with nAlb-MOG35-55 inhibited the clinical onset of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS).

SESSION X: TISSUE ENGINEEREING 2

4:15 PM – 4:40 PM **Invited Speaker: Daniel Abebayehu, UVA**

4:40 PM - 4:52 PM

Guiding Early Cell Mechanoresponse and Precise Matrix Deposition for Anisotropic Meniscus Tissue Engineering

Saitheja (Adi) Pucha, Emory University

Saitheja A. Pucha(1,2)*, Maddie Hasson(1,2), Hanna Solomon(1,2), Gail E. McColgan(1,2), Jennifer L. Robinson(3), Sebastian L. Vega(4,5), Jay M. Patel(1,2)
1.Atlanta VA Medical Center, Decatur GA. 2.Department of Orthopaedics, Emory University, Atlanta GA.
3.Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, WA.
4.Department of Biomedical Engineering, Rowan University, Glassboro NJ.
5.Department of Orthopaedic Surgery, Cooper Medical School of Rowan University, Camden, NJ.

adi.pucha@emory.edu, maddie.hasson@emory.edu, hannasolomon166@gmail.com, gemccolgan@gmail.com, jrobins1@uw.edu, vegas@rowan.edu, jay.milan.patel@emory.edu

To replicate the aligned nature of the meniscus via tissue engineering, advances have been made in fiber-reinforcement for recapitulation of its circumferentially-oriented architecture. However, most studies focus on macro-scale aspects, neglecting to consider the micro-scale cell-biomaterial interactions that govern matrix deposition/assembly towards organized neo-tissue. During development of the meniscus, cell alignment precedes aligned tissue deposition, highlighting the need to consider early response. Since the spatial response of cells within a fiber-reinforced microenvironment likely influences architecture of tissue, our objective was to elucidate patterns of cell response within these microenvironments. We utilized cell shape and mechanoresponse parameters, applying machine-learning strategies to better uncover these trends. To simulate a fiber-reinforced microenvironment, suture fragments were embedded within fibrin gels containing marrow-derived cells. Constructs were cultured for 3 days supplemented with TGF-β3 and varying concentrations of aprotinin (anti-fibrinolytic to tune remodeling). Cell and nuclear shape parameters, YAP nuclear ratio, and distance from suture were measured for individual cells following culture. Principal component analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) was applied to cluster cells into 3 groups based on 23 cell parameters. By analyzing cell clusters identified by PCA-AHC, significant differences in cellular conformity and YAP nuclear localization were revealed, yielding clusters of High Response (HR), Medium Response (MR), and Low Response (LR) cells. Spatial patterns between clusters were evident, with a general trend of decreasing response with increasing distance from fiber. Modulation of matrix stiffness showed that stiffness influenced HR cell mechanoresponse, with softer environments allowing for greater response. Reduction of fibrin remodeling by aprotinin caused HR cells to localize closer to the suture, showing that the range of cell sensing can be influenced by remodeling of surrounding matrix. Together, this provides evidence that, in a fiber-reinforced microenvironment, matrix stiffness mediates cell response (“how”), while remodeling mediates localization of responding cells (“where”). This study demonstrated patterns of cell responsivity in fiber-reinforced microenvironments, which can be utilized to organize precise matrix deposition in scaffolds for meniscus replacement. Ongoing work aims to correlate these cell mechanoresponses to

composition of aligned matrix to improve design of meniscus scaffolds at the micro-scale, optimizing macro-scale tissue organization.

4:52 PM - 5:04 PM

Engineering Synthetic Human Immune Organoids to Recapitulate Humoral Immune Response

ZHE ZHONG, Georgia Institute of Technology

Zhe Zhong1,2,3, Manuel Q. Perez1,3, Zhonghao Dai1,3, Valeria M. Juarez1,3, Eshant Bhatia1,3, Ankur Singh1,2,3*

1Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, Georgia 30332 USA

2George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332 USA

3Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332 USA

In response to viral infection and vaccination, naïve B cells are activated and begin to proliferate with the aid of cytokines and co-stimulatory signals from helper T cells, and these cells terminally differentiate into plasma cells and memory B cells within the specialized areas of lymphoid tissues known as germinal centers (GCs). A robust B cell maturation is crucial for producing high-quality antibodies and establishing enduring humoral immunity. Unfortunately, reproducing the human B cell maturation process ex vivo is difficult, mainly because the intricate biological activities and signals within the lymphoid microenvironment that direct B cell maturation are not fully understood. Despite advances in technologies that focus on human secondary lymphoid organs, no ex vivo systems have demonstrated the influence of lymphoid tissue microenvironment. Here, we created synthetic hydrogels that recapitulate the lymphoid tissue environment and promote the formation of germinal centers using B cells from tonsils and peripheral blood mononuclear cells (PBMCs). Our findings show that poly(ethylene glycol) hydrogels with maleimide functionality (PEG-4MAL) maintain the life of human B cells and generate responses to both live and inactive influenza viruses, as well as to commercial vaccines. When comparing organoids derived from human tonsils and PBMCs, we found that the PBMC-derived organoids sustained the production of germinal center B cells and plasma cells over 24 days, resulting in the formation of light zones and dark zones and differential responses to adjuvanted H1N1 vaccines. Investigating the T cell populations within these organoids, we detected the engagement between follicular helper T cells and B cells, suggesting the T cells' supportive role in B cell maturation. Flow cytometry confirmed the longevity of follicular T cells in PBMC-derived organoids for up to 16 days. Moreover, bulk RNA sequencing showed that the origin of the cell (tonsil versus PBMC) regulated the longevity of B cell maturation in biomaterials-based immune organoids.

5:04 PM - 5:16 PM

The Impact of Provisional Matrix Remodeling on Cartilage Repair Microenvironments

Hanna Solomon, Emory University

Maddie Hasson (Emory University) maddie.hasson@emory.edu

Xander Patton (Emory University)

wpatton30@gatech.edu

Sameh Labib (Emory University)

slabib@emory.edu

Jay Patel (Emory University

jay.milan.patel@emory.edu

Focal cartilage injuries are a common musculoskeletal malady, which is a cause of concern due to its lack of ability to regenerate on its own. Repair methods like microfracture (MFx) rely on marrow recruitment to the site of injury, forming a provisional clot that is remodeled. Unfortunately, the inferior fibrocartilage that forms often leads to poor long-term outcomes. Our objective was to investigate early provisional matrix remodeling and its impact on MFx environments and their functional regeneration.

To understand in vivo contraction and fibrosis, full thickness trochlear defects were created in 3 rabbits, followed by microfracture. At one week, defects demonstrated considerable contraction (~50% defect fill) and expression of fibrotic markers (alpha smooth muscle actin, Collagen type I). To study this further, marrow-derived cells (MDCs) were isolated from juvenile bovine femoral condyles and encapsulated within fibrin gels. Microgels (10L) were fabricated in an 8-well chamber slide and cultured for 3 days with our without aprotinin (fibrinolysis inhibitor; 100 KIU/mL) and/or TGF-β3 (chondrogenic agent; 10ng/ml), fixed/stained (Phalloidin, a-SMA, YAP/TAZ, fibronectin), and imaged with confocal microscopy. TGF-β3 increased micro-scale contraction of the fibrin network and promoted fibrotic gene expression. Aprotinin mitigated both this fibrin remodeling and fibronectin deposition. Interestingly, aprotinin and TGF-β3 both increased YAP/TAZ nuclear localization in MDCs, with an additive effect when both were applied. Finally, macrogels (100L) were cultured for 7 days in chemically-defined media (control, aprotinin, TGF-β3, aprotinin+TGF-β3) and imaged at the terminal point to measure contraction. Terminal gene expression of urokinase-type plasminogen activator (uPa), a-SMA, and plasminogen-activator inhibitor (PAI-1) were performed. Aprotinin partially reduced TGF-β3-mediated contraction; however, aprotinin and TGF-β3 both increased pro-fibrotic expression, a result that was additive in combination.

In summary, microfracture exhibited contraction and fibrosis as early as one week, and the addition of TGF-β3 led to increased fibrinolysis, contraction, and early fibrosis. Aprotinin reduced fibrin remodeling and fibronectin deposition, but surprisingly, both aprotinin and TGF-β3 synergistically increased YAP/TAZ activity, contrary to our expectations. Thus, antifibrinolytics may prevent clot contraction but exacerbate MDC fibrosis. Further studies of an alternative method that combat both contraction and fibrosis (PL) are currently being investigated.

POSTERS

Poster #: 1

Bacteroides Species-Mediated Enzymatic Degradation of Extracellular Matrix Components

Karen Mancera Azamar, University of Florida

Ana Maria Porras, Ph.D.

University of Florida

aporras@bme.ufl.edu

The extracellular matrix (ECM) is a structure composed of natural biomaterials as organized arrays of macromolecules. The ECM plays an active role in the severity and exacerbation of disease, while its dynamic remodeling is also controlled by diseased microenvironments and mechanisms through enzymatic degradation. Within the context of gastrointestinal environments, the interactions at the microbe-ECM biointerface between members of the gut microbiome and native colonic ECM are poorly understood. Bacterium within the Bacteroides genus are opportunistic pathogens of high abundance in the gut that encode an assortment of enzymes. An increased prevalence of specific Bacteroides species has been associated with the exacerbation and progression of diseases characterized by ECM remodeling, such as inflammatory bowel disease and colorectal cancer. Thus, we hypothesized that members of the Bacteroides genus can alter their microenvironment through enzymatic ECM degradation. The present work aimed to characterize degradation of individual natural ECM biomaterials by bacterial proteases and carbohydrate-active enzymes (CAZymes). We found that strains of B. ovatus and B. thetaiotaomicron degraded glycosaminoglycansusing hyaluronic acid and chondroitin sulfate substrates, with species-level differences. In contrast, we determined that crude supernatants from four strains of B. fragilis could degrade gelatin, collagen I, collagen IV, and elastin with strain-level differences. Meanwhile, filtered cell-free B. fragilis supernatants could only degrade collagen IV and elastin. When evaluating enzymatic activity in the presence of various protease inhibitors, our results suggest the involvement of cysteine proteases, serine proteases, and matrix metalloproteases. We also sought to test the impact of the growth environment on ECM degradation activity. Fluctuations in pH during culture influenced the extent of elastin and glycosaminoglycans degradation by B. fragilis or B. ovatus and B. theta, respectively. Overall, we concluded that Bacteroides species can degrade ECM components through the inducible production of proteases or CAZymes that include elastin, collagen I, collagen IV, hyaluronic acid, and chondroitin sulfate. Additionally, our results suggest that the synthesis of these enzymes is likely impacted by environmental conditions during bacterial growth. Our work highlights the importance of microbe-ECM biointerface interplay as an overlooked process leading to remodeling of natural biomaterials.

Poster #: 2

Engineering thermoresponsive biointerfaces: the role of polymer grafting architecture on surface wettability

Kelly Bukovic, University of Virginia

Sabrina Liskey: University of Virginia, sel7qy@virginia.edu

Steven R Caliari: University of Virginia, sc6au@virginia.edu

Rachel A Letteri: University of Virginia, rl2qm@virginia.edu

Thermoresponsive surfaces exhibiting temperature-dependent hydrophobicity allow for control over bioadhesive processes, offering an alternative to traditional enzymatic cell harvest techniques. Enzymatic harvest degrades cell surface proteins and extracellular matrix (ECM), negatively impacting cell viability and the regenerative capacity of cell-based therapies. In contrast, the switchable hydrophobicity of thermoresponsive surfaces enables the detachment of intact cell sheets simply by lowering the temperature. At physiological temperature, cells adhere, but detach as the temperature is lowered and the substrate becomes hydrophilic. To leverage thermoresponsive surfaces for effective cell sheet harvest, it is imperative to understand the interplay between thermoresponsive polymer chemistry and resulting surface properties which ultimately influence biological outcomes such as protein adsorption and cell behavior.

We synthesized thermoresponsive copolymers comprised of di(ethylene glycol) methyl ether methacrylate (DEGMA) and methacrylic acid (MAA) as thermoresponsive units and surface anchoring units, respectively. Whilst poly(N-isopropylacrylamide) (pNIPAAm) is often used to develop thermoresponsive surfaces, polymers of DEGMA demonstrate similar functional properties without thermal hysteresis behavior, which is anticipated to yield cleaner cell sheet release. To evaluate the impact of grafting architecture on temperature-dependent surface wettability, copolymers were synthesized using reversible addition fragmentation chain transfer (RAFT) polymerization to install surface anchoring units in block and random configurations with controlled molecular weight. The spatial distribution of tethering points is expected to impact thermoresponsive chain mobility, influencing the extent of surface hydration and thus, substrate wettability, which guides biointerfacial interactions with proteins and cells.

Libraries of random and block copolymers with varying mol% MAA were synthesized and characterized using 1H nuclear magnetic resonance (NMR) spectroscopy to evaluate molecular weight and monomer distribution. Random and block copolymers were grafted to aminated glass surfaces and available surface amines before and after polymer addition were investigated using ninhydrin assays. To evaluate surface wettability, contact angle measurements were conducted at physiological and refrigerator temperatures, with lower contact angles expected below the hydrophilic transition temperature. By advancing understanding of how polymer chemistry impacts wettability, this work informs the design of temperature-responsive biointerfaces to propel cell biomanufacturing.

Poster #: 3

Collagen-like syntax imparts order and closed-loop phase behavior to intrinsically disordered protein polymers

Maria Giraldo, Georgia Institute of Technology and Emory University

Jeremy Hanon, Emory University, jeremy.hannon@emory.edu

Mariell Pascual, Emory University, mariell.joy.pascual@emory.edu

Felipe Garcia Quiroz, Ph.D., Georgia Institute of Technology and Emory University, felipe.quiroz@emory.edu

Intrinsically disordered protein polymers (IDPPs) are an emerging class of repeat proteins that can undergo phase separation in response to biomedically relevant stimuli. IDPPs mimic intrinsically disordered proteins (IDPs), which exhibit large molecular-level conformational fluctuations. Native IDPs often drive phase separation to control the assembly of cellular structures. Their phase behavior comes in two flavors, differentiated by whether the phase transition occurs above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST). Collagen and elastin, two prototypical IDPs, represent these two extremes: UCST for collagen and LCST for elastin. These proteins share a disordered Pro- and Gly-rich scaffold but differ in the specific arrangement (syntax) of Pro and Gly residues and overall amino acid composition —with collagens uniquely featuring oppositely charged amino acids. Notably, the IDP domain of collagen drives the assembly of triple-helical domains, undergoing a disorder-to-order transition, a remarkable feature absent in engineered IDPPs. Inspired by these contrasting features, here we recombinantly synthesized IDDPs that incorporate collagen syntax and composition into elastin-like sequences. The resulting library consisted of over twenty IDPPs largely composed of matching elastin/collagen pairs that only differed in the underlying Pro-Gly syntax, ensuring that collagen-like IDPPs encoded perfect GXY repeats —a known syntactic element for triple-helix formation. Excitingly, computational examination of our designs by AlphaFold and Rosetta showed that collagen-like IDPPs uniquely fold into triple helical structures, whose stability is governed by the length of the IDPP and the choice of charged and aromatic residues. Contrasting the phase behavior of purified IDPPs, we discovered that the compositional bias of collagens encoded a novel dual LCST-UCST behavior (where LCST

Poster #: 4

Laminin I mediates resistance to Lapatinib in HER2-positive brain metastatic breast cancer cells in vitro

Sumiran Gurung, The University of Alabama, Tuscaloosa

Sumiran Gurung, Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL 35487, USA, email: skgurung@ua.edu

Lalita A. Shevde, Department of Pathology, O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35233, USA, email: lalitasamant@uabmc.edu

Shreyas S. Rao*, Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL 35487, USA, email: srao3@eng.ua.edu

The role of extracellular matrix (ECM) prevalent in the brain metastatic breast cancer (BMBC) niche in mediating cancer cell growth, survival, and response to therapeutic agents is not well understood. Emerging evidence suggests a vital role of ECM of the primary breast tumor microenvironment (TME) in tumor progression and survival. Possibly, the BMBC cells are also similarly influenced by the ECM of the metastatic niche; therefore, understanding the effect of the metastatic ECM on BMBC cells is imperative. Herein, we assessed the impact of various ECM components (i.e., Tenascin C, Laminin I, Collagen I, Collagen IV, and Fibronectin) on brain metastatic human epidermal growth factor receptor 2 (HER2)-positive and triple negative breast cancer (TNBC) cell lines in vitro. The highly aggressive TNBC cell line was minimally affected by ECM components exhibiting no remarkable changes in viability and morphology. On the contrary, amongst various ECM components tested, the HER2-positive cell line was significantly affected by Laminin I with higher viability and demonstrated a distinct spread morphology. In addition, HER2-positive BMBC cells exhibited resistance to Lapatinib in presence of Laminin I. Mechanistically, Laminin I-induced resistance to Lapatinib was mediated in part by phosphorylation of Erk 1/2 and elevated levels of Vimentin. Laminin I also significantly enhanced the migratory potential and replicative viability of HER2-positive BMBC cells. In sum, our findings show that presence of Laminin I in the TME of BMBC cells imparts resistance to targeted therapeutic agent Lapatinib, while increasing the possibility of its dispersal and clonogenic survival.

Poster #: 5

The Impact of Natural Extracellular Matrix Proteins on Quiescence Induction in In Vitro Cultured CCD-18 Colonic Fibroblasts

Zahra Mohammadalizadeh, University of Florida

Ana Maria Porras (Assistant Professor, University of Florida, email: aporras@bme.ufl.edu)

Intestinal fibrosis, a common complication in Crohn's disease (CD), is associated with the activation of fibroblasts, a key cell population in the intestinal wall. However, studying these cells in their quiescent state in vitro is challenging due to their propensity to undergo phenotype changes in response to the underlying substrate stiffness, like tissue culture polystyrene flasks (TCPS). Thus, conventional culture methods are inadequate for studying fibroblast activation processes. Our research aims to develop a protocol to induce quiescence in CCD-18 colonic fibroblasts in vitro, focusing on the role of natural extracellular matrix (ECM) proteins as cell culture coatings. Both ECM protein coatings and Vitamin D (VD) treatment have been suggested to mitigate pro-fibrotic effects in fibroblasts.

In our experiments, fibroblasts were cultured on different ECM coatings (collagen I, collagen III and laminin) or in VD-containing media with varying concentrations. We also assessed the effects of media supplementation with fibroblast growth factor (FGF). The fibroblast phenotype was evaluated by quantifying cell proliferation, viability, and α-smooth muscle actin (α-SMA) protein expression. Our results showed that treatment with 10 μM VD in conjunction with FGF led to a more pronounced fibrotic phenotype. Interestingly, under the influence of 10 μM VD alone, cells exhibited reduced α-SMA production. The choice of ECM coating on the culture plates also elicited diverse effects on cell activation, with lower concentrations of laminin exhibiting the capability to induce fibroblast quiescence. These findings highlight the important role that natural biomaterials can play in dictating fibroblast phenotype and underscore the challenges of developing a protocol to attain quiescence. Our data demonstrates diverse activity phenotypes in response to different treatments. To address this challenge, we are currently developing an optimal protocol by employing a full-factorial design of experiment (DOE), which considers the synergic impacts of ECM protein coatings, VD, and FGF to induce the maximum transition of fibroblast phenotype to the quiescent state. Our systematic approach will ultimately contribute to our understanding of fibroblast quiescence and yield a protocol that will allow us and other research groups to explore the mechanisms that trigger intestinal fibrosis.

Poster #: 6

Plodia interpunctella silk fibers: protein structure and composition influences on structure and cytocompatibility

Lauren Eccles, University of Florida

Lauren E. Eccles, UF Chemical Engineering, leccles@ufl.edu

Elizabeth L. Aikman, UF Chemical Engineering, elizabethaikman@ufl.edu

Jasmine B. McTyer, UF Chemical Engineering, jmctyer@ufl.edu

Isabel Matias Cruz, UF Chemical Engineering, i.matiascruz@ufl.edu

Adelyn L. Richgels, UF Chemical Engineering, richgelsa@ufl.edu

Whitney L. Stoppel, UF Chemical Engineering, whitney.stoppel@ufl.edu

Silk-based biomaterials, primarily derived from the silk fibroin protein of the Bombyx mori (B. mori) silkworm, have been studied for tissue engineering applications due to their advantageous mechanical properties, biocompatibility, and commercial availability. More recent efforts have aimed to expand the range of achievable silk-based biomaterial properties by investigating alternative sources of silk proteins that vary from B. mori silk in sequence and structure. These structural distinctions drive differences in physical and chemical properties of silk fibers, primarily due to the varying degree of crystalline content in the polymers. Crystalline content in silk fibroin is also the primary factor influencing the performance of silk biomaterials, translating to advantageous properties such as high elasticity, increased tensile strength, or enhanced bioactivity. For the development of alternative silk-based materials, we investigate silk from the Plodia interpunctella (P. interpunctella) silkworm. Early investigations into P. interpunctella silk have highlighted differences between the P. interpunctella silk fibroin proteins and B. mori silk fibroin proteins; however, P. interpunctella silks still largely lack development and characterization at the fiber level. This work evaluates the structural, thermal, mechanical, and cell-material properties of P. interpunctella silk as a raw material for biomaterial fabrication. As silk fibroin is the primary constituent in silk-based biomaterials, we explore how isolation of silk fibroin through degumming processes shifts properties and cytocompatibility from the non-degummed material. We utilize atomic force microscopy (AFM), dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR) to analyze material properties in addition to assessing proliferation and metabolic activity of normal human lung fibroblasts (NHLF) cells seeded on silk fiber sheets. Observed properties are used to identify links between silk fibroin protein sequence and fiber function in addition to forming hypotheses in how P. interpunctella silk-based biomaterials will perform in comparison to other natural biopolymers used in biomaterials. Ongoing work aims to develop methods to process P. interpunctella silk into material formats for medical applications in drug delivery and tissue engineering, utilizing the material characteristics determined here as a baseline for shifts in material performance.

Poster #: 7

Biodegradable Metallic nanoparticles incorporated nanofiber mesh: A potent fibrous platform for wound healing application

Narayan Bhattarai, North Carolina A&T State University

I prefer poster presentation only

Metal particles incorporated polymer matrices in various forms and geometry are found attracted materials platform for promoting wound healing and prevention of infection. However, the faith of these metal particles and their degraded products in the tissue environment is still unknown as both can produce cytotoxic effects and promote unwanted wound reactions. In this study, we synthesized and analyzed biodegradable metal particles, magnesium (Mg) and zinc (Zn) incorporated nanofiber mesh scaffolds for potential wound healing application. We first developed the coated metal particles with zein, a soluble protein from corn, and then imbedded those in polycaprolactone (PCL) nanofibers via electrospinning. We performed multi-modal evaluations of properties of the fibrous scaffolds' including physio-chemical properties and in vitro cellular responses. Several physicochemical properties such as fiber morphology, crystallinity, mechanical strength, hydrophilicity, degradation and release of metal ions, so forth were characterized. In vitro cellular response of the scaffolds was evaluated utilizing direct and indirect cytotoxicity assays and immunocytochemistry analysis with human dermal cells, Human umbilical vein endothelial cells. We provide evidence that the integration of metal particles in PCL nanofibrous scaffolds improved its physicochemical and biological functions. The immunocytochemistry analysis confirmed the elevation of vimentin and α-smooth muscle actin with the scaffolds suggesting that the fibroblast cells were highly differentiated into myofibroblasts. These fibrous scaffolds have significant potential in encapsulating and delivering both bio-active components and modulate the cellular activities that are involved in tissue repair and remodeling.

Poster #: 9

Combination therapy of microporous hydrogel scaffolds and supramolecular peptide assemblies to enhance wound healing

Pablo Cordero Alvarado, Duke University

Pablo Cordero Alvarado, p.cordero@duke.edu

Shamitha Shetty, shamitha.shetty@duke.edu

Alejandra Suarez-Arnedo, alejandra.suarez.arnedo@duke.edu

Tatiana Segura, tatiana.segura@duke.edu

Joel H. Collier, joel.collier@duke.edu

Disruption to the skin's barrier function triggers a coordinated cascade of immune processes aimed at restoring homeostasis. However, dysregulation of these immune responses may result in overactivation of inflammatory pathways and elevated secretion of pro-inflammatory molecules, ultimately resulting in delayed and poor wound healing. Supramolecular peptide nanofibers and Microporous Annealed Particle (MAP) scaffolds are promising strategies to modulate local immune activity for the safe and rapid treatment of inflammation without the need for additional immunostimulatory factors or recurrent administrations. Additionally, MAP scaffolds allow for rapid wound closure and significant regeneration to cutaneous wounds, including augmented regeneration of skin appendages, and increased tissue tensile strength. As a dual-arm strategy to modulate inflammation during wound healing, we have designed an immunotherapy utilizing MAP scaffolds and the supramolecular peptide nanofiber Coil29. Our MAP+Coil29 material elicits robust epitope-specific Th2-biased humoral and cellular immune responses, while also enabling rapid tissue regeneration following a single application at the wound site. Our work aims to modulate inflammatory pathways via local application of MAP+Coil29 scaffolds at the injury site of a murine wound healing excisional model. We hypothesize that in addition to a local Th2-biased response favoring regeneration, materials raising autologous antibody responses against inflammatory signaling pathways will result in further enhanced regeneration of cutaneous wounds, with longer lasting efficacy compared with current passive blockade technologies.

Poster #: 10

Engineered 3D In Vitro Bone Niche for Modeling Breast Cancer Metastasis in Bone

Weitong Chen, University of Georgia

WT. Chen, University of Georgia, wtchen@uga.edu
Ammad Farooq, University of Georgia, ammad.farooq1@uga.edu
Arjun Kotapalli, University of Georgia, arjun.kotapalli@uga.edu
Caroline Coggin, University of Georgia, cac58568@uga.edu
Thomas Ritchey, University of Georgia, thomas.ritchey@uga.edu
Tim Burg, University of Georgia, tburg@uga.edu
Karen Burg, University of Georgia, kburg@uga.edu

Introduction: Breast cancer patients often develop metastases in bone, causing severe morbidities. Understanding breast cancer bone metastasis (BCBM) in vivo remains challenging due to uncontrollable factors and complexities. An in vitro platform is crucial to systematically study BCBM mechanisms. This study involved the development of a 3D printed polyester-ceramic composite scaffold to explore interactions between breast cancer cells and the bone niche.1 This approach could offer new insights into preventing and treating BCBM.

Materials and Methods: Polylactide (PL, 45wt%) pellets and nano-β-tricalcium phosphate (TCP, 55wt%) were dissolved in dichloromethane. The solvent was evaporated, and the dried composite was extruded at 165°C into filament. Composite and PL filaments were 3D printed into porous scaffolds (7mm diameter ×5 mm height). The filament diameter, pore geometry, surface roughness, and water contact angle (n ≥3) were measured. Subsequently, 1×105 MCF7 breast cancer cells in 75µL media were seeded onto the scaffolds and adhesion was evaluated.

Results and Discussion: The fabricated scaffolds had pore sizes of 546.5 ± 30µm for both PL and PL/TCP groups, similar to the pore size of native cancellous bone (300-600 µm). The TCP scaffolds had a lower water contact angle (76.77 ± 0.85°) compared to the PL scaffolds (118.77 ± 1.97°), indicating improved hydrophilicity. Similarly, the surface roughness of the TCP scaffolds (10.8 ± 1.36µm) was significantly higher than that of the PL scaffolds (2.51 ± 0.31µm). As anticipated, the number of MCF7 cells adhering to the TCP scaffolds (7.6 ± 0.3 × 104) was higher than that in the PL scaffolds (6.3 ± 0.75 × 104), suggesting that enhanced hydrophilicity and surface roughness positively influenced cell adhesion.

Conclusions: The 3D printed bone scaffolds fabricated from novel filament (PL and 55wt% nTCP) were demonstrated as a first step toward building an in vitro BCBM model. Ongoing studies will study the use of the scaffolds to elucidate the interactions between osteoblasts and MCF7 cells within the niche.

References:

1. Chen W, et al, “Developing an Engineered In Vitro Bone Niche for Rapid Cell Adhesion and Early-Stage Interactions of Osteoblasts and Metastatic Breast Cancer Cells”, 2023 BMES Annual Meeting.

Poster #: 11

Plodia interpunctella silk: a pathway to sustainable and consistent biomaterials

Bryce Shirk, University of Florida

I would like to be considered for a Rapid Fire Oral Presentation

Silk, a naturally derived biopolymer produced by various arthropod species, has been highly valued throughout history for applications in textiles, cosmetics, and biomedical industries. Traditionally, sericulture has focused on the Bombyx mori species to increase manufacturing capacity and enhance silk fiber properties. However, these efforts have led to unintended variations in gene expression and silk protein production due to the cost-effective necessity of outdoor rearing practices. Seasonal changes and global warming concerns, which alter temperature and food source quality, further impact silk fiber consistency, posing a challenge for the commercialization of silk-based biomaterials. To address these issues, we explored the alternative silkworm species Plodia interpunctella, which can be reared indoors, allowing for controllable silk production. This study evaluates the parameter space for rearing P. interpunctella and its impact on silk fiber production and growth, assessing its potential for consistent, commercial-scale silk fiber production. We controlled temperature (24°C, 26°C, and 30°C), resource availability (larvae/gram diet), and population density (larvae/mL) to optimize silk fiber production and homogeneity. Our results indicated that higher temperatures accelerated insect growth, reducing their life cycles. Additionally, population density had the greatest impact on total silk production. Optimal conditions for maximizing silk production were found to be 24°C, with 180 larvae and 18 grams of food per 250 mL container. Under these conditions, only nine standard rearing boxes were needed to produce 5 grams of dry silk fibers in approximately 20 days. These findings suggest that P. interpunctella may be a promising alternative to B. mori due to its ease of laboratory rearing and controlled fiber reproducibility. Future work will investigate how the surface area within the rearing container affects total silk protein production. Additionally, we are genetically modifying the silk-producing organism to generate silk fibroin-like proteins for advanced biomaterial functionality. This controlled indoor rearing capacity allows for ethical and tightly regulated management of genetically altered species, paving the way for innovative biomaterials.

Poster #: 13

Launching Research - Online Delivery of Research Initiation Modules

Karen Burg, University of Georgia

Timothy C Burg, Department of Biomedical Sciences, University of Georgia, tburg@uga.edu

Introduction: We previously introduced a slate of research professional skills modules as a starting point for adaptation to a particular setting that can be successfully used in didactic or research settings [SFB 2019]. More recently, we found the need to develop them into an online repository to facilitate asynchronous learning.

My NRMN (my.nrmnet.net) is an online networking/mentoring platform to help connect Mentors and Mentees across the country. It is partially supported by the National Institutes of Health and has a strong biomedical focus.

Materials and Methods: The premise of the “Launching Research Part 1: Laboratory Readiness” was that students would benefit from the content that we have found useful with our own undergraduate and graduate researchers but it would be accessible through the online network and still mentor supported by the mentoring team. That is, students could complete the modules within the context of a conversation with an online mentor. We posed a series of pre and post questions to assess the incoming mindset of the learners and any change produced through the modules.

Results and Discussion: Questions such as Research is: 1) Finding new solutions to problems or to create new devices, 2) Testing and formulating new principles to improve understanding in a field, 3) Critically examining current ideas in your “field”, and 4) All of the above elicited the correct “All of the Above” from most students entering the course. We believe the value in the modules is in the details such as “A good email always starts with a "Hey So-and-So" greeting” 1) True or 2) False which showed improvement pre and post. It was surprising that the participants appeared to came into the course with a fairly sophisticated understanding of research.

Conclusions: Future work will be completed to refine the modules and to add additional content, following a customer discovery process. It appears viable to provide mentor augmented content in an online content.

Acknowledgments: Funding for this work was provided by the Harbor Lights Endowment.

References:

1. Burg TCB and KJL Burg. 2019. “Biomaterials Experiential Learning: Integrating Research and Professional Development”, Trans Society For Biomaterials.

Poster #: 14

Soft implantable printed bioelectronic system for wireless continuous monitoring of restenosis

Addy Bateman, Georgia Institute of Technology

Dr. Woon-Hong Yeo, Georgia Tech, whyeo@gatech.edu

Atherosclerosis is the most common underlying condition of cardiovascular disease, which remains the leading cause of death worldwide. This disease, where plaque builds on the inner walls of arteries causing blood vessels to narrow, is generally addressed through angioplasty and stenting. While implanting stents is a common procedure, a frequent complication is in-stent restenosis, where the artery re-narrows due to scar tissue. There are limited clinical options when diagnosing in-stent restenosis, as it can be asymptomatic until a severe blockage causes rapid degradation of a patient's condition.

To address this major clinical gap, we report an implantable vascular electronic device using a newly developed miniaturized capacitive strain sensor. A microneedle and capillary-based printing system is employed to achieve a high-resolution patterning of a soft, capacitive strain sensor. The sensor is made from alternating layers of polyimide and silver nanoparticle ink, then fully encapsulated in PDMS. The sensor is integrated with a wireless vascular stent to offer a battery-free, wireless monitoring system compatible with conventional catheterization procedures. The vascular stent is fabricated with enhanced laser cutting and electroplating settings to ensure low resistance and reliable antenna performance.

This sensor successfully fits within the stent to allow for seamless integration within a catheter, ensuring proper deployment into blood vessels. Restenosis was successfully diagnosed at variable restenosis stages using continuous monitoring of resonant frequency changes through pulsatile testing. Collectively, the arterial implantable bioelectronic system shows the potential for wireless, real-time monitoring of various cardiovascular diseases and stent-integrated sensing and treatments. This research demonstrates a fully printed, low profile strain sensor with high sensitivity to remotely detect restenosis within a stent. Furthermore, the device has clinical implications to provide high-risk patients with real-time monitoring of their health, providing them with more data to customize and continue their care.

Poster #: 15

Optimizing Delivery of Therapeutic Satellite Cells using a Platelet-like-Particle Laded Fibrin Scaffold in a Murine Model of Hind Limb Ischemia

Isabel Wallgren, Emory University

I prefer poster presentation only

Peripheral artery disease (PAD) occurs when atherosclerotic plaque builds up in the limbs, blocking blood flow to the region. While current interventions, like exercise regimens and management of underlying conditions such as diabetes and hypercholesteremia limit disease progression, invasive surgical intervention is often necessary to prevent the advancement of critical limb ischemia. An alternate, less invasive approach is based on promoting angiogenesis and arteriogenesis to strengthen the collateral vessel network, circumventing the blockage. The Hansen lab has demonstrated that satellite cells, skeletal muscle stem cells that repair muscle fibers and release growth factors, can be harnessed as a potential therapeutic for promoting tissue regeneration. The prior delivery method, encapsulating satellite cells in alginate, allowed for the release of angiogenic factors but prevented cells from moving into and repairing damaged muscle. To optimize this therapy in a mouse model of PAD, we hypothesize that a fibrin-based scaffold will allow the cells to more successfully promote angiogenesis and repair ischemic tissue compared to encapsulation in alginate. An in vitro study compared satellite cell viability when contained in fibrin gels of three stiffnesses (0.1, 0.25, 0.4 U/mL thrombin) and determined that while cells maintained high viability through day 14 in all groups, cell population increased as the concentration of thrombin increased. Another study explored the effect of initial seeding density on cell viability using 10k, 50k, and 100k cells per 250 uL clot in 0.1 and 0.4 U/mL thrombin. Preliminary results confirm that higher thrombin concentrations have greater proliferation and demonstrate that seeding 100k cells per clot is a feasible density for in vivo injections. Despite greater proliferation, higher thrombin clots hinder in vivo delivery by clogging the injection needle. We hypothesize that by utilizing the Brown Lab's composite fibrin-colloid scaffold with fibrin-binding platelet-like-particles (PLPs), we can use lower thrombin concentrations while still achieving higher stiffness. A current in vitro study is evaluating cell viability in PLP-laden fibrin clots containing 0.25 mg/mL PLPs with 0.1 or 0.25 U/mL thrombin, which will identify what groups to use for in vivo testing in a murine model of hind limb ischemia.

Poster #: 17

Engineering quiescent valve interstitial cells to explore the role of gut metabolites in valve disease

Samanvitha Deepthi Sudi, University of Florida

Dr. Ana Maria Porras, University of Florida

aporras@bme.ufl.edu

Tanmayee Kolli, University of Florida

tkolli@ufl.edu

Calcified Aortic Valve Disease (CAVD) is associated with diet-related shifts in the gut microbiome. Recent studies suggest that trimethylamine oxide (TMAO), a gut metabolite, may serve as a biomarker for atherosclerosis. We hypothesize that TMAO might also contribute to CAVD by activating valve interstitial cells (VICs), the primary cell type in the aortic valve given the share risk factors and molecular triggers.

To test this hypothesis, VICs isolated from female porcine aortic valves were cultured with 10% FBS on tissue culture plastic. TMAO treatments (25µM to 150µM) were then applied alongside control groups (untreated and transforming growth factor beta - TGF-β, an inducer of VIC activation). TMAO treatment had no significant effect on proliferation or α-SMA expression, markers of VIC activation. Conventional culture conditions spontaneously activate VICs into a myofibroblastic phenotype, complicating trigger identification.

We sought to assess if TMAO activates VICs under physiological conditions. We induced quiescence by our lab engineered protocol using a collagen-the primary biomaterial found in healthy aortic valves. To generate quiescent VICs (qVICs), VICs were cultured on collagen-coated plates (2µg/cm2) supplemented with 2% FBS, insulin, and fibroblast growth factor (FGF) for 10 days. We then treated these qVICs with TMAO (25µM to 150µM) for up to 5 days.TMAO treatment of qVICs increased proliferation and α-SMA expression (markers of activation), reaching levels comparable to activated VICs and TGF-β-treated cells.

TMAO treatment also increased qVIC production of extracellular matrix (ECM) proteins, reactive oxygen species, and angiogenic cytokines, suggesting that TMAO may also induce other hallmarks of CAVD beyond VIC activation. We were able to successfully identify the pathway utilized by this gut metabolite to initiate VIC activation. Inhibition of the Protein Kinase R-like ER kinase (PERK) pathway effectively attenuated the effects of TMAO on qVICs.

These results emphasize the significance of leveraging natural biomaterials to engineer physiologically relevant cell phenotypes. Our collagen-based protocol allowed us to establish the impact of TMAO treatment on healthy quiescent VICs. Overall, these findings suggest that TMAO may play a role in the development and progression of aortic valve disease, highlighting the importance of considering host-microbe interactions in cardiovascular disease progression.

Poster #: 18

Comparison of cartilage pre-digestion techniques for auricular cartilage tissue constructs

Nettie Brown, Georgia Institute of Technology

Nettie Brown, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, nettie.brown@gatech.edu
Vania Dominick, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, vdominick3@gatech.edu
Sai Kulkarni, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, sai.kulkarni@gatech.edu
Alana Frankel, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, afrankel31@gatech.edu
Scott Hollister, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, scott.hollister@bme.gatech.edu
Johnna Temenoff, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, johnna.temenoff@bme.gatech.edu

Auricular cartilage has limited regeneration capacity; therefore, researchers have explored reconstruction strategies like chondrocytes in 3D printed scaffolds, minced cartilage embedded in fibrin gels, and isolated chondrocytes encapsulated in hydrogels. As per these examples, different cartilage pre-treatment types can be used, so the goal of this study was to compare different levels of cartilage pre-digestion (isolated chondrocytes (isolated), partially digested minced cartilage (partially digested), minced cartilage (minced)) within poly (ethylene glycol) (PEG)-based hydrogels in terms of neo-cartilage formation within the constructs. Specifically, in this study, we developed a method for a partially digested minced cartilage matrix that we hypothesized can support chondrocyte phenotype maintenance due to the presence of native cartilage extracellular matrix (ECM).

Fresh auricular cartilage was cut into 1mm2 and 2mm2 pieces for the isolated and partially digested groups, respectively, and then treated with a 0.6 wt.% collagenase solution for 4 hours. All cartilage digestion levels were encapsulated in a 15 wt.% PEG-diacrylate (PEGDA) solution and crosslinked via radical polymerization. Cell viability was measured using Live/Dead staining (n=4). Over 4 weeks in vitro, gene expression of collagen 2 (COLII) and aggrecan (ACAN) was measured using PCR (n=4). In addition, hydrogels were stained for H&E and Safranin O and underwent immunohistochemistry (IHC: COLI, COLII, ACAN) (n=4).

All groups remained viable over 7 days. Isolated and partially digested groups showed a significant increase in COLII expression by week 2 (isolated:1018 ± 265, partially digested: 90 ± 56; (fold change over passage 2 chondrocytes)), while the isolated group expressed significantly more COLII (74 ± 16) and ACAN (7 ± 2) by week 4. H&E and Safranin O revealed a homogenous distribution of isolated chondrocytes and glycosaminoglycan deposition in the isolated group. IHC of the isolated group showed intense COLI, COLII, and ACAN staining around nuclei and additional ACAN deposition in the hydrogel matrix at week 4. This novel study provided a head-to-head comparison of different cartilage digestion levels as tissue sources for various cartilage tissue engineering applications.

Poster #: 19

Supramolecular peptide-protein granules for intracellular CRISPR-Cas9 protein delivery

Alex Adolphson, University of Florida

I would like to be considered for a Rapid Fire Oral Presentation

CRISPR-Cas9 protein has been revolutionary for both gene therapy and generation of engineered cell lines due to its ease of use and versatility. However, because of its large size, charge, and hydrophilicity, it does not readily cross the cell membrane. Current delivery methods either create pores in the cell membrane to allow it to cross or utilize nanomaterials such as lipid nanoparticles to allow for uptake via endocytosis. These methods are limited by toxicity and efficiency respectively. Here, we present a supramolecular peptide-protein delivery vehicle for intracellular protein delivery that seeks to address these challenges. This approach utilizes charge-complementary molecules known as CATCH(+) peptides and CATCH(-) fusion proteins. Alone, these molecules do not self-assemble due to electrostatic repulsion, but when combined form β-sheet fibrils. If a crowder is added such as polyethylene glycol (PEG), Tween-80 micelles, or excess CATCH(+) peptide, nanoscale CATCH(+/-) granules are formed at peptide concentrations ~10-fold lower than the critical fibrillization limit in dilute conditions (~200 mM). Granules are internalized in greater than 90% of treated cells within minutes by various suspension and adherent cell lines including fibroblasts, HEK293, T cells, dendritic cells, monocytes, and neutrophils with minimal cell death. Additionally, CRISPR-Cas9 protein that is endocytosed also needs to escape the endosome and traffic to the nucleus to reach its genomic target. MDA-MB-231 cells engineered to express galectin 8 fused to YFP were utilized to visualize endosomal escape of CRISPR-Cas9 protein granules. PEG, CATCH(+), and CATCH(-) fusion protein did not demonstrate endosomal escape alone. However, granules did show significant endosomal escape within minutes. Next, CRISPR-Cas9 targeting GFP for knockout was delivered to HEK293 cells engineered to express GFP. Granules had ~50% GFP knockout efficiency, which is comparable to other gold standard methods with minimal cytotoxicity. Collectively, these data establish CATCH(+/-) granules as a simple and flexible nanomaterial platform for cell engineering via intracellular protein delivery.

Poster #: 20

Tailoring nanoparticle design and transport by emulsion-mediated PEGylation

Alexander Heiler, Georgia Institute of Technology

Alexander J. Heiler, aheiler3@gatech.edu, Georgia Institute of Technology

Tae Hee Yoon, tyoon35@gatech.edu, Georgia Institute of Technology

Yunus Alapan, yunus.alapan@me.gatech.edu, Georgia Institute of Technology

Susan N. Thomas, susan.thomas@gatech.edu, Georgia Institute of Technology

How a drug delivery system (DDS) transports through the body to the intended target helps determine the effectiveness of the bioactive cargo. The DDS properties, like size and incorporation of poly(ethylene glycol) (PEG), are vital to navigating transport barriers within the body. For particles made using emulsion polymerization, like poly(propylene sulfide) nanoparticles (PPS-NP), the surfactant alters the particle properties as it is incorporated into the particle corona. Therefore, PEG-containing copolymer surfactants are used to synthesize and PEGylate PPS-NPs, controlling particle properties and PEGylation during synthesis, which then mediate biological transport, eliminating the need for post-synthesis modulation. PPS-NP were synthesized using a copolymer library at various concentrations. The copolymers differ in the lengths of the PEG and poly(propylene glycol) (PPG) components, yielding different copolymer properties, such as PEG chain length and overall hydrophilicity. NPs were characterized by thermogravimetric analysis (TGA) and dynamic light scattering (DLS) to determine composition, extent of surface PEGylation, and hydrodynamic size. Linear models were constructed to evaluate the correlations between copolymer and NP properties, with and without the influence of copolymer concentration. NPs synthesized using various copolymers exhibited properties dependent on copolymer concentration. After removing the dominating influence of copolymer concentration, NP size was most correlated to the PEG length and percentage of PEG in the copolymer. Meanwhile, the extent of PEGylation was most correlated to the hydrophilicity and PPG length, which controls the distance between PEG chains on the NP surface, demonstrating the influence of copolymer properties on the resulting NP properties. Next, NP diffusivity through collagen, an in vitro model of skin interstitium, was evaluated by fitting the fluorescence of fluorophore-labeled NPs throughout the collagen to a diffusion model. The NP diffusivity depended on copolymer concentration, PEG length, and PPG length. Diffusion is a size-dependent process, agreeing with the correlation to copolymer concentration, which strongly mediates the NP size. Furthermore, the PEG and PPG lengths, which modulate the size and density of the PEG chains on the NP surface, additionally influence diffusivity, mediating the interactions between the NP and collagen. The copolymer properties that influenced changes in NP properties also manifested changes in NP transport.

Poster #: 22

Royal Jelly's 10-Hydroxy-2-decenoic-acid Imparts Potential Antimicrobial Properties to High Density Polyethylene through Immersion

Elizabeth Matlock-Buchanan, University of Memphis

I would like to be considered for a Rapid Fire Oral Presentation

High Density Polyethylene (HDPE) is a common polymer material currently used to fabricate food containers, medical consumables, and implants. Unfortunately, HDPE is prone to contamination by microorganisms through attachment and formation of biofilm colonies. Biofilm formation may result in severe infections, which then may cause implant rejection, repeated surgeries, and may even lead to death. 10-Hydroxy-2-decenoic-acid (10-H2DA) is an antimicrobial unsaturated fatty acid and is the main lipid component found in the honeybee's royal jelly.10-H2DA has been reported to have antitumor, antimicrobial, and other inhibitory properties. Here the therapeutic is investigated as a dispersal signaling molecule, or DSM, to inhibit the spread of microbes and eventual formation of biofilm colonies on the polymer HDPE. HDPE coupons were immersed in a solution of 10 mg of 10-H2DA fatty acid to 1 mL of ETOH and then air dried. Characterization included FTIR, contact angle, and an HPLC analysis of the three-day elution study in phosphate buffered saline, or PBS. The HDPE coupons were immersed in 200 µL of PBS with the entire solution removed and frozen at each time check. Fresh PBS was then added back to each coupon until the next time check with nine time checks total up to 72 hours from initial immersion in therapeutic. Results of FTIR and contact angle analyses confirm that the 10-H2DA therapeutic was adsorbed to the surface without chemical conjugation. Elution studies showed that approximately 75% of the adsorbed fatty acid was released in the first three hours, with an additional 20% eluted within the first six hours. This almost complete initial release happened because the therapeutic only adhered through hydrophobic interactions. With 95% of the original amount of the 10-H2DA therapeutic released within the first six hours. This simple coating strategy could be used to provide early protection from microbes without using traditional antibiotics.

Poster #: 23

Fabrication of antibody-loaded microparticles for sustained-release immunotherapy of ovarian cancer

Karla Robles, Tennessee State University

John Paul Libanati - Vanderbilt University, john.paul.libanati@vanderbilt.edu

Todd Giorgio - Vanderbilt University, todd.d.giorgio@vanderbilt.edu

Richard Mu - Tennessee State University, rmu@tnstate.edu

Low success rates have been reported in the systemic administration of checkpoint-inhibitor immunotherapy of ovarian cancer. We hypothesize that through the localized administration of an antibody reservoir, treatment can be sustained and concentrated in the tumor region. In this study, we present the design and development of antibody-loaded particles towards sustained-release immunotherapy. Coaxial electrospray (CES) is an emerging technology in the encapsulation of biomolecules because it's a one-step process that can achieve high encapsulation rates. The encapsulation of the fluorescently-labeled human immunoglobulin G (IgG-FITC) antibody was achieved in PLGA microspheres using CES.

Poster #: 24

Engineered Hexameric Coiled-Coil Fusion Protein as Potential Potent COVID-19 Therapeutic and Prophylactic

Linh Mai, Georgia Institute of Technology

Linh Mai: Georgia Tech School of Chemical and Biomolecular Engineering; lmai35@gatech.edu

Wei Lv: Georgia Tech School of Chemical and Biomolecular Engineering

Narayanaiah Cheedarla: Emory School of Medicine; narayanaiah.cheedarla@emory.edu

Andrew Neish: Emory School of Medicine; aneish@emory.edu

Julie Champion: Georgia Tech School of Chemical and Biomolecular Engineering; julie.champion@chbe.gatech.edu

After almost 7 million deaths worldwide and its lasting effects on everyday life, COVID-19 is here to stay. One of the most common approaches to neutralize the disease's pathogen, SARS-CoV-2, is through blocking the Receptor Binding Domain (RBD) region of the viral spike protein from associating with Angiotensin Converting Enzyme 2 receptor on human cells, thus preventing cell entry and viral replication. Due to the speed at which SARS-CoV-2 mutates, however, the customary approach of using highly specific monoclonal antibodies (mAbs) to target this region proves to be challenging, due to their size, production complexity and cost of production. To address this problem, we proposed using Designed Ankyrin Repeat Protein (DARPin) – smaller, less complex antibody-mimetic proteins. To achieve multivalency, we genetically fused and displayed these proteins on CC-HEX, a self-assembling hexameric coiled coil nanocarrier. The fusion proteins are modular and were efficiently produced in E.Coli, making them much more adaptable to viral mutations. Here, we demonstrated potent neutralization of SARS-CoV-2, across multiple variants (Wuhan, Delta, Omicron) through pseudovirus assay. Additionally, these fusions proteins conserve their structure and neutralizing ability after aerosolization using a vibrating mesh nebulizer. Through this work, we hope to establish HEX-DARPin proteins as potential prophylactics and therapeutics for COVID-19.

Poster #: 25

Peptide-hydrogel properties influence the development of anti-drug antibody against immobilized biopharmaceuticals

Lucas Melgar, University of Florida

I would like to be considered for a Rapid Fire Oral Presentation

Biomaterial approaches for biopharmaceutical delivery can directly address first-pass challenges including targeting to disease site and extending half-life. However, prolonged drug exposure time also increases the likelihood of emergence of anti-drug antibodies (ADAs) against the foreign biological macromolecule, compromising its therapeutic effects. We have developed a hydrogel platform for protein immobilization based on pairs of cationic and anionic peptides that co-assemble into supramolecular fibrils known as "CATCH(X+/Y-)". Changing the number (X,Y = 2, 4, or 6) or identity (X = K/R; Y = D/E) leads to changes in CATCH(X+/Y-) stiffness and structure. CATCH(+/-) hydrogels are injectable and retained at subcutaneous injection sites for more than 2 weeks with weak, rapidly resolving inflammation. Using a subcutaneous repeated injection model to deliver a therapeutic enzyme, we show that ADA development depends on CATCH(X+/Y-) material properties and formulation conditions. In contrast with similar peptide fibril systems, increased ADA was not associated with a particular CATCH(X+/Y-) hydrogel charge state; instead, net neutral pairs were more immunogenic than net cationic or anionic pairs. Increased stiffness of CATCH(X+/Y-) hydrogels also correlated to higher antibody titers than did softer gels, irrespective of overall charge. CATCH(X+/Y-) in a free-flowing "sol state" nanofiber formulation resulted in lower antibody titers compared to the hydrogel state, although, this was accompanied by a decreased residence time at ~7 days. CATCH(6K+/6D-) mixtures formed unique spherulitic structures in the sol state (i.e., "microspheres"), which had increased residence time on par with that of the hydrogel state. CATCH(6K+/6D-) microspheres showed the lowest ADA titers even with this increased residence time. Collectively, these data demonstrate that antibody development against an immobilized enzyme is highly dependent on the characteristics of the CATCH(X+/Y-) carrier including its charge, stiffness, residence time, and architecture, which can be ultimately tuned to increase pharmacokinetics of the biopharmaceutical without negative impacts on immunogenicity.

Poster #: 26

Thermosensitive Hydrogel for Local, Sustained Release to Lymphatic Vessels

Maya Levitan, Georgia Institute of Technology

Maya Levitan: Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology; Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University; maya.levitan@gatech.edu

Alex Heiler: Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology; School of Chemical and Biomolecular Engineering, Georgia Institute of Technology; aheiler3@gatech.edu

Susan Thomas: Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology; Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University; Winship Cancer Institute, Emory University; George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology; susan.thomas@gatech.edu

Statement of Purpose: Lymphedema is a chronic disease characterized by accumulation of interstitial fluid, impaired lymphatic vessel pumping, and fibrosis, leading to swelling, infections, and limited mobility. It affects over 130 million people worldwide, but despite its prevalence, there is no pharmacological treatment. Microcystic lymphatic malformations, another disease of lymphatic vessels, is characterized by the growth of infiltrative lesions containing lymph or blood which can cause deformation, pain, and organ dysfunction. While rapamycin has been explored as a treatment, long term use of this drug at high enough doses to induce lesion regression may not be feasible given the side effects. Therefore, there is a need in both diseases for a local, sustained release drug delivery platform which delivers immune modulatory drugs directly to lymphatic vessels. In this work, we present a thermosensitive hydrogel (F127-g-gelatin) made from the biocompatible polymers gelatin and Pluronic® F127, which are widely used in humans. This injectable hydrogel degrades into drug-loaded micelles which are optimally sized for lymphatic uptake, enabling locoregional delivery of drugs to lymphatic vessels. A variety of drugs, including rapamycin, can be loaded into F127-g-gelatin by simple mixing.

Methods: In vitro release of rapamycin-loaded F127-g-gelatin was assessed through degradation and drug release studies. A stability study of F127-g-gelatin was conducted on polymer stored dry or dissolved at 4.5 wt% in PBS at several temperatures for up to 6 months. Gelation temperature and degradation of the hydrogel were measured at each time point.

Results & Conclusion: F127-g-gelatin loaded with various concentrations of rapamycin can sustainably release the drug over 8-10 days without influencing the degradation pattern of the hydrogel, potentiating its use for treating lymphatic malformations. Moreover, the degradation and drug release behavior of rapamycin-loaded F127-g-gelatin was unaffected by storage at -20 °C. The stability study revealed that gelation and degradation of F127-g-gelatin remains stable under dry storage at -80 °C, -20 °C, and room temperature and under storage in PBS at -80 °C and -20 °C. The hydrogel's stability when loaded with drugs and stored under various conditions opens opportunities for its use in collaborative projects and heightens its potential for translatability.

Poster #: 27

Pulsatile Drug Delivery Platform for Use in Mental Health and Drug Addiction

Parker Brewster, University of Mississippi

Glenn Walker, gmw@olemiss.edu

Thomas Werfel, tawerfel@olemiss.edu

The opioid crisis and treatment-resistant depression (TRD) are pressing public health challenges that demand innovative solutions. Our research focuses on developing a pulsatile drug delivery platform, which holds promise for delivering medications with precision and control. This platform utilizes bioresorbable microdevices that employ a microfluidic "fuse" made from surface-eroding cellulose acetate phthalate (CAP) and Pluronic® F-127 (P) polymer composites. This technology enables automated interval delivery of pharmacotherapies, a critical advancement in the treatment of these conditions.

For TRD, our goal is to demonstrate the capability of our implants to deliver a range of release schedules, including daily, every 72 hours, and weekly doses. We aim to accommodate multiple release schedules within a single device, offering personalized treatment options. The long-term integrity of these microfabricated implants will be thoroughly assessed, both in vitro and in vivo. We will focus on the bioactivity of various antidepressants, such as brexpiprazole, olanzapine, and esketamine, upon their release from the device. This approach addresses the need for consistent and controlled drug delivery, potentially improving patient adherence and treatment outcomes.

In addressing opioid addiction, our platform seeks to provide a fully biodegradable device capable of delivering tapering dosages of fentanyl, alongside withdrawal-symptom-reducing medications like lofexidine and buprenorphine. This innovative approach aims to mitigate the risks associated with opioid treatment, such as abuse and overdose, by ensuring precise and controlled drug delivery. The long-term integrity and bioactivity of synthetic opioids released from these implants will be rigorously evaluated, ensuring their safety and effectiveness.

Preliminary in vitro studies have shown promising results, with our devices demonstrating the ability to release fluorescent model drugs in a controlled manner, closely mimicking the desired release profiles of actual medications. These findings highlight the potential of our drug delivery platform to revolutionize the treatment of TRD and opioid addiction. By providing precise and consistent drug delivery, our technology offers a transformative approach to treatment, enhancing adherence and reducing the potential for abuse.

Poster #: 28

Endosomolytic Polymersomes Enhance Intracellular Delivery of Nucleic Acid Therapeutics for Improved Anticancer Immune Responses

Payton Stone, Vanderbilt University

Alexander J. Kwiatkowski, Ph.D.; Vanderbilt University; alexander.kwiatkowski@vanderbilt.edu

Olga Fedorova, Ph.D.; Yale University; olga.fedorova@yale.edu

Anna M. Pyle, Ph.D.; Yale University; anna.pyle@yale.edu

Cancer immunotherapy has revolutionized the fields of oncology and drug delivery, with immune checkpoint blockade (ICB) demonstrating remarkable potential as an alternative to traditional treatment regimens. Although ICB has demonstrated disease control in certain cancers, it has also indicated limited therapeutic efficacy in poorly immunogenic cancers. Through the activation of specific cellular pattern recognition receptors (PRRs), the immunosuppressive tumor microenvironment (TME) of these cancers can be reprogrammed to a more immunogenic, 'hot' phenotype with larger populations of infiltrating T cells, directly correlating with improved responses to ICB. Indeed, activation of the retinoic acid-inducible gene I (RIG-I) pathway has been found to elicit a downstream signaling cascade resulting in the production of type I interferons which can induce this shift in the TME. Unfortunately, most RIG-I-activating therapeutics are not able to freely cross the cell membrane where they are required to activate this cytosolic PRR. The goal of this work is to optimize the loading of 5'-triphosphorylated RNA (3pRNA), a potent RIG-I agonist, within pH-responsive polymeric nanocarriers via a flash nanoprecipitation (FNP) process and assess intracellular delivery and therapeutic efficacy in a cancer model. To accomplish this, we analyzed the effect of copolymer properties on nanocarrier physical properties, RNA loading, cytotoxicity, endosomolytic activity, and RIG-I activation in vitro. We then validated the ability of the system to mitigate disease progression in a murine cancer model by intratumorally administering our 'lead' formulation and monitoring tumor volume, weight loss, and survival over the course of the experiment. With this FNP platform, we highlight the potential for tailored production of a variety of polymeric nanocarriers for potential translatable use in other drug delivery and immunomodulatory applications.

Poster #: 29

Implantable controlled-release chemotherapeutic drug-eluting mesh for postoperative abdominal cancer tumor eradication

Ummay Mowshome Jahan, NC State University

Dr. Martin W. King, NC State University, martin_king@ncsu.edu

Hypothermic intraperitoneal chemotherapy (HIPEC) is a potent therapeutic approach for several abdominal cancers, including colorectal, ovarian, gastric and appendiceal cancer as well as peritoneal carcinomatosis. This approach involves surgical tumor removal followed by the localized application of heated chemotherapeutic drugs within the abdominal cavity to eliminate residual cancer cells. Our study focuses on developing an implantable chemotherapeutic drug-releasing mesh that will eradicate any remaining cancer cells during the period of postoperative scar tissue formation.

The mesh has a tubular braided structure fabricated from resorbable poly(lactic acid-co-glycolic acid (PLGA) multifilament yarns on a specialized 16-bobbin braiding machine. Subsequently after heat-setting, the mesh is coated with a doxorubicin-loaded PLGA hydrogel. The braided mesh exhibits a favorable degradation profile over four weeks, ensuring compatibility with the postoperative healing process. ToF-SIMS analysis confirms a uniformly dispersed coating of doxorubicin across the mesh's surface. Moreover, in an in-vivo toxicity

assessment involving the implantation of the drug-free mesh in a mouse model, no significant inflammatory reactions were observed, confirming the mesh's biocompatibility.

Our ongoing work focuses on optimizing the hydrogel-coated mesh to fine-tune the release kinetics of the doxorubicin. We are also assessing the chemical and mechanical properties of the mesh, enabling us to better understand its long-term stability and efficacy. In addition, the drug kinetics of the hydrogel-loaded mesh are being evaluated. To assess the mesh's therapeutic potential, we are conducting in vitro experiments using various cancer cell models and exploring various in vivo models to validate our findings. This research represents a significant step toward enhancing the precision and effectiveness of HIPEC procedures for abdominal cancer treatment. The development of this controlled drug-releasing mesh holds promise for improving patient outcomes by providing a targeted and

sustained delivery system for chemotherapeutic drugs, ultimately contributing to the advancement of abdominal cancer therapy.

Poster #: 30

Glycopolymeric Nanoparticles for Targeted Delivery to Tumor-Associated Macrophages: Biodistribution, Toxicity and Anticancer Efficacy

Veeresh Toragall, The university of Mississippi

Veeresh Toragall, Oluwaseyi T Shofolawe-Bakare, and Thomas A. Werfel

Department of Biomedical Engineering, University of Mississippi, University, MS, USA

Veereshtoragall@gmail.com

tawerfel@olemiss.edu

Tumor-Associated Macrophages (TAMs) play crucial roles in tumor progression and immune suppression, making them attractive targets for cancer therapy (promoting an anti-tumor immune response or inhibiting pro-tumorigenic activities). Further, the impact of TAM-mediated efferocytosis as an emerging immune checkpoint on the tumor microenvironment phenotype. Therefore, directing therapies towards TAMs holds greater promise in enhancing the efficacy of cancer treatments while reducing off-target effects. Hence, we fabricated polymeric mannose (PMAM) nanoparticles (GNPs) that target macrophages and show controlled drug release. We also examined for their targeted delivery, toxicity, biodistribution and anticancer efficacy in 4T1 induced tumor mice model. The formulated GNPs showed the ability to release cargo in a pH-dependent manner which makes them ideal for endosomal drug delivery. Further, in vivo biodistribution studies showed the GNPs were capable of internalization by TAMs compared to tumor cells in the TME. Also, toxicity study revealed that the GNPs (< 3 mg/kg BW) did not show any sign toxicity in healthy animals and was safe to use. Finally, in vivo studies showed that GNPs has significantly reduced tumor volume compared to free UNC2025, showing greater therapeutic efficacy in triple negative breast cancer model. We believe that glycopolymer-based nanoparticles, which inhibit efferocytosis, hold potential as a standalone cancer immunotherapy and as a complementary agent to enhance response rates to checkpoint immunotherapy.

Poster #: 31

Programmably Degradable Hydrogels for Optimizing Monoclonal Antibody Release

Xi Ying, Georgia Institute of Technology

Xinyi Sheng, xsheng37@gatech.edu

Wenting Shi, swt1215@gatech.edu

M.G. Finn, mgfinn@gatech.edu

Monoclonal antibodies have revolutionized treatment paradigms across a range of diseases, owing to their specificity and efficacy. However, their therapeutic potential is often constrained by conventional delivery methods, leading to suboptimal dosing and increased side effects. This study introduces an approach to control the release dynamics of Immunoglobulin G (IgG) through hydrogel encapsulation and/or tethering, focusing on drug delivery mechanisms that address these challenges. By employing hydrogels for the sustained release of therapeutic agents, this research proposes a promising strategy for improving drug efficacy and patient compliance while reducing adverse effects. We investigate the use of oxanorbornadiene (OND)-modified dextran hydrogels, utilizing the retro-Diels-Alder (rDA) mechanism for programmable gel degradation, which leads to highly tunable release profiles. Our findings suggest that IgG release kinetics can be meticulously controlled by varying the types of OND molecules with different rDA half-lives, the degree of substitution (DS) in OND-dextran complexes, polymer weight percentages, and polymer chain lengths. These insights contribute to the design of drug delivery systems with programmable dosing profiles. Preliminary results indicate that IgG can be released sustainably for up to three weeks, demonstrating both traditional first-order and intriguing non-first-order release kinetics. This work highlights the potential of hydrogel-based platforms in patient-centric therapy and presents a novel method for the programmable delivery of monoclonal antibodies.

Poster #: 32

Biofunctionalized gelatin hydrogels support development and maturation of iPSC-derived cortical organoids

Andrew Kjar, Vanderbilt University

Andrew Kjar, andrew.kjar@vanderbilt.edu, Vanderbilt University

Mia R. Haschert, mia.r.haschert@vanderbilt.edu, Vanderbilt University

Alexis Yates, alexis.yates@vanderbilt.edu, Vanderbilt University

A. Joey Simmons, alan.j.simmons@vanderbilt.edu, Vanderbilt University

Gabriella Robertson, gabriella.l.roberston@vanderbilt.edu, Vanderbilt University

Corinne W. Curry, corinne.e.warlick@vanderbilt.edu, Vanderbilt University

Nicole T. Marguerite, nicole.t.marguerite@vanderbilt.edu, Vanderbilt University

Vivian Gama, vivian.gama@vanderbilt.edu, Vanderbilt University

Ken S. Lau, ken.s.lau@vanderbilt.edu, Vanderbilt University

Jonathan M. Brunger, jonathan.m.brunger@vanderbilt.edu, Vanderbilt University

Ethan S. Lippmann, ethan.s.lippmann@vanderbilt.edu, Vanderbilt University

Human neural organoid models have become an important tool for studying neurobiology. However, improving the representativeness of neural cell populations in such organoids remains a major effort. In this work, we compared Matrigel, a commercially available matrix, to an N-cadherin peptide-functionalized gelatin methacryloyl hydrogel (termed GelMA-Cad) for culturing cortical neural organoids. After robust material characterization by atomic force microscopy, scanning electron microscopy, and NMR, we cultured induced pluripotent stem cells in the material. We then employed full-mount and section immunostaining and single-cell RNA sequencing to characterize the resulting cell fates after cortical organoid differentiation.

We determined that both crosslinking conditions and peptide presentation can tune cell fate and diversity in gelatin-based matrices during differentiation. Of particular note, cortical organoids cultured in GelMA-Cad produce higher numbers of neurogenic and ciliated radial glia, which mapped to developmental human progenitor states. Moreover, in GelMA-Cad, upper layer and deep layer neurons collectively represented 27% of the detected cells at day 120 (15% and 12%, respectively). In contrast, at day 120, upper layer and deep layer neurons comprised <1% cells detected in Matrigel controls. We also observed enrichment of choroid plexus epithelial cells in higher crosslinking conditions, providing compelling evidence that matrix properties can influence neural organoid differentiation. In transcriptomic comparisons, GelMA-Cad embedded organoids had lower signatures related to stress pathways, a known problem with conventional Matrigel techniques. Taken together, outcomes from this work showcase GelMA-Cad as a simple and defined hydrogel alternative to Matrigel for neural organoid culture, marking it as a valuable resource.

Poster #: 33

Aptamer-functionalized Nucleic Acid-Collagen Complexes: An Advanced Bioactive Matrix for Angiogenesis

Barbara Stewart, University of Central Florida

I prefer poster presentation only

Introduction

The increasing need for organ regeneration has led researchers to pursue the development of biomaterial technologies to restore native tissue-like structures and provide an extracellular matrix (ECM)-like environment. We seek to facilitate the enhanced promotion of angiogenesis which is crucial for cell survival and tissue functionality.

The term nucleic acid-collagen complexes (NACCs) describes a wholly native biomaterial that potentially offers localized, and targeted bioactivity toward angiogenesis through function-specific DNA aptamers. NACCs are a versatile and fibrous system that arises from the spontaneous self-assembly of single-stranded DNA (ssDNA) with collagen and can be functionalized with any desired oligonucleotide sequence. They have been shown to offer targeted bioactivity that does not rely on the use of growth factor supplementation, and the presence of micro- and nano-fibers within their architecture confers structural support for endothelial cells.

Methods and Results

To evaluate the ability of NACCs to stimulate endothelial cells towards angiogenesis, we used a known DNA aptamer sequence that acts as a vascular endothelial growth factor (VEGF) agonist. This sequence selectively binds to the VEGFR-2 receptor of endothelial cells and activates it. We performed sprouting and tubulogenesis assays to assess the emergence of endothelial cell tubules and sprouts within the biomaterial. Phase contrast and confocal microscopy indicated increased tubular network area and number of sprouts, compared to control NACCs functionalized with a random ssDNA sequence not known to have any bioactivity. Additionally, we used a reverse transcription polymerase chain reaction (rt-PCR) gene array to quantify gene expression of key signal transduction pathway-focused genes involved in modulating the angiogenic process. In aptamer-functionalized NACCs, we saw the enhanced expression of genes such as matrix metalloproteinase 2 (MMP-2), angiopoietin 2 (ANGPT-2), and cadherin 5 (CDH-5).

Conclusion and Significance

Our results give us more reasons to keep focusing our studies on controlling angiogenesis since it can mean a dramatic change in immunotherapies, cancer research and personalized medicine. The significance of our work is that we will keep working to position NACCs as dynamic biomaterials for translational applications.

Poster #: 34

Recombinant Fusion Proteins as Versatile Tools for Protocell Development with Embedded Sensing Functions

Bornita Deb, University of Florida

Adriana LaVopa, University of Florida, alavopa@ufl.edu

Emma McDougal, University of Missouri, eomcdougal@gmail.com

Jackson Powers, university of Florida, powersj@ufl.edu

Carl Denard, University of Florida, cdenard@ufl.edu

Yeongseon Jang, University of Florida, y.jang@ufl.edu

In the pursuit of elucidating the rules of life, one promising avenue is the bottom-up creation of cell-mimetic structures, called 'synthetic cells. These synthetic cells can be engineered to imitate the structure, functions, and properties of natural cells, achievable through various approaches. Among all functions, sensory capabilities are crucial for the operation of living cells, enabling them to communicate and respond to external stimuli by performing appropriate actions. Recombinant protein self-assembled vesicles open new opportunities to incorporate functionally folded, sensory proteins into membranes with high modularity.

Here, we develop self-assembled protein vesicles that can sense and respond to specific signaling molecules by leveraging the ternary complex system involving FKBP-Rapamycin-FRB. The sensory protein domain, either FRB- or FKBP-, was genetically fused to a fluorescent protein-leucine zipper fusion protein (i.e., FRB-mCherry-ZE or FKBP-sfGFP-ZE). These sensory-fluorescent-leucine zipper fusion proteins self-assemble into vesicles with a counter leucine zipper fused with elastin-like polypeptide (ZR-ELP) by forming amphiphilic protein building blocks in water at the temperature above the lower critical solution temperature of ELP. We have successfully created vesicles self-assembled from FRB-mCherry-ZE and FRB-sfGFP-ZE with ZR-ELP. The rapamycin-induced interaction of these vesicles reveals the critical role of signaling molecule concentration in the modulation of vesicle intercommunication, where vesicle aggregation serves as an indicator of sensory activity. To characterize the vesicle's sensory response to rapamycin, we utilized dynamic light scattering to analyze their size and aggregation. At the same time, fluorescence and confocal microscopy techniques offered insights into their spatial distribution and aggregation behavior. Co-localization studies further quantified their interactions, enhancing our understanding of the vesicle's functionality for sensing. This paves the way for further exploration of various potential applications of protein vesicles to recapitulate minimal cellular functions.

Poster #: 35

Comparing the use of brain-derived and synthetic phosphatidylserine in lipid-polymer hybrid particles for modulating macrophage inflammation

Caroline Rucker, University of South Carolina

Kidochukwu Atube, Chemical Engineering Department, University of South Carolina, katube@email.sc.edu

Michael Gower, Chemical Engineering and Biomedical Engineering Departments, University of South Carolina, gowerrm@mailbox.sc.edu

Macrophages are immune cells that respond to inflammatory stimuli throughout the body. Macrophage dysfunction contributes to many aspects of chronic inflammatory disease. Thus, macrophages are an important target for therapies to resolve chronic inflammation. In previous work, we successfully synthesized poly(lactide-co-glycolide) (PLG) particles surface-functionalized with phosphatidylserine (PS). On the surface of cells undergoing apoptosis, PS functions as an “eat me” signal and a cue to resolve inflammation. Accordingly, macrophages bind and take up PS-bearing apoptotic cells through cell-surface receptors, and subsequently initiate an anti-inflammatory response. Likewise, we have shown that PS-presenting PLG (PS:PLG) particles are readily taken up by lipopolysaccharide (LPS)-inflamed bone marrow-derived macrophages (BMDMs) and decrease the section of inflammatory cytokines TNF-a and IL-6 while increasing the release of IL-10, an anti-inflammatory cytokine. However, for those studies, the PS used was derived from porcine brain, which is not recognized as safe by the FDA due to concerns involving the transmission of infectious encephalopathies such as mad cow disease. Hence, in this study, we sought to use 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), a synthetically derived PS, and compare its efficacy to the porcine brain-derived PS. Using transmission electron microscopy to investigate the organization of PS on the particle surface, we observed that PS:PLG particles made with DOPS (DOPS:PLG) presented a unique flower-like morphology when compared to PS:PLG, which presented a spherical morphology. Despite this difference, DOPS:PLG particles achieved the same loading of PS as measured by NMR. Importantly, we found that DOPS:PLG particles were able to target and modulate LPS-induced inflammation in BMDMs with the same efficacy as particles made with brain-derived PS. These findings advance the use phosphatidylserine-PLG hybrid particles as a potential therapeutic for patients with chronic inflammation. Future studies will investigate the use of these DOPS:PLG particles to deliver small-molecule drugs that can further modulate the anti-inflammatory effects of the particles.

Poster #: 36

Three-Dimensional Printed Piezoelectric Breast Cancer Bone Metastasis Model

Erika Landree, University of Georgia

Cheryl Gomillion, Ph.D.

School of Chemical, Materials, & Biomedical Engineering, University of Georgia

ctgomill@uga.edu

Bone has been identified as the most prevalent site for breast cancer metastasis; however, the cause is not fully understood. The mechanosensitive property of bone cells plays an important role in breast cancer metastasis to bone. In vivo, bone tissue experiences mechanical loading and creates electrical signaling as a result of this piezoelectric effect. Evidence suggests that breast cancer cells undergo increased proliferation and migration in the presence of electrical fields caused by the electrical signaling of bone cells. Three-dimensional (3D) in vitro models are widely used in cancer research; however, current models lack this piezoelectric component. An accurate, biomimetic model that mimics in vivo signaling is necessary to better understand the process of breast cancer metastasis to bone. This may be accomplished by introducing a piezoelectric polymer into the model, that yields an electrical field when mechanical stimulation, such as ultrasound, is applied. This study aimed to evaluate a novel 3D in vitro piezoelectric breast cancer bone metastasis model by observing the interactions between metastatic breast cancer cells and the 3D bone microenvironment. Our model consists of a 3D-printed scaffold made of polycaprolactone (PCL), demineralized bone matrix, and polyvinylidene fluoride (PVDF). The scaffolds are fabricated using 3D pneumatic printing. The high levels of sheer stress caused by pneumatic printing induce the formation of β phase content in the PVDF. Preliminary studies evaluated the piezoelectric properties of PVDF-loaded scaffolds and osteogenic differentiation within the model. Results showed an increase in piezoelectric properties when pneumatic printing was used for scaffold fabrication; osteogenic differentiation increased when cells were cultured within the dynamic model. Ongoing work includes the investigation of triple negative breast cancer cell migration within the bone model, with and without ultrasonic stimulation.

Poster #: 37

Multi-domain disordered proteins drive robust intracellular self-assembly through convergent phase transitions

Ian Sicher, Georgia Institute of Technology

I prefer poster presentation only

Intrinsically disordered proteins (IDPs), both synthetic and endogenous, undergo phase transitions to scaffold cellular structures. Sequence-level molecular information distinguishes IDPs that phase separate above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST). IDPs with a singular LCST or UCST phase-separation sequence grammar dominate the known landscape of IDP-driven phase transitions in nature and across "smart" biomaterials. We and others recently reported UCST-LCST diblock IDP polymers (IDPPs) with useful in vitro self-assembly, which were engineered by specifying one domain as the sole driver of phase separation. However, the intracellular behavior of multi-domain IDPPs with competing phase-separation grammars remains unexplored. To fill this gap, here we recombinantly synthesized and characterized a library of nine novel multi-domain IDPPs that integrate potent UCST and LCST grammars at either the repeat or the domain levels. Across the library, we used two UCST motifs and one LCST motif, and systemically varied UCST/LCST ratios. We successfully purified these multi-domain IDPPs with good yield (e.g., ~100 mg/L of culture) through a highly scalable phase separation-driven protocol. Surprisingly, temperature-dependent UV-visible absorbance measurements in a physiological buffer revealed that all multi-domain IDPPs converged towards LCST-type phase transitions, even for IDPPs with high UCST/LCST ratios. Moreover, increasing UCST content in multi-domain IDPPs consistently lowered the LCST cloud point temperature well below 37 °C. Fusing these at the gene level to a green fluorescent protein and using live-cell microscopy to probe their intracellular self-assembly, we saw robust formation of liquid-like biomolecular condensates in human cells. IDPPs with convergent LCST cloud points close to 37 °C appeared diffuse in the cytosol or formed small condensates. Multi-domain IDPPs with repeat-level integration of competing UCST-LCST grammars consistently led to prominent formation of multiple micron-sized condensates per cell. Our ongoing experiments are probing the ability of intracellular multi-domain IDPPs to disconnect from their single-grammar IDPP counterparts (either diffuse in the cytosol or phase separated). Because known intracellular IDPs predominantly exhibit UCST phase transitions, our findings suggest that multi-domain IDPPs with convergent LCST phase separation behavior are exciting new building blocks for de novo engineering of organelle-like condensates in human cells.

Poster #: 38

Comparison of alginate and fibrin hydrogels for cell delivery

Jared Albert, North Carolina State University & UNC- Chapel Hill

I prefer poster presentation only

Alginate is a commonly used biomaterial due to its natural origin and low cytotoxicity levels. Alginate is widely used for cell encapsulation due to its favorable gelation properties. Some examples of cell encapsulation utilizing alginate hydrogels include encapsulation of islet cells to avoid immune recognition for treating diabetes and encapsulation of satellite cells to increase angiogenesis in a hind limb ischemia model. However, encapsulation does not allow for cell permeation and engraftment with the surrounding microenvironment, which minimizes cell survival and functionality. Fibrin matrices represent an alternative biomaterial for cell encapsulation and delivery. Fibrin is also naturally derived and is generated by adding thrombin to fibrinogen. Thrombin activates fibrinogen to produce insoluble fibrin fibers which form a fibrillar fibrin matrix. Since fibrin matrices are naturally generated in the body and have been shown to allow cell infiltration and engraftment during wound healing, it has great promise as a biomaterial. We hypothesize that the fibrin matrices will lead to better long-term cell survival compared to alginate hydrogel encapsulation. Human dermal fibroblasts were either encapsulated within a 2% alginate hydrogel or within a fibrin matrix comprised of water, HEPES buffer, 2 mg/mL fibrinogen, and 0.1 or 0.25 U/mL thrombin. 3 hours or 2 days following encapsulation, NucBlue Live and NucGreen Dead ReadyProbe Reagent stains were used to assess cell viability. The hydrogels and matrices were imaged using a Leica MICA WideFocal with 10X lens on confocal settings and the percent of living cells was determined. Results show an increase in cell viability for cells in fibrin matrices created with 0.1 U/mL thrombin compared to fibrin matrices created with 0.25 U/mL thrombin or alginate hydrogels. On-going studies are evaluating how incorporation of a fibrin-specific targeting platelet-like particle into the fibrin matrices, which are known to retract clots and dynamically and regionally stiffen the microenvironment, further influence cell viability.

Poster #: 39

Evolution of Stem Cell Production: Multi-sensor Array & Wireless Electronics for Real-time Cell Culture Monitoring

Jimin Lee, Georgia Institute of Technology

Jimin Lee, Georgia Institute of Technology, jimin.lee@me.gatech.edu (as a presenting author)

Woon-Hong Yeo, Georgia Institute of Technology, whyeo@gatech.edu (as a corresponding author)

Large-scale manufacturing of stem cells, crucial for personalized medicine, faces challenges in consistency, quality, and scalability due to limitations in current bioreactor technologies. Herein, we introduce a novel smart bioreactor system that integrates wireless, multivariate sensors into a flexible cell bag, enabling real-time, in situ monitoring of critical culture parameters such as pH, dissolved oxygen and glucose levels, and temperature. This innovation supports long-term monitoring over 30 days, facilitating dynamic assessment without interfering with the cell culture environment. Experimental results validate system accuracy, sensitivity, and reliability under continuous operation, showcasing its potential to transform stem cell manufacturing processes.

This presentation will highlight the system's impact on stem cell research and therapy, emphasizing its capacity for high-throughput manufacturing to produce high-quality stem cells. Future work aims to expand this technology to larger-scale operations, further advancing stem cell manufacturing capabilities.

Poster #: 40

Control of Enzyme Cascade Reactions within Synthetic Cell-like Multicompartment Protein Vesicles

Jooyong Shin, University of Florida

Jooyong Shin

-Department of Chemical Engineering, University of Florida

-jooyong.shin@ufl.edu

Yeongseon Jang

-Department of Chemical Engineering, University of Florida

y.jang@ufl.edu

The intricate mechanisms of living cells, characterized by their multi-compartmentalization, regulate enzyme cascade reactions for the essential processes of life such as metabolism or signal transduction. Replicating these systems has been considered as one of the most critical tasks in synthetic cell exploration. Moreover, the development of artificial organelles holds significant importance in synthetic biology, as these structures mimic the compartmentalization seen in living cells, enabling precise control and optimization of biochemical processes. Globular protein vesicles (GPVs), vesicles self-assembled from globular fusion proteins, emerge as a promising platform in synthetic cell development, leveraging protein's biocompatibility and functions as the building blocks. Particularly, functional globular protein incorporated compartments represent promising artificial organelle platforms, offering the ability to localize enzymes within a defined space, thus enhancing efficiency and functionalities. In this study, we demonstrate the formation of multicompartment protein vesicles housing octopine dehydrogenase (ODH) incorporated vesicles and coacervates as artificial organelles. By pairing with pyruvate kinase, we perform enzyme cascade reactions to simulate pyruvate metabolism under anaerobic conditions in vitro. We foresee that the promise of these synthetic cell-like structures in mimicking vital cellular processes among specific confinements with recombinant fusion proteins will pave the way for progress in synthetic cell engineering and biotechnology.

Poster #: 41

Structurally decoupled hyaluronic acid-based 3D model to elucidate the impact of matrix metalloproteinase-mediated invasion of brain metastatic breast cancer cell spheroid

Kasra Goodarzi, The University of Alabama

Kasra Goodarzi (kas-ra Goo-dar-zi)

Email: kgoodarzi@crimson.ua.edu

Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL, USA

Shreyas S. Rao* (she-ri-yas ra-o)

Email: srao3@eng.ua.edu

Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL, USA

Polymeric hydrogels have been extensively employed to study cancer cell-matrix interactions as they provide cancer cells with a relevant three dimensional (3D) context evidenced in vivo and enabling us to maintain the cellular phenotype in vitro. The ability to tune hydrogel properties has enabled recapitulation of several key aspects of the tumor microenvironment in vitro. In the context of breast cancer, it is known that cancer cells invade the tissue at the primary and metastatic site by degrading the native extracellular matrix (ECM) using matrix-metalloproteinases (MMPs) resulting in disease progression. Efforts have been made to model MMP-mediated invasion of cancer cells by incorporating MMP-cleavable crosslinks in the hydrogel structure, however, to our knowledge, model systems enabling effective decoupling between hydrogel mechanical properties and mesh size, while incorporating MMPs into the hydrogel matrix have not been reported. To address this need, we fabricated a structurally decoupled hyaluronic acid (HA) based 3D biomimetic model to specifically investigate the invasion of metastatic breast cancer cells mediated by MMPs. The hydrogels were fabricated using varying ratios of biologically sensitive (i.e., MMP cleavable peptide) and insensitive crosslinkers (i.e., Dithiothreitol (DTT) or polyethylene glycol dithiol (PEGDT)) to investigate the impact of incorporated MMP-cleavable peptides on the invasion of encapsulated MDA-MB-231Br metastatic breast cancer spheroids. We found that HA hydrogels crosslinked with various ratio of DTT/MMP or PEGDT/MMP exhibited comparable mechanical and physical properties as tested via rheological measurements, swelling ratio analysis, estimated mesh size, and permeability measurements. However, their degradation rate in the presence of collagenase enzyme was significantly altered and directly related to the concentration of MMP-cleavable peptide used to crosslink the hydrogel. Consistent with this, encapsulated MDA-MB-231Br spheroids in HA hydrogels with MMP sensitivity showed more invasiveness than those without MMP after 14 days of culture. Further, F-actin staining revealed invaded cells with a well-developed actin cytoskeleton and presence of invasive protrusions at the periphery of spheroids within HA hydrogels containing MMP cleavable peptides as opposed to those without MMP-cleavable peptide incorporation. Overall, these structurally decoupled HA hydrogels provide a platform to study MMP-mediated invasion of metastatic breast cancer spheroids.

Poster #: 42

Enzymatically-Degradable Hydrogel Microcarriers Modulate Secretome of Mesenchymal Stromal Cells

Keshav Shah, Georgia Tech

Keshav R. Shah, Georgia Tech: keshav.shah@gatech.edu

Johnna S. Temenoff, Georgia Tech: johnna.temenoff@bme.gatech.edu

Culture of mesenchymal stromal cells (MSCs) on enzymatically-degradable hydrogel microcarriers (μCs) would enable more efficient cell harvest following in vitro expansion and facilitate in vivo cell delivery. However, the effects of enzymatically-degradable μCs on modulating the secretory properties of MSCs, a main mechanism of action in vivo, remain underexplored . Therefore, we aimed to determine the effect of enzymatically-degradable hydrogel microcarrier formulation on MSC numbers and soluble factor secretion compared to standard non-degradable poly(styrene)-based microcarriers (Synthemax).

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 overnight incubation in collagenase (assessed via phase contrast microscopy). Additionally, PEG-VPM μCs (n=7) were subjected to microscale mechanical testing (20μN preload applied over 1 min followed by compression at 0.5% strain/s). PEG-VPM μCs exhibited a compressive modulus of 54.3 ± 15.8 kPa.

Changes in cell number and secretory activity were evaluated following culture of human MSCs (RoosterBio) for 4d on both PEG-VPM and Synthemax μCs (n=8). Via PicoGreen assay, fold changes in numbers of MSCs 24h and 4d after seeding on PEG-VPM μCs did not differ from that on Synthemax. Multiplex ELISA revealed significant differences in the abundance of secreted factors: MSCs cultured on PEG-VPM secreted 2.1-fold higher levels of the pro-regenerative factor hepatocyte growth factor compared to Synthemax, and the immunomodulatory cytokine interleukin-10 was secreted by MSCs on PEG-VPM but not Synthemax. Additionally, MSCs on PEG-VPM secreted significantly lower levels of several pro-inflammatory factors, including growth-regulated oncogene, interleukin-6, and interleukin-8.

In a follow-on study, pro-inflammatory cytokine licensing (used to augment cytokine secretion by MSCs) was investigated for its impact on MSC numbers on microcarriers. MSCs were cultured on PEG-VPM μCs, Synthemax μCs, and planar poly(styrene) with or without IFN-γ (0, 10, or 100ng/mL) for 4d (n=8). MSC numbers on each surface did not significantly vary with IFN-γ concentration.

Together, these results suggest that hydrogel carrier properties can influence soluble factor secretion from MSCs, and MSC adhesion is maintained in the presence of pro-inflammatory cytokine licensing. Further development of this system may advance both the manufacturing and therapeutic delivery of MSCs.

Poster #: 43

Independent tuning of hydrogel stiffness, viscoelasticity, and integrin engagement to study fibroblast-macrophage crosstalk

Leilani Astrab, University of Virginia

Leilani Astrab1, Lily Burns1, Erica Hui2, Steven R. Caliari1,2

1) Dept. of Biomedical Engineering, University of Virginia

2) Dept. of Chemical Engineering, University of Virginia

la4re@virginia.edu; lgb3em@virginia.edu; eh3ja@virginia.edu; sc6au@virginia.edu

Cells engage with their surrounding extracellular matrix (ECM) via integrin binding, which provides structural support and mechanical stimuli. These cues are converted to biochemical signals through the process of mechanotransduction, allowing cells to both sense and respond to their environment. Studies have shown that dysregulated changes in ECM mechanics can lead to diseases like cancer and fibrosis; however, traditional cell culture platforms for studying these diseases do not capture physiologic mechanics or components of ECM.

To better understand the contribution of changing ECM properties in pathogenesis, our group and others utilize hydrogels to recapitulate the microenvironment of native and diseased tissues. By modifying chemical motifs on a hyaluronic acid backbone, we leverage thiol-ene click chemistry to tune mechanical properties like stiffness and viscoelasticity. Additionally, we can incorporate various cell adhesion motifs that mimic those found in native ECM. While many material-based culture methods utilize the fibronectin-derived RGD peptide, studies show that this motif has lower binding affinity compared to longer peptide/protein fragment domains and can non-specifically bind multiple integrin subunits. Furthermore, these limitations in cell culture methods convolute the investigation of cell-cell interactions. In the context of lung fibrosis, where multiple mechanosensitive cells like macrophages and fibroblasts are driving disease and mechanics of ECM are changing throughout disease progression, there is a need to develop tunable platforms that decouple these factors.

To distinguish integrin-mediated cell activation from substrate mechanics, we have utilized fibronectin-derived fragments that demonstrate preferential integrin engagement (αvβ3 vs. α5β1). Incorporating these fragments into our previously developed, hyaluronic acid-based system allows us to independently tune stiffness, viscoelasticity, and integrin binding. Our group has shown that fibroblasts seeded on substrates that preferentially engage αvβ3 integrin demonstrate increased spreading, actin stress fiber formation, and focal adhesion size, indicative of myofibroblast activation. Here, we found that cadherin 11, a cell-cell adhesion molecule implicated in fibrotic macrophage-fibroblast crosstalk is upregulated in fibroblasts seeded on hydrogels with preferential αvβ3 engagement. We have previously found that fibroblasts co-cultured with M2 macrophages exhibit increased spreading, collagen-1, and cadherin-11 expression regardless of hydrogel mechanics, and anticipate that these trends will uphold on hydrogels with different integrin-specificities.

Poster #: 45

Neural-muscle cell co-cultures in aligned collagen-glycosaminoglycan (CG) scaffolds for skeletal muscle tissue engineering

Ryann Boudreau, University of Virginia

Ryann D. Boudreau, University of Virginia, Department of Biomedical Engineering, zns8mx@virginia.edu

Sarthak Pathak, University of Virginia, Department of Chemical Engineering, kyn6hw@virginia.edu

Geshani Bandara, University of Virginia, Department of Chemical Engineering, bek2hm@virginia.edu

Steven R. Caliari, University of Virginia

Department of Chemical Engineering

caliari@virginia.edu

Volumetric muscle loss (VML) is the traumatic removal of skeletal muscle exceeding natural wound healing capabilities. Treatments are unable to overcome the detrimental effects of VML, including reduced force generation capacity and impaired locomotion. Biomaterials are emerging as promising therapeutic alternatives for skeletal muscle tissue engineering due to their ability to support cellular alignment, neural connection, and electrical excitability necessary for integrated myogenesis and neurogenesis.

We previously created biomaterials for skeletal muscle tissue engineering utilizing a directional freeze-drying process to fabricate aligned and highly porous collagen-glycosaminoglycan (CG) scaffolds made to mimic muscle architecture both in ECM composition and in microstructural organization. While previous work showed that these scaffolds could support in vitro myogenesis and limited functional recovery in vivo, we were interested in further exploring if these scaffolds could support simultaneous myogenesis and neurogenesis for improved functional regeneration. Inspired by recent studies on 2D substrates demonstrating expedited myogenesis when co-culturing muscle cells with neural cells, we hypothesized that seeding muscle-derived cell (MDC) and neural stem cell (NSC) co-cultures into a 3D aligned scaffold would be beneficial for skeletal muscle tissue engineering.

Primary rat MDC-NSC co-cultures were cultured to assess the promotion of robust myotube maturation and NMJ formation within 2D controls and 3D CG scaffolds. Myotube diameter, fusion index, and neuromuscular junction (NMJ) formation were quantified utilizing immunocytochemistry. Results show evidence of co-cultures positively impacting myogenesis on 2D surfaces with neural cells promoting faster maturation of MDCs. Ongoing work is assessing the influence of incorporating skeletal muscle-derived extracellular matrix components (e.g. laminin), with or without electrical stimulation, on further improving myogenesis.

Poster #: 46

Engineering Hydrogels to Investigate the Role of Extracellular Matrix Cues on Natural Killer Cell Functions

Suzanne Lightsey, University of Florida

Blanka Sharma, blanka.sharma@bme.ufl.edu, University of Florida

Madison Temples, templesmadison18@gmail.com, University of Florida

Benjamin Kaufman, bkaufman1@ufl.edu, University of Florida

Gabriella Flinn, flinngabriella@ufl.edu, University of Florida

Natural killer (NK) cells are of interest for immunotherapy due to their ability to detect and kill cancer cells without prior antigen priming. For NK cells to exert their cytotoxic functions, direct contact with cancer cells is needed; however, NK cells that extravasate to the tumor largely remain in the stroma of solid tumors and only a small fraction come into direct contact with tumor cells. The biochemical and mechanical properties of solid tumors are known to play a role in NK cell infiltration and activation; however, it remains unclear if these properties act as a barrier or a substrate. Traditionally, 2D in vitro culture systems are used to perform mechanistic studies of NK cell functions; however, these culture systems do not replicate the 3D tumor microenvironment and, incidentally, correlate poorly with in vivo and clinical studies. The Sharma Lab has established a 3D poly(ethylene) glycol (PEG)-based hydrogel system in which the biochemical and mechanical properties can be independently manipulated to study the effect of tumor extracellular matrix (ECM) on NK cell function. Through the inclusion of hyaluronic acid (HA), a glycosaminoglycan prevalent in lung tumors, we observed increased NK cell infiltration, suggesting that HA may act as a substrate in the tumor microenvironment. Alternatively, when we increased the stiffness of our hydrogel system, we observed a decrease in NK cell infiltration suggesting that solid tumors may utilize mechanotransduction pathways to avoid detection. Future studies will investigate the influence of tumor ECM components, like HA and stiffness, on NK cell activation and elucidate the mechanisms involved.

Poster #: 47

Microporous Particle Scaffolds for Mesenchymal Stromal Cell Expansion and Enhanced Immunomodulatory Function

Thomas Spoerer, University of Georgia

Thomas Spoerer (tms85694@uga.edu):
- School of Chemical, Materials and Biomedical Engineering, University of Georgia, Athens, GA, USA
- Regenerative Bioscience Center, University of Georgia, Athens, GA, USA
Charlotte Williams (charlotte.williams2@uga.edu)
- School of Chemical, Materials and Biomedical Engineering, University of Georgia, Athens, GA, USA
Ross A. Marklein (ross.marklein@fda.hhs.gov)
- School of Chemical, Materials and Biomedical Engineering, University of Georgia, Athens, GA, USA
- Regenerative Bioscience Center, University of Georgia, Athens, GA, USA

Background: Despite promising preclinical studies, challenges in reproducibly manufacturing therapeutic mesenchymal stromal cells (MSCs) at scale hinder their clinical translation. Hydrogel-based culture systems, like microporous particle (MP) scaffolds, have shown to offer a scalable solution while also enhancing MSC function(1,2). To establish MP scaffolds as an alternative to traditional MSC biomanufacturing, we expand MSCs within MPs and compare their immunomodulation to cells grown on traditional tissue culture polystyrene (TCPS). Our findings show MP scaffolds allow for scalable MSC expansion and enhance their immunomodulatory function.

Methods: VF was assessed by incubating MPs with fluorescent dextran (2000kDa) that diffuses into MP pores, and three regions of interest (ROI) were imaged using confocal microscopy (Zeiss LSM900). For cell culture, MSCs from three donors and two tissue sources (RB98-adipose, RB71-bone-marrow, RB62-adipose – RoosterBio) were cultured in 96-well plates within MPs using chemically defined media (CDM). Cell counts were acquired by quantifying dsDNA via PicoGreen. Immunomodulation was assessed via an indoleamine 2,3-dioxygenase (IDO) assay(3). Briefly, MSCs were grown in 2D or 3D for 24 hours, exposed to pro-inflammatory cytokines (50 ng/mL TNFα and IFNϒ) for 24 hours, and subsequently assayed for IDO activity.

Results: MPs exhibited consistent VF (~33%) across three replicates, demonstrating reliable scaffold preparation. After 4 days of MSC expansion in MPs, the three donors proliferated 2X, on average, although significant differences were observed between the three donors. Coefficients of variation (CV) were notably low for each donor in MPs (RB98: 2.17%, RB71: 1.65%, RB62: 1.81%), possibly due to assurance of consistent VF across scaffolds. IDO activity was significantly higher in MSCs within MPs for both control and primed conditions across all donors, excluding the primed RB71 group. These results highlight MP's potential to expand MSCs and enhance their immunomodulatory function in the presence and absence of priming with pro-inflammatory cytokines.

Conclusion: MP scaffolds demonstrated the ability to serve as a viable MSC expansion platform and enhance their immunomodulatory function across multiple donors.

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Poster #: 48

Hydrogel microcarriers reduce development of MSC senescence in small and large scale culture

Zoe Mote, Georgia Institute of Technology

Zoe Mote zmote3@gatech.edu (GT), Elizabeth Ross eross36@gatech.edu (GT), Haaris Jilani hjilani3@gatech.edu (GT), Johnna Temenoff johnna.temenoff@bme.gatech.edu (GT)

Mesenchymal stromal cells (MSCs) have great potential as a regenerative medicine therapy due to their immunomodulatory capacity. However, to reach therapeutic doses, large scale culture in bioreactors is required. One challenge hindering MSC scale-up is development of replicative senescence during expansion, which leads to growth arrest and decreases the quality of the cell product. To effectively scale up MSC culture in bioreactors, microcarriers are necessary prodive adequate surface area. Therefore, the hypothesis of this study is that PEGDA-based microcarriers can serve as substrates to reduce senescence of MSCs cultured in suspension bioreactors. PEGDA microcarriers incorporating the integrin engaging peptide RGD were fabricated using a microfluidic system. Commercially available plastic microcarriers (Synthemax) were used as a control. MSCs from two donors were seeded at 5000 cells/cm2 and cultured for 6 days in agitated 6-well plates or in duplicate PBSmini 0.1L bioreactors. Media was assayed for lactate (n=2-3), cell yields were quantified with picogreen (n=3) and cell counts (n=2), and cells were stained for -gal (n=4-6). Cells from bioreactors were co-cultured with macrophages and TNF- concentrations were assayed using an ELISA (n=5). In well plate cultures, similar trends in lactate accumulation and cell yields were seen between PEGDA and Synthemax microcarriers. When stained for senescence-associated -galactosidase, cells on Synthemax showed increased staining compared to cells on PEGDA with nearly 50% of cells staining positive for -gal compared to less than 10% on PEGDA. In bioreactors, lactate accumulation was similar between microcarrier types, but cell yields indicate that PEGDA may support improved growth at larger scale, while -gal staining trends were maintained. After bioreactor expansion, all cells significantly suppressed secretion of TNF- from macrophages, and PEGDA-cultured cells suppressed to a degree that was not significantly different than the negative control, indicating that PEGDA carriers support MSCs that retain immunomodulatory capacity. -galactosidase results indicate that PEGDA based microcarriers may be used to reduce development of senescence in MSC culture. From these studies, PEGDA microcarriers were shown to serve as an equivalent or superior surface vs commercially-available microcarriers for MSC culture at both small and larger scale.

Poster #: 49

T follicular helper-like cell differentiation, encapsulation, and culture in hydrolytically degradable microgels

Adya Verma, Georgia Institute of Technology

Valeria M. Juarez (valeria.juarez@gatech.edu) 1, Ana Mora Boza (aboza3@gatech.edu) 2, Adya Verma (averma320@gatech.edu) 1, Andrés J. García (andres.garcia@me.gatech.edu) 1,2, Ankur Singh (ankur.singh@me.gatech.edu) 1, 2

Organizations:

1 Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA

2 Woodruff School of Mechanical Engineering and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, 315 Ferst Drive, 2310 IBB Building, Atlanta, GA, USA

T follicular helper (Tfh) cells provide functionally crucial signals to B cells in secondary lymphoid tissues, like the lymph node, during an adaptive immune response. However, obtaining high quantities of functional Tfh cells to model these responses ex vivo can be difficult solely from primary tissue isolation. Here, we obtained naïve CD4+ T cells from a bulk human peripheral blood mononuclear cells (PBMC) sample and evaluated their encapsulation and maintained culture within hydrolytically degradable microgels. We then explored their differentiation into Tfh-like cell phenotypes using cytokine stimulation with and without biomaterial-cell interactions. Our microgels are generated using a microfluidic droplet generator composed of a PEG-4MAL biomaterial at <100 μm diameter. Optimized concentrations of ethylene glycol bis-mercaptoacetate (EGBMA), a hydrolytic crosslinker, within the microgel allows for highly tunable degradation kinetics. Isolated CD4+ T cells from human donors were resuspended in macromer densities of PEG-4MAL polymer solution and pre-functionalized with a fibronectin-mimic RGD peptide and FITC for in vitro tracking of microgels. Microgel degradation, swelling kinetics, and T cell viability in vitro were monitored by optical microscopy, and confocal imaging. T cell microgels were cultured with IL-12 and Activin-A to support differentiation into more Tfh-like phenotypes. Using flow cytometry, we evaluated the recovered Tfh cells and observed increases in CD40L expression after 5 days in culture, a key signal that Tfh cells provide to B cells. Media from cultured 2D and microgel conditions were also evaluated for secretion of IL-21 and other key T cell cytokines using ELISA and Legendplex. This research will establish foundational knowledge of T cell differentiation within hydrogels and will ultimately be applied to generate a functional T cell zone mimicking the human lymph node for exploration of adaptive immune responses in vitro.

Poster #: 50

SA-FasL Microgels Designed to Promote Immune Tolerance

Angelica Torres, Georgia Institute of Technology

Angelica Torres, Georgia Institute of Technology, alt7@gatech.edu

Ana Mora-Boza, Georgia Institute of Technology, aboza3@gatech.edu

Stephen Linderman, Georgia Institute of Technology, slinderman6@gatech.edu

Sophia Kioulaphides, Georgia Institute of Technology, skioulaphides3@gatech.edu

Alex Heiler, Georgia Institute of Technology, aheiler3@gatech.edu

Paul Archer, Georgia Institute of Technology, paul.archer@me.gatech.edu

Susan Thomas, Georgia Institute of Technology, susan.thomas@gatech.edu

Esma Yolcu, University of Missouri, esma.yolcu@health.missouri.edu

Haval Shirwan, University of Missouri, haval.shirwan@health.missouri.edu

Andres Garcia, Georgia Institute of Technology, andres.garcia@me.gatech.edu

Type 1 Diabetes (T1D) affects 1.6 million Americans and accounts for over $15 billion in annual health care costs. T1D is characterized by the irreversible, autoimmune destruction of the insulin producing β-cells in the pancreas. Without the production of insulin, blood glucose levels increase causing life-threatening complications. Allogeneic transplantation of islets is the only treatment with curative intent. However, this is limited by lack of available donors and by graft failure in most patients within 5 years due to both acute and chronic rejection of the islets. Current immunosuppressive drugs contribute to graft failure by direct toxicity to transplanted islet β cells and by worsening peripheral insulin resistance. Therefore, there is a need to create methods to induce tolerance of transplanted islets to develop a cure for T1D. Previously demonstrated by our lab, we can co-deliver 200µm microgels that present Streptavidin-Fas Ligand (SA-FasL) to promote the immune acceptance of transplanted islets in non-human primates. Additionally, these 200µm microgels still induce mouse A20 cell apoptosis 2 months after being manufactured demonstrating their off-the-shelf potential. However, SA-FasL is only presented on the surface of the microgels so a downfall of the 200µm gels is that most of the space that they take up in the graft is not used to deliver SA-FasL. Therefore, we have developed smaller (50µm) microgels with SA-FasL that induce the apoptosis of mouse A20 cells like that of the larger microgels. Promoting immune acceptance of islets with these microgels pushes the field closer to developing a cure for T1D.

Poster #: 51

Exploring Effects of Surface Conjugated poly(ethylene) glycol on Pancreatic Islets: Assessing T cell Migration in a 3D Co-culture environment

Damea Pham, University of Florida

Damea Pham, University of Florida, dameapham@ufl.edu

Magdalena M. Samojlik, University of Florida, samojlik@ufl.edu

Irayme Labrada-Miravet, University of Florida, ilabrada@bme.ufl.edu

Cherie L. Stabler, University of Florida, cstabler@bme.ufl.edu

Background: Grafting long-chain poly(ethylene) glycol to pancreatic islets increases graft efficacy in both murine and non-human primate models. However, specific mechanisms for how PEG impairs graft rejection have yet to be fully elucidated. Herein, we utilized an in vitro immune screening platform to gain insight into how PEGylation modulates immune attack. Using an antigen specific system, we can interrogate impacts of PEG grafting on T cell mobility.

Methods: Pancreatic islets were derivatized with linear PEG (5000 Da, JenKem, Inc.) as previously described(2). OTI-GFP CD8 T cells were isolated, cultured, and activated for 48 hours as previously described before being cultured with free or PEGylated mOVA or B6 islets in decellularized pancreatic ECM hydrogel, prepared as described elsewhere and adapted for culture of immune cells and pancreatic islets3. Time-lapse confocal imaging of activated T cells and PEGylated islets in 3D culture was conducted over 16 hours. Murine antigen-specific models were compared to murine non-specific models to elucidate differences in migration patterns. T cell migration parameters quantified following time-lapse imaging using the ImageJ TrackMate plug-in. To evaluate PEGylated islet viability and function, Live/Dead imaging was performed using a LIVE/DEAD Viability Cytotoxicity Assay kit and static glucose stimulated insulin secretion (GSIS) assays were performed as described elsewhere.

Results: Interrogation of the impact of linear PEG grafting on effector CD8 T cell mobility found that effector CD8 T cells cultured with untreated islets show no differences in migration parameters when compared to T cells cultured with linear PEG formulations in either antigen-specific or non-specific models. This indicates that effector CD8 T cells are not repelled by the PEG grafted to the islet surface.

Conclusions: Both antigen specific and non-specific systems reveal that PEGylation has no impacts on effector CD8 T cell migration patterns following their interaction with the PEGylated islet. Thus, observed benefits of PEGylation in animal models is unlikely due to repelling of already activated immune cells. Future work will further investigate if PEGylation plays more a role in impairing the activation of T cells.

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Poster #: 52

PEG-Crosslinked Vinyl Azlactone Hydrogels with Tunable Architecture for Sequential Release of Multiple Immunomodulating Drugs

Emily Rasmussen, University of Mississippi

Adam Smith, aes@olemiss.edu, University of Mississippi

Thomas Werfel, tawerfel@olemiss.edu, University of Mississippi

Immunotherapy provides an exciting alternative to traditional chemotherapeutics for cancer treatment. However, current immunomodulating approaches to cancer treatment are hindered by their poor pharmacokinetics after intravenous administration and accumulation at off-target sites. Therefore, we proposed the use of an implantable hydrogel for the controlled release of multiple immunotherapeutic cargos in concert. Using poly(2-vinyl-4,4-dimethylazlactone) (pVDMA) and functionalized PEG crosslinkers, we were able to release multiple drug classes at different rates dependent on crosslinking composition and density. Further, we demonstrated burst release of a small molecule and sustained release of a monoclonal antibody from the same hydrogel.

Crosslinking density and composition were varied to construct a library of hydrogels with various properties. The gels were then incubated in buffer and measurements were taken to quantify the degradation and release of therapeutic agents over time.

We were able to release multiple drug classes from the hydrogels including small molecule, polymer-drug conjugate, protein, antibody, and nanoparticle. By tuning the crosslinking density and composition, differential release profiles were achieved for the drug classes dependent on their size and the composition of the hydrogel. Notably, while small molecules, polymer-drug conjugates, and proteins achieved near-complete release within an initial three-day window, antibodies manifested sustained release kinetics over a span of two weeks. Furthermore, nanoparticle release profiles exhibited pronounced sensitivity to crosslinking density, with densely crosslinked gels demonstrating reduced release efficiency compared to their lower crosslinked counterparts. Additionally, we have been able to demonstrate dual release of a small molecule and antibody from the same hydrogel, indicating the hydrogel could be used for immunotherapy that requires the release of multiple drug classes at different rates.

The ability to finely control therapeutic release across various drug classes presents an exciting avenue for the development of multifunctional therapies, capable of eliciting specific, localized immune responses. Overall, the programmable hydrogel platform holds promise for advanced materials with tunable degradability and controlled release, offering broad utility in biomedical applications. Further studies will measure the anti-tumor effects of immunotherapeutic agents released from these hydrogels in a mouse model.

Poster #: 53

Antigen-loaded extracellular vesicles to regulate adaptive immune responses and drive immunological tolerance

Hayden Pagendarm, Vanderbilt University

Youn Jae Jung (youn.jae.jung@vanderbilt.edu) - Vanderbilt University

Alexander Kwiatkowski (alexander.kwaitkowski@vanderbilt.edu) - Vanderbilt University

Christopher Lofts (christopher.a.lofts@vanderbilt.edu) - Vanderbilt University

Taylor Patino (taylor.m.patino@vanderbilt.edu) - Vanderbilt University

Jacob Schulman (jacob.a.schulman@vanderbilt.edu) - Vanderbilt University

Karan Arora (karan.arora@vanderbilt.edu) - Vanderbilt University

John T. Wilson (john.t.wilson@vanderbilt.edu) - Vanderbilt University

Autoimmune diseases are driven by adaptive immune responses that are inappropriately directed towards an autoantigen. Current treatments primarily rely on broad immunosuppression, which leaves patients susceptible to viral infection and cancer progression without providing a permanent cure. Tolerogenic vaccines capable of tolerizing the immune system towards a particular autoantigen posit a potential treatment without the drawbacks of broad immunosuppression. Unfortunately, the clinical success of such therapies has been limited by their inabilities to: 1) adequately deliver sufficient antigen to the lymphatics, where T cell priming occurs and 2) spatiotemporally co-deliver antigen and tolerizing adjuvant to reprogram antigen presenting cells (APCs) towards a suppressive phenotype. To this end, we have developed an extracellular vesicle (EV)-based platform for the co-delivery of antigens and immunoregulatory cues. EVs are cell membrane enclosed nanoparticles (~50-200 nm) secreted by all cells as a means of intercellular communication via the transfer of various biomolecules. Additionally, many types of EVs are intrinsically immunosuppressive and involved in maintenance of immune tolerance. In this work, we aimed to exploit the size-dependent lymphatic trafficking and intrinsic immunosuppressive effects of exogenously administered EVs to induce tolerogenic T cell priming by APCs, leading to CD4+ regulatory T cell (Treg) proliferation and CD8+ effector T cell (Teff) deletion/anergy. EVs were isolated from HEK293SF-3F6, B16.F10, hTERT-MSC, and BeWo cells and functionalized with dibenzocyclooctyne (DBCO) moieties using a DBCO-PEG12-TFP Ester linker. Azide-functionalized moieties were ligated to DBCO-EVs via overnight incubation. EVs were characterized using NTA, western blot, and TEM. EVs were screened for immunosuppressive potential in vitro using bone marrow-derived dendritic cells (BMDCs). BMDCs were challenged with lipopolysaccharide (LPS) and then treated with EVs. Flow cytometry was used to quantify expression of CD80, CD86, CD40, and MHC II. The ex vivo biodistribution of EVs was evaluated by labeling DBCO-EVs with azide-Cy5 and interrogating the tissue- and cell-specific delivery using IVIS and flow cytometry, respectively. Peptide antigens were synthesized with N-terminal azido groups using solid phase peptide synthesis (SPPS). Antigen-loaded EVs were evaluated in vivo using OT-I and OT-II adoptive T cell transfer models as well as an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS).

Poster #: 54

Utilizing protein nanosheets as a scaffold for therapeutic proteins in cancer immunotherapy.

Ishmamul Hoque Sadab, Georgia Institute of Technology

Ishmamul Hoque Sadab, Yusef Amir Haikal, Raka Chakraborty, John Blazeck, Julie A. Champion.

School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, 950 Atlantic Drive NW, Atlanta, GA, 30332 USA

isadab3@gatech.edu ; yhaikal3@gatech.edu ; rchakraborty37@gatech.edu ; john.blazeck@chbe.gatech.edu ; julie.champion@chbe.gatech.edu

The efficacy of intratumorally administered cancer immunotherapies relies on the specificity and localization of therapeutic macromolecules within the tumor microenvironment. To this end, we propose protein nanosheets (pNS) for controlled and prolonged presentation of therapeutic proteins in the tumor. pNS are self-assembled two-dimensional protein materials with thickness less than 100 nm. Our synthesis methodology entails aqueous self-assembly through a straightforward process, wherein two fusion amphiphilic proteins are mixed, followed by end-to-end rotation for a predetermined duration. The distinct hydrophilic and hydrophobic moieties in the fusion proteins contribute to the alignment of protein assemblies at the air-water interface during pNS synthesis. The pNS are compatible with different sized globular proteins spanning 19.39-86.80 kDa and can be synthesized out of two different globular proteins. Modulating protein quantity during synthesis, along with rotation time and speed yields pNSs with approximate size ranges, definite pNS concentration and different concentrations of globular proteins on their surface. These pNS show lower diffusivity in mice-derived tumors imposed by their micro-scale size. They exhibit enzymatic activity comparable to soluble control and are structurally stable in the human pooled serum for a prolonged time. The versatility in the pNS synthesis process coupled with their limited diffusivity, high loading capacity and better stability make them a suitable platform for localized delivery of proteins and enzymes for tumor-targeted cancer immunotherapy.

Poster #: 55

Smart Prodrug Synthesis for Controlled Release of Active Sting Agonists

Karan Arora, Vanderbilt University

Karan Arora, Vanderbilt University ;karan.arora@vanderbilt.edu

Jacob A Schulman, Vanderbilt University ;jacob.a.schulman@vanderbilt.edu

John T Wilson Vanderbilt University; john.t.wilson@vanderbilt.edu

Controlled drug release can be achieved through prodrug development, which enhances the therapeutic efficacy but reduces off-target effects. A new avenue for synthesis of smart prodrugs that are capable of releasing active sting agonists in a controlled manner is proposed herein. By following the principles of prodrug design and adopting stimuli-responsive linker molecules, we hope to achieve precise spatiotemporal control over drug release and consequently promote therapeutic outcome. Our strategy involves conjugation of sting agonists to biocompatible carriers by means of cleavable linkers that respond to certain environmental cues such as enzymes or external stimuli such as light, pH etc. These prodrugs become active in a spatiotemporal fashion. This leads into the selective liberation of an active sting agonist upon administration. Such a controlled release system maximizes not just drug efficiency but also minimizes systemic exposure thereby limiting toxicity. By pursuing this, we anticipate significant advancements in immunotherapy research.

Poster #: 56

Engineered hydrogel platform for the study of B cell-stromal interactions in Germinal Centers.

Manuel Quinones Perez, Georgia Institute of Technology

Zhe Zhong, Georgia Institute of Technology, zzhong47@gatech.edu

Christopher Carlson, Georgia Institute of Technology, christopher.carlson@gatech.edu

Valeria Juarez, Georgia Institute of Technology, valeria.juarez@gatech.edu

Stephanie Vagelos, Georgia Institute of Technology, svagelos3@gatech.edu

Alejandro Molina, Georgia Institute of Technology, amolina34@gatech.edu

Ankur Singh, Georgia Institute of Technology, ankur.singh@me.gatech.edu

Successful immune responses to vaccines or pathogens are characterized by the presence of high affinity antibodies and immunological memory. B cells, who are responsible in the production of humoral immune responses, can generate high affinity antibodies through Germinal Center (GC) structures found in Secondary Lymphoid Organs. However, the cells involved in the GC reaction go beyond B cells and T cells, it also involves non-hematopoietic (defined by the lack of CD45 expression) cells commonly named stromal cells. These cells are responsible for the maintenance of the lymphoid niche by secretion of survival factors, production of extracellular matrix (ECM), lymph node expansion and contraction, and antigen presentation. Although much of stromal immunology knowledge has been generated using animal models, not much success has been made when translating these findings in human ex vivo models. Therefore we propose the use of a synthetic or semi-synthetic hydrogel system to better understand B cell-stromal immunology. Stromal cells were obtained from tonsil tissue removed from pediatric patients and were processed to a single cell suspension with or without stromal cells prior to hydrogel encapsulation. First we conducted studies using a PEG-4MAL hydrogel system with ECM derived cues in the presence or absence of stromal immune cells. Our findings showed that in the presence of stromal cells the number of B cells, T cells and antibody secreting cells (defined by CD19+CD27++CD38++) was significantly increased. We also noted an increase in the concentration of B cell survival cytokine IL6 and chemokine CXCL12 after 12 days in culture in our hydrogel system in the presence of stromal cells. Due to the presence of collagen in native tissue, we hypothesized that the presence of collagen would further increase the survival of stromal cells and further increase the survival of other immune cells as well. Our findings demonstrated that when collagen is included in our synthetic hydrogel system, we saw a 2-fold expansion in B cells and stromal cell populations is increased. All together our results highlight the benefit of a combined semi-synthetic system for studying of B cells and stromal cell interactions ex-vivo.

Poster #: 57

Development of Self-Assembled Protein Vesicles Displaying Alpha C Antigen as a Novel Group B Streptococcus Maternal Vaccine for Enhanced Protection

Mariela Rodriguez-Otero, Georgia Institute of Technology

Mariela Rodriguez-Otero, Georgia Institute of Technology, m.rodriguez@gatech.edu

Julie A. Champion, Georgia Institute of Technology, julie.champion@chbe.gatech.edu

Protein vesicles self-assembled from recombinant fusion proteins are biocompatible, tunable, and stable, making them candidates for vaccine applications. Recombinant protein vesicles are self-assembled from Globule-Zipper-ELP protein complexes, where “Globule” is a globular protein, zipper is a coiled coil, and ELP is a thermoresponsive elastin-like-polypeptide that exhibits lower critical solution temperature phase behavior. Protein vesicles exhibit stability at physiological conditions with tunable size and allow protein antigens to be displayed on the surface with tunable antigen concentration and conserved antigen structure. We hypothesize that a protein vesicle-based subunit vaccine will enhance the strength of cellular and humoral immune responses compared to soluble antigen. In this work, a protein vesicle maternal vaccine was designed against Group B Streptococcus (GBS), as it is the leading cause of life-threatening infections in newborns. Stable vesicles were developed using Alpha C, a GBS antigen, and an ELP containing tyrosine at 5 guest residue positions. These vesicles exhibited long-term stability at physiological conditions, and by increasing the ZE/ZR ratio, the diameter was reduced to 50 nm to facilitate drainage into lymphoid organs and allow direct interaction of vesicles with follicular B cells for enhanced humoral responses. Alpha C vesicles elicited enhanced bone marrow dendritic cell activation of MCH II+ and CD86+ markers when compared to soluble Alpha C. Upon evaluation of the viability of functional protein vesicles displaying a GBS antigen, this vaccine design will be tested in mice and results of vaccination comparing Alpha C vesicles and soluble Alpha C will be presented, including humoral and cellular immune responses.

Poster #: 58

Adjuvanted lymph node-resident and lymph node-migratory antigen presenting cells differently influence the T-cell response to immune checkpoint blockade

Samuel Lucas, Georgia Institute of Technology

Samuel N. Lucas, Georgia Institute of Technology, slucas30@gatech.edu

Margaret P. Manspeaker, Georgia Institute of Technology, mmanspeaker3@gatech.edu

Alisyn R. Bourque, Georgia Institute of Technology, bourque.a@gatech.edu

Susan N. Thomas, Georgia Institute of Technology, susan.thomas@gatech.edu

A key research problem in cancer immunotherapy is developing further understanding of factors that drive T-cell antitumor responses to immune checkpoint blockade (ICB). Increasingly, tumor-draining lymph nodes (TdLNs) have been understood to play a critical role in the T-cell response to ICB. This is because TdLNs facilitate the co-mingling of T-cells, tumor antigen, and antigen-presenting cells including conventional dendritic cells (cDCs) to enable T-cell activation and differentiation. cDCs can be delineated into resident cDCs, which access tumor antigen draining into the TdLN through lymphatic capillaries, and migratory cDCs, which take up tumor antigen in the periphery before trafficking to the TdLN. Previous work in our lab has used an engineered particle system to implicate resident and migratory cDCs in promoting distinct T-cell responses to antigen presentation in the TdLN, with resident cDCs associating with a more stem-like CD8+ T-cell phenotype and migratory cDCs associating with a more effector-like CD8+ T-cell phenotype. These phenotypes of CD8+ T-cell are known to respond differently to ICB. Inspired by this, we hypothesize that separately adjuvanting TdLN-resident and TdLN-migratory cDCs will enable delineation of their relative contributions to T-cell response to ICB. To investigate this, we react pyridyl disulfide-functionalized poly(propylene) sulfide nano- or microparticles with the TLR9 agonist CpG to create 30 nm CpG-NPs that can drain to TdLNs via lymphatics and 500 nm CpG-MPs that are transported to TdLNs by migratory cDCs. These CpG-NPs/MPs are injected through various routes of administration into B16F10 tumor-bearing mice and analyzed to determine their biodistribution (IVIS, flow cytometry) and their immunomodulatory and therapeutic effects when combined with ICB (immune phenotyping, tumor growth, survival). Preliminary results using 30 nm CpG-NPs in combination with ICB indicate that CpG-NP delivery to TdLN-resident cDCs is accompanied by an increase in cDC1 presence and maturation in the TdLN. This in turn couples with ICB to induce increased differentiation of stem-like CD8+ T-cells to effector-like CD8+ T-cells in the TdLN. Furthermore, this differentiation effect is accompanied by an increase in mobilization of antigen-experienced PD-1+ CD8+ T-cells into the blood when adjuvanting LN-resident cDCs in the TdLN but not the non-draining lymph node (NdLN).

Poster #: 59

Supramolecular peptide-protein granules for tunable intracellular protein delivery

Stephanie Herrera, University of Florida

I prefer poster presentation only

The ability to deliver active proteins across the cell membrane and into the cytosol would provide access to druggable targets not available in the extracellular environment. However, proteins do not efficiently cross the cell membrane to enter the cytosol on their own. Here we will present the development of supramolecular peptide-protein granules for intracellular protein delivery. This approach utilizes charge-complementary molecules known as, “CATCH(+) peptides” and “CATCH(-) fusion proteins”. Alone, CATCH(-) and CATCH(+) remain in the soluble state, but combined form b-sheet fibrils. After introducing a crowder to the mixture, such as polyethylene glycol (PEG), Tween-80 micelles, or excess CATCH(+) peptide, 100-200 nm “CATCH(+/-) granules are formed at peptide concentrations ~10-fold lower than the critical fibrillization limit in dilute conditions (~200 mM). CATCH(+/-) granules are rapidly internalized by various adherent and suspended mammalian cell types including fibroblasts, HEK293, T cells, dendritic cells, monocytes, and neutrophils. Different CATCH-fusion proteins can be co-assembled into multicomponent granules with tunable composition. Multicomponent granules allow for co-delivery of both an “effector” (i.e., a protein that confers therapeutic function) along with a “selector”, where the latter can be used to enrich the sub-population of cells that have internalized the CATCH(+/-) granule. CATCH(+/-) granule internalization demonstrates greater delivery efficiency and lower cell death than other state-of-the-art methods, such as cell penetrating peptides or electroporation. Collectively, these data establish CATCH(+/-) granules as a simple and flexible nanomaterial platform cell engineering via intracellular protein delivery.

Poster #: 60

Multilayered Cryogel for Macrophage Modulation Toward Anti-Tumor Immunity

Sydney Henriques, Vanderbilt University

Evan B. Glass, PhD; Vanderbilt University; evanbglass15@gmail.com
Ori Z. Chalom, Vanderbilt University; ori.z.chalom@vanderbilt.edu
Kristen L. Hoek, PhD; Vanderbilt University; kristen.l.hoek.1@vanderbilt.edu
Sarah M. Goldstein, Vanderbilt University; sarah.m.goldstein@vanderbilt.edu
Andrew J. Wilson, PhD; Vanderbilt University Medical Center; andrew.j.wilson@vumc.edu
Renjie Jin, PhD; Vanderbilt University; renjie.jin@vanderbilt.edu
Fiona E. Yull, DPhil; Vanderbilt University; fiona.yull@vanderbilt.edu
Todd D. Giorgio, PhD; Vanderbilt University; todd.d.giorgio@vanderbilt.edu

The tumor microenvironment (TME) presents multiple dysregulated signals polarizing macrophages towards M2 functions, thus suppressing anti-tumor immunity. The spatiotemporal regulation of drug release in the TME is critical to tumor associated macrophage (TAM) activation toward M1 functions and the restoration of functional immunosurveillance. Immunomodulators, such as cytokines, offer promise in TME modulation and promote immune cell infiltration. To prevent toxicity from the systemic delivery of these immunomodulators, localized delivery is necessary. We have implemented an injectable cryogel (hydrogel fabricated at -20oC) to act as a delivery depot for inflammatory cytokines IFN-γ and IL-12, as well as CCL2, a chemokine that attracts pro-tumor, M2-like macrophages. The aim of this cytokine-loaded cryogel is to draw M2-like TAMs from the tumor and repolarize them toward M1-like inflammatory functions to induce anti-tumor immunity.

We first 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. This localized treatment primes the TME for subsequent T cell-based immunotherapies like immune checkpoint blockade.

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 inflammatory 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 induce an inflammatory tumor microenvironment to suppress tumor growth and prime the tumor to be susceptible to other therapies, such as immune checkpoint blockade.

Poster #: 61

Optimization of siRNA Nanoparticles with Custom Polymeric Surfactants

Amelia Soltes, Vanderbilt University

Nora Francini, Vanderbilt University, nora.francini@vanderbilt.edu

Ellie Okonak, Bucknell University, ellie.okonak@gmail.com

Shrusti Patel, Vanderbilt University, shrusti.s.patel@vanderbilt.edu

Richard D'Arcy, Vanderbilt University, richard.darcy@vanderbilt.edu

Carlisle DeJulius, Vanderbilt University, carlisle.r.dejulius@vanderbilt.edu

Shubham Gulati, Vanderbilt University, shubham.gulati@vanderbilt.edu

Craig Duvall, Vanderbilt University, craig.duvall@vanderbilt.edu

Small interfering RNA (siRNA) is a promising therapy for regulation of specific gene expression, but endolysosomal escape presents a barrier against intracellular bioavailability and silencing potency. Here, siRNA-loaded nanoparticles (si-NPs) are conceptualized, synthesized, and screened for intracellular 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 disruption for endosome escape. New custom polymeric surfactants have been developed with increased anchoring to the si-NP core to promote stability while maintaining gene knockdown activity and cytocompatibility. The surfactants are diblock copolymers with an anchoring block that includes varied density and length of alkyl side groups in order to investigate and optimize the contributions of hydrophobic interactions in anchoring to the si-NP core.

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. PLGA, PDB, and siRNA were dissolved in acetonitrile and mixed with the surfactant dissolved in deionized water in the CIJ mixer. Dynamic light scattering (DLS) was performed to evaluate the size, polydispersity index (PDI), and zeta potential of the si-NPs, which were found to have a narrow size distribution and neutral charge. The viability and gene silencing activity of the si-NPs loaded with luciferase-targeting siRNA were then assessed in luciferase-expressing MDA-MB-231 cells 48 hours after treatment. Luciferase knockdown was achieved while maintaining cell viability. Further studies are ongoing to determine a lead si-NP formulation.

Poster #: 62

Development of ingestible molecular probes for non-invasive detection of sucrase-isomaltase deficiency

Carly Kimpling, Georgia Institute of Technology

I prefer poster presentation only

Sucrase-isomaltase (SI) is an important intestinal brush border glycosidase that breaks down dietary sucrose into the monosaccharides, glucose and fructose, for absorption in the small intestine. Individuals with congenital sucrase-isomaltase deficiency (CSID) have an intolerance to sucrose and experience chronic post-prandial symptoms such as gas, bloating, diarrhea, and pain due to fermentation of intact sucrose by the microbiome in the large intestine. CSID is currently diagnosed via endoscopy and collection of biopsy samples from the small intestine, which are used in ex vivo assays to measure SI activity. However, less invasive diagnostic tests are needed as CSID usually presents at an early age in pediatric patients. Breath tests are non-invasive and rapid alternatives and enable diagnosis via detection of disease-indicating volatile organic compounds in breath (i.e. breath biomarkers). In this work, we sought to develop a method to induce the exhalation of breath biomarkers reflecting intestinal SI activity for non-invasive detection of CSID. Glucose-based probes were synthesized containing covalently bound volatile reporter molecules. We hypothesized that ingested probes would release volatile reporters upon degradation by active SI in the small intestine and that liberated reporters would diffuse from the intestinal lumen into blood circulation and be exhaled after pulmonary gas exchange. Probe specificity for sucrase activity was confirmed by reacting probes with sucrase and other common intestinal glycosidases and analyzing the reaction headspace for signal from volatilized reporters using mass spectrometry. Volatile reporter signal was only observed when probes were reacted with sucrase. Non-specific probe degradation was also not observed in tissue homogenates from the mouse GI tract. When probes were administered via oral gavage into healthy mice, breath signal from exhaled reporters was observed during the time the probe transited the small intestine (0-1.5 h after oral gavage). No signal was observed from mice administered vehicle controls. Pre-treatment of mice with sucrase inhibitors reduce the induced breath signal, indicating that the breath signal is specifically driven by intestinal sucrase activity. Altogether, we have developed ingestible probes that can be used for breath-based assessment of intestinal sucrase activity to provide a non-invasive alternative to CSID diagnosis via endoscopy and biopsy.

Poster #: 63

An asparagine-rich polypeptide tag for the assembly of protein gels

Eric Hill, University of Florida

Stephen Michel, University of Florida, smichel7845@ufl.edu

Natasha Sequeira, University of Florida, nsequeira@ufl.edu

Benjamin Keselowsky, University of Florida, bkeselowsky@bme.ufl.edu

Gregory Hudalla, University of Florida, ghudalla@bme.ufl.edu

Certain amino acid sequences give rise to polypeptides that form supramolecular networks in response to specific external stimulus. Here, we have identified an asparagine rich polypeptide sequence, (GGGSGGGSGGNWTT)10 or "NGT," that when fused to superfolder green fluorescent protein (NGTsfGFP) or NanoLuc luciferase (NGTnL), assembled into supramolecular network at reduced temperatures. These fusion proteins were recombinantly expressed in Origami B (DE3) competent cells and purified in acceptable yields as soluble protein. Both NGTsfGFP and NGTnL retained the activity of the fused protein, demonstrated by NGTsfGFP emitting green fluorescence in blue light, and NGTnL emitting blue light in the presence of the chemical substrate furimazine. Oscillatory rheology characterized the resulting materials as a weak viscoelastic gel which exhibited self-healing after mechanical disruption and softening with increasing temperature. Gel formation was disrupted through mutation of the asparagine residues in the NGT sequence, installation of glucose onto the asparagine residues via Actinobacillus plueropneumoniae N-glycosyltransferase, or use of a chaotropic agent, suggesting a role of asparagine hydrogen-bonding in supramolecular network formation. Due to gel formation being mediated by NGT, NGTsfGFP and NGTnL could co-assemble into a two-component gel. This multifunctional gel emitted both green and blue light in the presence of furimazine, demonstrating bioluminescence resonance energy transfer between the nL and sfGFP domains. These data position NGT as a temperature-responsive polypeptide tag that can be used to create functional biomaterials from soluble fusion proteins synthesized by cell-based hosts.

Poster #: 64

Hemostatic and Wound-Targeting Antibacterial Silver-Composite Injectable Microgels Prevent Biofilm Growth in Synthetic Wound Media

Ethan Pozy, NC State University

I would like to be considered for a Rapid Fire Oral Presentation

The risk of bacterial infection is increased following traumatic injury due to both the disruption of physical barriers to the environment and dysregulation of the immune system. An estimated 65-80% of human infectious diseases are caused by biofilm-forming bacteria that incorporate extracellular material into networks that protect the pathogen from immune cells. Bacteria in biofilms undergo epigenetic and metabolic changes that greatly reduce the susceptibility of the microbes to antibiotic treatment. These biofilms are implicated in infected and non-healing chronic wounds. We have developed a platelet-mimicking nanosilver microgel composite comprised of a polyNIPAM microgel loaded with silver nanoparticles (AgULCs) that demonstrate antibiotic activity. These microgels are decorated with fibrin-specific antibodies to form platelet-like particles (AgPLPs) that bind to and increase the density of fibrin clots present at injury and augment hemostasis. Incubation with AgULCs and AgPLPs have been shown to halt the growth of S. aureus in broth microdilution assays, and incubation with AgULCs has demonstrated a dose-dependent reduction and prevention of S. aureus biofilm biomass and metabolism in a synthetic wound media that has been shown to drive similar genetic expression to bacterial wound infections in vivo. AgPLPs present a potential therapeutic platform to improve traumatic injury outcomes with hemostatic and antibacterial functionality.

Poster #: 65

The Art of War: S. aureus Biofilm Defensive Tactic Provides Offensive Opportunity

Grant Scull, The Joint Department of Biomedical Engineering at UNC-Chapel Hill and North Carolina State University

Grant Scull, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Adrian Aligwekwe, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Ysabel Rey, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Drew Koch, Comparative Medicine Institute, NC State University, Raleigh, NC, College of Veterinary Medicine, NC State University, Raleigh, NC;

Kimberly Nellenbach, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Ana Sheridan, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Sanika Pandit, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Jennifer Sollinger, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC;

Joshua G. Pierce, Comparative Medicine Institute, NC State University, Raleigh, NC, Department of Chemistry, NC State University, Raleigh, NC;

Matthew Flick, UNC-Chapel Hill School of Medicine, Chapel Hill, NC;

Jessica Gilbertie, Edwad Via College of Osteopathic Medicine, Blacksburg, VA;

Lauren Schnabel, Comparative Medicine Institute, NC State University, Raleigh, NC, College of Veterinary Medicine, NC State University, Raleigh, NC;

Ashley C. Brown, Joint Department of Biomedical Engineering, NC State University and UNC-Chapel Hill, Raleigh, NC, Comparative Medicine Institute, NC State University, Raleigh, NC

Introduction:

S. aureus is a bacterial species that is highly infectious upon breach of the dermis. Bacteria persist in the wound site by generating a protective biofilm matrix. S. aureus secretes virulence factors that: recruit the host's machinery to produce fibrin via coagulase; manipulate fibrin into a protective biofilm matrix via Clumping Factor A; and commence biofilm degradation for dissemination via staphylokinase. Fibrin biofilms reduce antimicrobial therapeutic efficacy and stymie the immune system. Despite these defensive capabilities, the fibrin-binding tactic employed for biofilm formation is exploitable. We have

developed fibrin-based nanoparticles (FBNs) that can load antimicrobial therapeutics like vancomycin for dermal biofilm administration. FBNs can penetrate and be incorporated into the biofilm matrix by bacteria, providing a targeted delivery vehicle that increases therapeutic contact time.

Methods/Results:

FBNs were synthesized via enzymatic thrombin-fibrinogen reaction, followed by fibrin clot straining and sonication. FBNs were characterized by atomic force microscopy and NanoSight nanoparticle tracking analysis for dry height (~300nm) and diameter (~15nm), and hydrodynamic diameter (~215nm). Particles were loaded with vancomycin via a “breathing in” method and release was tracked over 144hrs, with a ~70% loading efficiency and sustained release profile. FBNs were incubated with S. aureus USA300 WT and ΔclfA strains, and demonstrated interaction with WT but not ΔclfA. A fibrin biofilm was formed over 24hrs by inoculating 30% human plasma and 546-labeled fibrinogen in RPMI media with GFP+ Newman strain, and resultant biofilms were treated with 647-labeled FBNs for 24hrs. FBNs were seen interacting with sequestered bacteria via confocal. A viability assay using vancomycin-free and -loaded FBNs (vFBNs) was conducted as in the prior assay. After 48hr treatment incubation, biofilms were stained with BacLight Green and imaged via plate reader and confocal. Green:red signal was compared, and 1024μg/ml vFBNs had a significantly greater efficacy than free drug. Similar results were seen in a murine dermal wound infection model.

Discussion:

S. aureus is an invasive pathogen that forms protective biofilms. vFBNs infiltrate fibrin biofilms, promoting enhanced bacterial killing compared with free drug in vitro and in vivo. vFBNs significantly reduce the required therapeutic dose to treat infection, limiting off-target effects of antimicrobials.

Poster #: 66

Sweet Corn Phytoglycogen Dendrimers as a Lyoprotectant for Dry-State Protein Storage

Junha Park, University of Florida

I would like to be considered for a Rapid Fire Oral Presentation

Protein biotherapeutics typically require expensive cold-chain storage to maintain their fold and function. Packaging proteins in the dry state via lyophilization can reduce these cold-chain requirements. However, formulating proteins for lyophilization often requires extensive optimization of excipients that both maintain the protein folded state during freezing and drying (i.e., “cryoprotection” and “lyoprotection”), and form a cake to carry the dehydrated protein. Here we show that sweet corn phytoglycogens, which are glucose dendrimers, can act as both a protein lyoprotectant and a cake-forming agent. Phytoglycogen (PG) dendrimers from 16 different maize sources (PG1-16) were extracted via ethanol precipitation. PG size was generally consistent at ~70-100 nm for all variants, whereas the colloidal stability in water, protein contaminant level, and maximum density of cytocompatibility varied for PG1-16. 10 mg/mL PG1, 2, 9, 13, 15, and 16 maintained the activity of various proteins, including green fluorescent protein, lysozyme, β-Galactosidase, and horseradish peroxidase, over a broad range of concentrations, through multiple rounds of lyophilization. PG13 was identified as the lead excipient candidate as it demonstrated narrow dispersity, colloidal stability in phosphate-buffered saline, low protein contaminants, and cytocompatibility up to 10 mg/mL in NIH3T3 cell cultures. All dry protein-PG13 mixtures had a cake-like appearance and all frozen protein-PG13 mixtures had a Tg’ of ~ -26 ℃. The lyoprotection and cake-forming properties of PG13 were density-dependent, requiring a minimum density of 5 mg/mL for maximum activity. Collectively these data establish PG dendrimers as a new class of excipient to formulate proteins in the dry state.

Poster #: 67

Materials characterization of a self-assembling elastin-like polypeptide fusion as a potential platform for immunotherapies

Kat Lazar, Duke University

Kat M. Lazar

Duke University department of Biomedical Engineering

kml105@duke.edu

Yulia Shmidov

Duke University department of BME

yulia.shmidov@duke.edu

Rachel L. Strader

Duke University department of BME

rachel.strader@duke.edu

Shamitha Shetty

Duke University department of BME

shamitha.shetty@duke.edu

Joel H. Collier

Duke University department of BME

joel.collier@duke.edu

Ashutosh Chilkoti

Duke University department of BME

chilkoti@duke.edu

Self-assembling polypeptides are a unique category of biomaterials that undergo spontaneous organization into nano- or microstructures due to the combination of precise interactions, such as hydrogen bonds, ionic and electrostatic interactions, π-stacking, and hydrophobic effects. Many of these self-assembling materials are stimuli-responsive and undergo morphological changes based on external cues such as temperature, pH, or enzymatic activity. Stimuli-responsive self-assembling materials have grown increasingly popular as platforms to improve vaccine delivery and immunogenicity by increasing plasma residence time, improving uptake, or targeting lymphoid tissues.

We have identified a novel self-assembling fusion protein, termed K2(E36)115, which forms a diverse array of nano and microstructures in a concentration and temperature dependent manner. The protein is

comprised of a short, zwitterionic peptide (K2) derived from an immunomodulatory class of drugs called Glatiramoids, as well as (E36)115, a long and hydrophobic elastin-like polypeptide (ELP). ELPs are a family of repetitive proteins that undergo lower critical solution temperature (LCST) phase behavior, wherein they transition from a soluble to coacervate state upon heating above a characteristic transition temperature (Tt). Below its Tt, K2(E36)115 forms a heterogeneous assembly of vesicles with variable size and configuration (unilamellar, multilamellar, multicompartmental), but same lamellar thickness. Above its Tt, it forms large coacervates that, remarkably, maintain a multilamellar structure.

In this work, we aimed to elucidate the self-assembling behavior of K2(E36)115 to identify lead formulations for further application as immunotherapies. We used dynamic light scattering, UV-Vis spectrophotometry, and cryo-electron microscopy to characterize the temperature and concentration dependence of nanoparticle assembly. To investigate the contributions of specific amino acids to ionic and π-stacking interactions, we designed a library of fusion proteins with single amino acid alterations in the parent peptide K2 and evaluated them for disruption in nanoparticle formation. In our ongoing work, we aim to characterize a second library of fusion proteins where the ELP component has varied length and hydrophobicity, to investigate how the features of the hydrophobic block influence morphology. Together, this information provides insight into the molecular characteristics governing the self-assembly of (K2)E36115 and guides further investigation into the development of a tunable, stimuli-responsive platform for immunotherapy development.

Poster #: 68

Engineering multiplexed breath biomarkers for rapid respiratory pathogen identification

Khoi Le, Georgia Institute of Technology

Presenting Author

Khoi Le

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

khoi30@gatech.edu

Co-Authors

Vishal Manickam

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

vamanickam@gatech.edu

Thomas Li

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

tli446@gatech.edu

Leslie W. Chan, Ph.D.

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, 30332, USA

leslie.chan@gatech.edu

Respiratory infections are caused by a wide variety of pathogens, and pathogen identification is a crucial step for informed, targeted treatment. However, pathogen identification takes days due to time-intensive clinical assays that require culturing of microbes from patient samples. To eliminate this step, we are developing a diagnostic test that leverages existing pathogen growth inside the infected lungs. Together, the pathogen and host produce a repertoire of proteases in the infected tissue microenvironment that can be used for pathogen classification. To harness pulmonary protease activity for rapid pathogen

identification, we have developed nanosensors that can be administered into the lungs to induce exhalation of reporters for lung protease activities.

Nanosensors are comprised of volatile reporter molecules tethered to a nanocarrier via protease-cleavable peptide linkers. Upon peptide cleavage, liberated reporters vaporize and are subsequently exhaled for quantification in breath via mass spectrometry. Thus, nanosensors harness disease-associated protease activities to produce breath biomarkers. Biomarker signatures comprised of the activity of multiple proteases provide superior disease specificity to that of single proteases. Therefore, for pathogen identification, we have established multiplexed sensing capabilities in which nanosensors for orthogonal proteases are barcoded with volatile reporters of distinct mass.

To test our approach, we established three mouse models of lung infection with common respiratory pathogens: Staphylococcus aureus, Pseudomonas aeruginosa, or Klebsiella pneumoniae. Cleavage assays combining bronchoalveolar lavage fluid collected from infection models and a fluorogenic peptide substrate library revealed 10 orthogonally-cleaved peptides that can be used for pathogen identification. The 10-plex includes peptides that are cleaved by host and pathogen proteases. Using established linker chemistries, peptides were VOC-barcoded to develop a nanosensor array. Reaction of the nanosensor array with proteases in vitro confirmed that nanosensors are cleaved by targeted proteases with high specificity and that the magnitude of reporter signal reflects protease activity levels. Most importantly, multiplexed protease sensing in vitro was possible using the nanosensor array. We are currently assessing the ability of nanosensors to generate pathogen-specific breath biomarkers in mouse infection models. Success of this work will result in a breath test for rapid pathogen identification to facilitate informed, targeted treatment of lung infections.

Poster #: 69

Thrombin-sensitive platelet-like-particles for injury site-specific shape change

Krista Hilmas, North Carolina State University/UNC-Chapel Hill

Preventable mortality rates in the battlefield are primarily caused by combat-associated traumatic hemorrhage and trauma-induced coagulopathy. Consequently, hemorrhage control and resuscitation has been labeled a primary focus area of research towards material development for 'on-field' mitigation of combat trauma. First-generation platelet-like particles (PLPs) have been developed to induce clot contraction and stability by binding to fibrin fibers and exerting a strain on fibers as they collapse into an energetically favorable conformation, enhancing hemostatic benefits and wound healing outcomes. PLPs, however, are morphologically slower than native platelets due to their inability to respond to injury site-specific agonists. Therefore, second-generation thrombin-sensitive platelet-like particles (tsPLPs) have been developed to change shape in response to thrombin exposure at a wound site. Thrombin-sensitive nanogels (TSNs) are synthesized by crosslinking N-isopropylmethacrylamide (NIPMAM) with a thrombin-cleavable peptide sequence. These TSNs are then conjugated with a fibrin-binding motif through EDC/NHS chemistry to create tsPLPs that bind to fibrin and are amenable to shape change upon exposure to thrombin at a wound site. This study evaluated TSNs synthesized with a decreased initiator content, from 34.7% to 7.5% APS initiator, to increase the yield of TSNs and allow for greater scale up. TSNs were then evaluated before and after exposure to thrombin for changes in size and morphology. TSNs were incubated with thrombin (75U/mL) at room temperature, and the solution was spiked with Thrombin (100U/mL) daily. Aliquots of TSN/thrombin solution were taken at t=2hr, 4hr, 24hr, 48hr, and 96hrs for characterization by Atomic Force Microscopy (AFM) and NanoSight. Results showed an increase in size upon exposure to thrombin, with a size peak at the 2-hour timepoint, and a decrease in size at later timepoints, indicating degradation of TSNs by thrombin. Furthermore, tsPLPs were evaluated for their ability to induce clotting in an APTT assay and were found to have similar clotting times to first-generation PLPs. In conclusion, TSNs exhibit shape change deformation abilities, indicated by initial swelling of particles and further degradation, and tsPLPs retain clot contraction abilities similar to first-generation PLPs, without exposure to high levels of thrombin. Ongoing studies are evaluating particle deformation's effect on wound healing efficacy.

Poster #: 70

Optimizing a Nanoparticle Drug Delivery System to Treat Preterm Labor

Lauren Link, Vanderbilt University

Christopher J. Hansen, Vanderbilt University, christopher.j.hansen@vanderbilt.edu

Emaa Elrayah, Middle Tennessee State University, eae3b@mtmail.mtsu.edu

Michelle Guzman, Vanderbilt University, michelle.guzman@vanderbilt.edu

Jennifer Herington, Vanderbilt University Medical Center, jennifer.l.herington@vumc.org

Todd Giorgio, Vanderbilt University, todd.d.giorgio@vanderbilt.edu

Biomaterials have a slew of uses across many medical disciplines. Among these applications, drug delivery methods have received ample attention for their obvious utility in treating complex diseases, mitigating off-target effects, and improving pharmacokinetics and pharmacodynamics. Poly(lactic-co-glycolic) acid (PLGA) has garnered great popularity – with FDA-approval this biopolymer is among the most used biomaterial in drug delivery applications. With robust heterogeneity across the field, our work aimed to characterize formulation parameters for PLGA nanoparticles (NPs) that tune particle size, loading efficiency, and surface modification while maintaining particle morphology and surface charge. We compare two formulations, using a simple oil-in-water emulsion technique. One with dicholoromethane (DCM) and polyvinyl alcohol (PVA), and the other with ethyl acetate (EtAc) and D-α-tocopheryl polyethylene glycol succinate (Vit E-TPGS). With serial centrifugation we show an ability to isolate multiple particle cohorts by size with tight polydispersity (PDI). However, the resulting EtAc/Vit E-TPGS NPs produced more variable NPs (PDI:0.30) in comparison to the DCM/PVA NPs (PDI:0.20). For drug loading, we successfully encapsulated two lipophilic molecules, indomethacin and nifedipine, in high efficiency (82±15% and 90±4%, respectively) without major effect on NP size. Using succinyl ester, we have shown preliminary studies coupling either antibody or small-peptide molecules to the particle surface. We used a 3x4 matrix approach to test both reactive to non-reactive polymer ratios and peptide to NP mass ratios. We show that greater peptide to NP mass ratio and lower reactive to non-reactive polymer ratios produce NPs with more peptide bound per particle, however this is accompanied by lower coupling efficiency. Moreover, we novelly report coupling efficiency on the NPs directly through either dot blots (for antibodies) or Ellman's assay (for small-peptide molecules). In future studies, we plan to explore another common chemistry, maleimide click-chemistry, with our peptide molecules and compare their efficiencies and total coupling to succinyl ester methods as reported here. This effort is to provide a resource to varying methods in the field to streamline development of these useful drug delivery systems.

Poster #: 71

Nanofibrous hydrogel preparation via electrospinning for drug delivery and wound healing

Shiqi Wei, Georgia Institute of Technology

Shiqi Wei (swei86@gatech.edu)

Reina Funatomi (rfunatomi3@gatech.edu)

Blair Brettmann (blair.brettmann@mse.gatech.edu)

With its high surface area and porous structure, a nanofibrous scaffold helps with protein adsorption, binding of ligands, diffusion of molecules, biomimicking of the extracellular matrix (ECM), and promoting stem cell differentiation. Electrospinning is a polymer processing method to obtain nonwoven fiber mats with fiber diameters ranging from tens of nanometers to hundreds of micrometers. This research presents a nanofibrous hydrogel incorporating hyaluronic acid (HA) prepared via electrospinning, a method that holds promise for drug delivery and wound healing applications. The process involves optimizing the components, including the effective polymer (HA), the carrier polymer (polyethylene oxide) that improves spinnability, the crosslinker (polyethylene glycol diacrylate), and the photo initiator (Irgacure 2959) to create an electrospun fibrous unwoven mat with continuous and uniform fibrous morphology. This mat is crosslinked using ultraviolet (UV) light and swelled under deionized water to form the nanofibrous hydrogel. The nanoscale morphology of the electrospun fiber mat, the mechanical properties of the nanofibrous hydrogel, and the release rate of drug molecules from the hydrogel under acid, base, or neutral solutions related to the formulation of polymer solutions for electrospinning are further studied. We find that a higher ratio of PEO improves fiber morphology but decreases the mechanical properties of the crosslinked hydrogel. Because cells can sense the stiffness of the surrounding matrix and respond accordingly, the tuning of mechanical properties of fibrous hydrogel facilitates versatile adaptation to the physiological environment. This hydrogel system has the potential for the application of wet healing dressing to reduce scar formation, degradable bioscaffold for long-term treatment of knee pain caused by osteoarthritis (OA), and cell therapy by promoting Mesenchymal Stem Cells (MSC) differentiation.

Poster #: 72

Addressing Immune Suppression in Acute Respiratory Distress Syndrome (ARDS) with Synthetic Nanoparticle Antibodies (SNAbs)

Shivani Vyas, Georgia Institute of Technology

Acute respiratory distress syndrome (ARDS) is a severe condition characterized by acute lung inflammation and respiratory failure, and results in a hospital mortality rate of approximately 40%.Immunosuppressive myeloid cells, termed myeloid-derived suppressor cells (MDSCs), play a vital role in shaping immune balance in acute respiratory diseases. MDSCs are involved in the pathogenesis of ARDS due to their contribution to the amplification of pulmonary inflammation and tissue damage. Current therapies utilized to deplete MDSCs, ranging from small molecule inhibitors to monoclonal antibodies (mAbs), encounter challenges in specifically targeting MDSCs, resulting in broad off-target effects and limited efficacy. The development of novel immunomodulatory therapies targeting MDSCs can potentially mitigate disease severity in these conditions. We have developed synthetic nanoparticle antibodies (SNAbs) to target and deplete MDSCs and restore beneficial host immune responses in models of ARDS. SNAbs are multivalent, bifunctional gold nanoparticles (AuNPs) featuring MDSC-targeting ligands and antibody-fragment crystallizable (Fc)-mimicking ligands. By bridging MDSCs with immune effector cells, SNAbs have demonstrated the capability to induce the depletion of MDSCs. We administered SNAbs to ARDS-challenged murine lung cells in vitro and through aerosolized delivery to a mouse model of ARDS. Following flow cytometry and cytokine analysis, we demonstrated that SNAbs can deplete MDSCs, reduce immunosuppressive mediator expression, and restore beneficial T cell responses.

Poster #: 73

Synthetic biomaterials as carrier for limbal stem cells derived from induced pluripotent stem cells for ocular surface regeneration.

Nasif Mahmood, North Carolina State University

Nasif Mahmood (nmahmoo3@nscu.edu);

Mohamed R Eletmany (mrmoham2@ncsu.edu); Ummay Mowshome Jahan (ujahan@ncsu.edu); Ahmed El-Shafei (amelsha@ncsu.edu);

Brian C. Gilger (bgilger@ncsu.edu)

Jessica M. Gluck (jmgluck@ncsu.edu)

Limbal stem cell deficiency (LSCD) is a debilitating ocular surface disorder occurring due to loss or dysfunction of limbal stem cells (LSCs). Existing treatments for LSCD include transplantation of autologous limbus from the healthy eye in unilateral cases, or allogeneic limbal tissue from living or cadaveric donors when both eyes are affected using human amniotic membrane , the innermost layer of the placenta as the carrier. Scarcity, lack of reproducibility, risk of disease or infection transmission of amniotic membrane have made research necessary into alternative biomaterials compatible with LSCs. Also, treatment of LSCD is further exacerbated by limited supply of healthy LSCs especially in the case of bilateral LCSD. To address the existing limitations, we designed a graft with the essential properties and combining it with a viable source of LSCs to develop a treatment for LSCD. LSCs derived from induced pluripotent stem cells (iPSCs) have the advantage of being an unlimited and non-immunogenic source. We developed a xeno-free and chemically defined protocol for differentiating iPSCs to LSCs and observed the expression of relevant markers (PAX6, p63α,CK12). Electrospun synthetic membranes were laser perforated, plasma-treated, and biofunctionalized with Collagen-IV to mimic the corneal microenvironment with sufficient mechanical strength to withstand surgical manipulation and minimize the obstruction of vision. Laser perforations with different diameters and spacing allowed for regulation of the mechanical properties and light transmittance of the construct. The light transmittance of the construct after perforation was increased to 60%. Plasma treatment allowed more homogenous and profuse covering with Collagen-IV compared to non-plasma treated membranes which improved cell adhesion to the scaffold by providing binding sites that receptors of cells can interact with. We have made progress developing an iPSC-LSC seeded synthetic graft which has potential to address existing limitations of LSCD treatment.

Poster #: 74

One Step, Catalyst-Free Synthesis of a Mechanically Tailorable Organohydrogel and its Derived Underwater Superoleophobic Coatings

Aasma Sapkota, University of Georgia

Aasma Sapkota† aasma.sapkota@uga.edu

Arpita Shome† arpita.shome@uga.edu

Natalie Crutchfield† natalie.crutchfield@uga.edu

Joseph Christakiran Moses† joseph.moses@uga.edu

Isabel Martinez† isabel.martinez@uga.edu

Hitesh Handaα† hhanda@uga.edu

Elizabeth J. Brisbois †* ejbrisbois@uga.edu

† School of Chemical, Materials, & Biomedical Engineering, University of Georgia, Athens 30602, United States

αPharmaceutical and Biomedical Sciences Department, College of Pharmacy, University of Georgia, Athens, GA 30602, United States

*Corresponding author

Organohydrogels are an emerging class of soft materials that comprises the mechanical durability of organogels and the biocompatibility of hydrogels for prospective biomedical applications. In this work, we have introduced a facile, catalyst-free one-step chemical approach to develop an organohydrogel with impeccable anti-biofouling properties following the amine-epoxy ring opening reaction under ambient conditions. Hence fabricated mechanically tailorable organohydrogels have prospective applications in medical devices, drug delivery, and tissue culture. The affinity of the current organohydrogel to both organic solvents and water was exploited to incorporate the nitric oxide donor, S-nitroso-N-acetylpenicillamine (SNAP) from ethanol, and subsequently, the water-sensitive NO-releasing organohydrogels were observed to demonstrate excellent antibacterial activity against E. coli and S. aureus with 99.71% and 87.31% reduction in planktonic bacteria, respectively, without eliciting any cytotoxicity concerns. Moreover, the reported organohydrogel with remarkable water uptake capacity was extended as a coating on different medically relevant polymers to demonstrate transparent underwater superoleophobicity. Thus, the reported facile organohydrogel and its derived underwater anti-fouling coating can open avenues for utility in biomedical, energy, and environmental applications.

Poster #: 75

Combination Biomedical Therapeutics Utilizing Nitric Oxide and Zinc Molybdate

Sarah Wilson, University of Georgia

Sarah N. Wilson - School of Chemical, Materials, and Biomedical Engineering, The University of Georgia. snw60730@uga.edu

Yi Wu - School of Chemical, Materials, and Biomedical Engineering, The University of Georgia. yi.wu27@uga.edu

Natalie Crutchfield - School of Chemical, Materials, and Biomedical Engineering, The University of Georgia. natalie.crutchfield@uga.edu

Myddleton Parker - School of Chemical, Materials, and Biomedical Engineering, The University of Georgia. myddleton.parker@uga.edu

Elizabeth Brisbois - School of Chemical, Materials, and Biomedical Engineering, The University of Georgia. ejbrisbois@uga.edu

Hitesh Handa - School of Chemical, Materials, and Biomedical Engineering, The University of Georgia. hhanda@uga.edu

Antimicrobial resistance (AMR) has been increasing at a steady pace for the last decade. It has been estimated that in the United States alone, AMR causes 2.9 million infections and 35,000 deaths annually. This threat has encouraged research into alternative therapies apart from conventional antibiotic use. Nitric oxide (NO) is an alternative therapy that has been extensively researched for its relevance as a broad-spectrum antimicrobial that pathogens cannot grow resistant to. In addition to synthetic NO donor molecules, researchers can utilize catalysts that take advantage of naturally occurring molecules to generate NO consistently over long periods. Materials containing metal ion coatings have shown dose-dependent NO generation as well as bactericidal killing. Herein, a common NO donor, S-nitroso-N-acetylpenicillamine (SNAP), was blended into a medical-grade polymer to create NO-releasing films. The films were then coated with a polymer solution containing zinc molybdate ($ZnMoO_4$) to create NO-releasing and -generating films with a bimetallic oxide outer layer confirmed with scanning electron microscopy (SEM) and elemental analysis. Bimetallic oxides represent multiple metal complexes with the ability to catalyze NO generation and kill bacteria similarly to mono-composite metals while maintaining high biocompatibility, something single metal ions do not display. The combination materials showed a release of relevant levels of NO (0.5-4.0 Flux) for at least 7 days while the $ZnMoO_4$-only films displayed dose-dependent generation of NO comparing the 1 and 3 wt% $ZnMoO_4$. Additionally, the inclusion of $ZnMoO_4$ on the surface of the material alone showed a 51.02% killing efficiency towards E. coli. Even more so, the combination of a NO-releasing and -generating surface showed an almost two-log reduction in adhered bacteria colonies, representing a 98.53% reduction efficiency. Further biological testing was completed to determine the biocompatibility of the combo material. When exposed to human fibroblast cells for 24 h, there was no visualized toxicity towards the cells from any of the material groups. This NO + bimetallic oxide material displayed comparable antibacterial and NO-generation properties as materials utilizing mono-composite metal ions while maintaining high biocompatibility, even showing proliferative effects. These characteristics make it an intriguing class of materials for use in the biomedical field.

Poster #: 76

Investigating in Vitro Angiogenesis in a Verapamil-Releasing 3D Printed Polymeric Scaffold

Amanda Bradley, University of Florida

BACKGROUND

Angiogenesis is key for successful tissue integration of cellular implants. Although not the intended use, verapamil has been shown to have an impact on in vivo vascularization. In order to improve the vascularization of porous 3D implants, this long-term study observes the direct impact of verapamil drug release from polymeric scaffolds on the formation of in vitro blood vessels.

METHODS

A reverse-cast method was used to generate 3D printed PDMS scaffolds loaded with verapamil (bulk concentration: 0%, 0.05%, and 0.25%). Negative space was filled with fibrin, NHLFs, and GFP-HUVECs, and media was replaced every 24h. Scaffolds were confocal imaged on d7, 14, and 21 and analyzed for percent vessel coverage in scaffold pores in ImageJ. Concurrently, verapamil elution data was collected daily and analyzed by multiple reaction monitoring (MRM).

RESULTS

Verapamil was successfully integrated into and released from a PDMS construct. Over 21 days, both 0.05% and 0.25% scaffolds display a first-order release profile with peak concentrations of 83.5 ng/mL and 1375.4 ng/mL respectively. After 8 days, both scaffolds reach steady-state averaging a daily release of 24ng/mL (0.05%v) and 356.6 ng/mL (0.25%v). Control and 0.05% scaffolds had significant increases in vessel coverage from d7-14, both having more coverage on d14 than 0.25% scaffolds. By d21, vessel coverage across all groups was the same. Visually, at d14 and 21, 0.05% scaffolds have thicker and more highly branched vessels.

CONCLUSIONS

Verapamil was successfully integrated into a PDMS scaffold, yielding first-order release with varied magnitude based on verapamil loading. Verapamil appears to have minimal impact on vessel coverage in vitro, however, there appears to be a shift in vessel thickness, length, and branching which future work aims to quantify.

Poster #: 77

PLG microparticles encapsulating retinoic acid accelerate muscle recovery in mice after hindlimb casting-induced muscle atrophy.

Candice Cheung, University of South Carolina

Kelsey Krusen, University of South Carolina, kkrusen@email.sc.edu

Khufu Holly, University of South Carolina, kmholly@email.sc.edu

Paul Ward Pratz, University of South Carolina, robertpw@email.sc.edu

R. Michael Gower, University of South Carolina, gowerrm@mailbox.sc.edu

Muscle atrophy is the loss of muscle mass from disuse, aging, or chronic illness. The recovery process starts with an activation of local and infiltrating immune cells, which creates a local inflammatory environment before attenuating towards an anti-inflammatory, wound-healing stage. The inflammatory stage utilizes cytokines secreted by pro-inflammatory macrophages such as interleukin-6 (IL-6) and is important for immune cell recruitment, removal of cellular debris, and to activate stem cells. However, IL-6 impedes repair systems if sustained and must be attenuated by anti-inflammatory cytokines and muscle-regenerative factors such as insulin-like growth factor-1 (IGF-1), produced by anti-inflammatory macrophages. We have shown that all-trans retinoic acid (ATRA) induces stem cell differentiation and induces macrophages to produce IGF-1 in culture. However, ATRA has poor oral bioavailability and a short half-life in in vivo systems. Thus, we developed particles for extended release. For this study, we hypothesized that ATRA delivery into the fascia of muscle would accelerate muscle growth after immobilization-induced atrophy in mice.

C57bl/6 male mice were given casts immobilizing the hindlimbs for 10 days. Upon cast removal, particles containing a high, medium, or low dose of ATRA or saline were injected into the intermuscular space between the gastrocnemius and tibialis anterior muscles. Mice were allowed to recover for 3-7 days, after which mice were euthanized and hindlimb muscles were collected for biochemical or histological analysis. H&E shows fiber cross-sectional area is larger in high- and low-loaded ATRA-PLG groups compared to saline at the 3- and 7-day timepoints. To understand the mechanism for increased fiber recovery, ELISAs of muscle lysate show IL-6 decreased at the 3-day timepoint in a dose-dependent manner, with a 50% decrease in the low-dose treatment group. Solei sections were immunofluorescently stained for macrophage markers CD206 (pan-macrophage), CD68 (anti-inflammatory), and CD163 (anti-inflammatory). Taken together, local delivery of ATRA-PLG may be a potential treatment for accelerating recovery after muscle atrophy. To further elucidate the mechanism for which ATRA is accelerating recovery, we intend to determine whether ATRA impacts signaling pathways associated with muscle growth and stem cell activation (proliferation, differentiation) using western blotting and immunofluorescent techniques.

Poster #: 80

Impacts of beta-sheet crystallinity on the mechanical properties of chemically and physically crosslinked silk fibroin hydrogels

Elizabeth Aikman, University of Florida

Elizabeth L. Aikman, University of Florida Department of Chemical Engineering, elizabethaikman@ufl.edu

Marisa O Pacheco, University of Florida Department of Chemical Engineering, marisa.pacheco@ufl.edu

Hannah K Bagnis, University of Florida Department of Chemical Engineering, hannah.bagnis@ufl.edu

Isabelle K Gerzenshtein, University of Flordia Department of Microbiology and Cell Science, ikaela.gerzensht@ufl.edu

Whitney L Stoppel, University of Florida Department of Chemical Engineering, whitney.stoppel@ufl.edu

Silk fibroin (SF), obtained from Bombyx mori silkworm cocoons, is a natural biopolymer used in the formation of biomaterials due to its biocompatibility, controllable degradation rate, and tunable physical properties. In SF hydrogels, molecular weight, concentration, and crystallinity are all tunable parameters that affect the mechanical and physical properties of the resulting structures. SF is known to form physical crosslinks via secondary structure formation within the protein, resulting in ordered beta-sheet structures. Organization of beta sheet structures via hydrogen bonding yields crystalline regions amongst amorphous polymer chairs, decreasing optical transparency and increasing mechanical strength over time. Crystalline beta-sheet structures can be intentionally induced via application of shear forces or by increasing the temperature, but will form even without intentionality as the biopolymers interact within the hydrogel, aiming to minimize free energy. To interfere with the free energy landscape, we investigated the impact of solution parameters (concentration and molecular weight) on resulting hydrogel mechanical properties through shear rheology. Furthermore, we evaluated the extent to which chemical crosslinking minimizes physical crosslinking, exploring horseradish peroxidase enzymatic crosslinking. Physical crosslinking (beta-sheet content) was assessed through Fourier transform infrared (FTIR) spectroscopy and optical transparency. Gelation kinetics reveal the temporal profile of gelation for physical and chemical crosslinking strategies. Results confirm that SF molecular weight has the greatest impact on viscosity and the greatest impact on physical crosslinking dynamic moduli. The gelation kinetics of the chemical crosslinking chemistries are faster than the physical crosslinked hydrogels; however, the storage and loss moduli of the physically crosslinked hydrogels is significantly larger than the chemically crosslinked materials. Understanding the relationship between chemical and physical crosslinking in SF hydrogels enables temporal control over mechanical properties for future soft tissue engineering applications. Temporal control can also be modulated through additional chemical crosslinking methods in SF hydrogels, including methacrylation or norbornene functionalization.

Poster #: 81

Using 3D Engineered Tissue Model for Investigating the Role of CXCL7 in Colon Cancer Progression

Jannatul Ferdous Nipa, Auburn University

Additional Authors: Hadeel Aldhowayan2, Kwaghtaver S. Desongu1, Michael W Greene2, Elizabeth Lipke1.

Department/Program: 1. Department of Chemical Engineering, Auburn University. 2. Department of Nutritional Sciences, Auburn University.

Colorectal cancer (CRC) ranks among the top three prevalent cancers worldwide, with a high incidence in the US. 3D engineered tissue models are used for studying cancer behavior, enabling replication of tumor microenvironments which enhance our insight into tumorigenesis and therapeutic development. For our study, we used our 3D engineered tissue model to study the impact of chemokine ligand CXCL7 on CRC cells (HT-29 cell lines) by using transfected CRC cells with CXCL7 and an empty vector used as control. CXCL7 contributes to driving progression of various types of cancer, including CRC. Our preliminary findings suggest that CXCL7 chemokine promotes CRC cell proliferation, consistent with the clinical data of CRC patients. We examined the effect of CXCL7 on the proliferation and viability, and the change of colony and overall tissue size. The transfected cells were encapsulated in poly (ethylene glycol) (PEG)-fibrinogen hydrogels to provide a 3D environment for the cells. Cancer cell proliferation was assessed by measuring EdU incorporation in DNA after 8 days of encapsulation. Viability was observed on day 1, day 8 and day 15 post-encapsulation. 3D engineered tissues were cultured for 30 days to assess temporal differences between tissues formed with CXCL7 transfected and vector control HT29 cells. From the experimental outcomes we observed that engineered tissues formed with cells expressing CXCL7 displayed significantly higher growth and proliferation compared to the vector-transfected engineered tissues. Additionally, colony formation in CXCL7 transfected tissues showed noticeable increase in size when compared to the vector-transfected tissues on day 8 and day 15 (significantly on day 15) of culture. The viability of cells in the tissues formed with CXCL7 transfected cells was higher than that in the tissues formed with vector transfected cells (day 8 and day 15 of culture). Overall, this study demonstrated that CXCL7 expression impacts cellular behavior inside 3D engineered tissue, including HT29 cell colony development, viability and proliferation. This evaluation of the impact of CXCL7 on CRC growth within a 3D tissue engineered model opens opportunities for further investigation into it's potential as a therapeutic target and biomarker.

Poster #: 82

Hydrophilic Reactive Oxygen Species-Degradable Scaffolds for Wound Healing

Joshua McCune, Vanderbilt University

Prathana Patil(1), Jeffery Davidson(2), Scott Guelcher(1&3), and Craig Duvall(1)

1. Department of Biomedical Engineering, Vanderbilt University

2. Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine

3. Department of Chemical and Biomolecular Engineering, Vanderbilt University

prarthana.patil@vanderbilt.edu

scott.guelcher@vanderbilt.edu

craig.duvall@vanderbilt.edu

jeff.davidson@vanderbilt.edu

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. In pilot studies, these scaffolds have demonstrated exceptional healing responses in excisional wound models with more robust tissue infiltration, vascularization, and re-epithelialization. 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.

Poster #: 84

Large Engineered Cardiac Tissue Production using Tissue Engineered Pluripotent Stem Cell Microspheres as Building Blocks

Justin Harvell, Auburn University

Mohammadjafar Hashemi, Dept. Chemical Engineering, Auburn University, (mzh0111@auburn.edu). Della Kelly, Dept. Chemical Engineering, Auburn University, (DAK0025@auburn.edu). Kevin D. Roehm, CFDRC, Huntsville, AL, (kevin.roehm@cfd-research.com). Prabhakar Pandian, CFDRC, Huntsville, AL, (prabhakar.pandian@cfd-research.com). Elizabeth A. Lipke, Dept. Chemical Engineering, Auburn University, (elipke@auburn.edu).

This study evaluated the use of differentiating 3D microspheroidal engineered heart tissues (µEHTs) as building blocks for macro-scale EHT production (>1 cm3). Currently, EHT production methods are complex and not easily scalable; advances are required for moving to manufacturing scale cell production and tissue assembly. We have previously shown that µEHTs can be formed by encapsulating human induced pluripotent stem cells (hiPSCs) in poly(ethylene glycol) fibrinogen (PF) hydrogel microspheres and differentiating these hiPSC-laden engineered tissue microspheres in scalable, suspension culture. Here, to investigate the ability to form larger EHTs from the µEHTs, µEHTs were assembled into millimeter scale tissues on differentiation days 3, 5, and 7. As a control, millimeter scale engineered tissues were also formed directly from hiPSCs as previously reported. Secondary encapsulated tissues were produced by adding differentiating µEHTs to PF precursor solution and photocrosslinking in millimeter scale molds. µEHTs were successfully encapsulated and remained viable to form millimeter scale tissues. For all tested secondary encapsulation timepoints, µECTs fused together to form larger composite tissues and underwent successful cardiac differentiation forming EHTs. Tissue contraction was observed in EHTs formed at all secondary encapsulation timepoints by day 20 and contraction/relaxation velocity temporally increased through day 40. All tissues appropriately responded to beta-adrenergic stimuli, one indicator of functional maturation. µEHTs encapsulated on day 5 had higher contraction and relaxation velocities compared to days 3 and 7, suggesting that day 5 secondary encapsulation yields better cardiac tissue function. To test macro-scale large tissue production using this method, µECTs from day 5 were secondarily encapsulated within a perfused large tissue bioreactor (1 cm3). Macro tissues were successfully formed from day 5 µECTs within the bioreactor and after five days of culture, cells remained viable when in proximity to the perfusion channels and showed positive staining for the cardiac marker alpha sarcomeric actinin. Overall, µECTs from differentiation day 3, 5, and 7 can successfully be employed as building blocks to produce larger tissues through secondary encapsulation in PF; the approach established here was demonstrated for use in macroscale CT production and has potential applications to 3D EHT printing.

Poster #: 85

3D Printable Biomaterials for Tissue Engineering Applications

KENAN SONG, UGA

Kenan Song,

University of Georgia (UGA)

kenan.song@uga.edu

Yuxiang Zhu,

Arizona State University (ASU)

yuxiang.zhu@asu.edu

Pelvic organ prolapse (POP) is a prevalent condition among women, characterized by the descent of pelvic organs into the vaginal canal due to weakened pelvic floor muscles and connective tissues. Current treatments for POP often involve surgical intervention, including the use of tissue scaffolds to provide support and promote tissue regeneration. However, existing scaffolds face limitations in customization, biocompatibility, and mechanical strength. This research project proposes leveraging 3D printing technology to address these challenges and develop personalized, biocompatible, and mechanically robust tissue scaffolds for POP treatment. By utilizing advanced 3D printing techniques, such as material extrusion and digital light processing, we aim to fabricate scaffolds with tailored structures and properties, optimizing their effectiveness in supporting pelvic organs and promoting tissue regeneration. This innovative approach holds promise for revolutionizing POP treatment by providing patients with bespoke solutions that enhance treatment outcomes and improve quality of life.

Poster #: 86

Engineered 3D In Vitro Model of Colorectal Cancer in Insulin-Sensitive and Insulin-Resistant Microenvironments

Kwaghtaver Desongu, Auburn University, Auburn, AL

Kwaghtaver S. Desongu(ksd0028@auburn.edu) 1, Peter Abraham(pta0003@auburn.edu) 1, Ifeoluwa Odeniyi (iao0004@auburn.edu) 2, Grace Hester (geh0019@auburn.edu) 1, Jannatul F. Nipa (jzn0050@auburn.edu) 1, Michael W. Greene (mwg0006@auburn.edu) 2, Elizabeth A. Lipke (eal0003@auburn.edu) 1

1. Department of Chemical Engineering, Auburn University,
2. Department of Nutritional Sciences, Auburn University

This work establishes an insulin-resistant (IR) 3D engineered colorectal cancer (3D-eCRC) tissue model to examine the role of IR in colorectal cancer (CRC) progression. CRC remains the third-most common cancer and the second-leading cause of cancer related deaths in the US. CRC has been epidemiologically linked with obesity; IR and inflammation could underlie this link. However, the mechanism is not well understood. To better understand this link, in vitro CRC models that more accurately mimic IR and associated inflammation are needed. Here, we developed a 3D-eCRC model that incorporates an IR microenvironment and examined the impact on CRC cell growth and migration.

To form 3D-eCRC tissues, HT29 CRC cells were encapsulated in poly(ethylene glycol) fibrinogen. To maintain cell viability in insulin-sensitive (IS) and IR culture conditions, 3D-eCRC tissues were cultured in media containing 50% CRC media and 50% of IS conditioned media (CM), IR CM, or control media.

To obtain IS CM, 3T3 L1 fibroblasts were differentiated into adipocytes. To obtain IR CM, we treated the differentiated matured adipocytes with tumor necrosis factor alpha and 1% hypoxic conditions, replaced the media, and then collected the CM after 24 hours.

On day 15 of culture, cell viability within the 3D-eCRC tissues in the control, IS and IR conditions was 51%, 54%, and 54%, respectively. IS and IR CM stimulated cell proliferation within the 3D-eCRC tissues; percentages of cells positive for the cell proliferation marker Ki67 were 6.3%, 16.5% and 12.0% in control, IS, and IR conditions, respectively. Examination of the morphology of resulting cell colonies revealed that 3D-eCRC tissues cultured in control media had smooth, round colonies, whereas IS and IR tissues showed rough, fragmented colonies.

Additionally, cells in IS and IR 3D-eCRC tissues were more migratory than control. From day 14 to day 15 of culture, 23,000, 29,000, and 44,000 cells migrated out of control, IS, and IR 3D-eCRC tissues, respectively.

In conclusion, our results suggest that IS and IR conditions impacted cell proliferation, migration, colony morphologies.

Future studies will examine gene expression and tissue staining to further understand the roles of IS and IR microenvironments on 3D-eCRC tissue properties.

Poster #: 87

Engineered Vascularizing Hydrogel to Deliver Stem Cell-Derived Beta Cells

Mariana Rodriguez, Georgia Institute of Technology

Mariana Rodriguez; Georgia Tech; mariana26@gatech.edu

Angélica Torres; Georgia Tech; alt7@gatech.edu

Sophia Kioulaphides; Georgia Tech; skioulaphides3@gatech.edu

Rachel Choi; Georgia Tech; rchoi37@gatech.edu

Michelle Quizon; Georgia Tech; mquizon3@gatech.edu

Graham Barber; Georgia Tech; gbarber7@gatech.edu

Andrés García; Georgia Tech; ag120@gatech.edu

Type 1 Diabetes (T1D) is a chronic, autoimmune disease which results in the irreversible destruction of β-cells. The insulin produced by the β-cells is responsible for the regulation of blood glucose levels and without them, the blood glucose levels become dangerously high and multiple long-term health complications can arise. The direct injection of exogenous insulin is the current standard to regulate glucose levels but requires constant monitoring and still does not prevent the long-term complications of T1D which leads to an average 18-year reduction in life span. The transplantation of allogeneic islets shows promise as a cure, but donor availability and long-term survival of the cells have prevented its success. Induced pluripotent stem cell-derived β-cells (SCβ-cells) have emerged as an alternative cell source for implantations due to their scalability. Furthermore, the subcutaneous space is a clinically relevant transplant site that is minimally invasive compared to the intrahepatic site but has low vascularization. Hydrogels made from 4-armed maleimide-terminated poly(ethylene glycol) (PEG) are useful for controlled vasculogenic endothelial growth factor (VEGF) delivery. Subsequently, islet vascularization and engraftment in the epididymal fat pad and subcutaneous space. Changes in the polymer backbone and crosslinker affect the mode of degradation and delivery of the VEGF. Additionally, the dose of VEGF within the hydrogel needs to be optimized to ensure the formation of functional vessels. Therefore, we conducted experiments to assess the capability of different PEG/VEGF gel formulations in promoting vascularization in the subcutaneous space by transplanting SCβ-cells with different PEG/VEGF gels and evaluating the survival and engraftment of the cells. Information about vessel density and insulin secretion within the grafts will be used to select the most promising gel formulations to then be used in a diabetic mouse model.

Poster #: 88

Direct-Ink Write 3D printing of therapeutic poly (N- vinyl caprolactam) and hyaluronic acid-based scaffolds

Samina Yasmeen, Clark Atlanta University

I would like to be considered for a Rapid Fire Oral Presentation

Layer-by-layer printing is an ideal choice for fabricating 3D scaffolds using bioinks that support cellular and tissue growth such as photocrosslinkable methacrylated hyaluronic acid (meHA) however, it has limited printing properties. Therefore, there is a need to design hybrid bioinks to address this issue. In this work, we prepared bioinks containing trihybrid PVCL-meHA-allyl α-tocopherol (PMATP), PVCL-meHA-allyl carvacrol (PMCAR), PVCL-meHA-allyl curcumin (PMCURC), and PVCL-meHA-allyl catechin (PMCAT), consisting of polyvinyl caprolactam (PVCL) (P), meHA (M), and one of the functionalized antioxidants (ANTOX). Allevi 3D bioprinter was used to print bioink at 17°C, 45 psi, 6 mm/s, 30% crosslinking intensity, and a 37°C printing bed temperature. Fourier Transform Infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) were employed to determine the crosslinking and morphological features, respectively. The 3D scaffolds were evaluated for swelling behavior printability antioxidant activity, thermoresponsive and rheological properties. High-resolution scaffolds (PMANTOX) were printed by covalent crosslinking of PVCL, meHA, and ANTOX, providing the bioink showed rapid sol-to-gel transition at 37 °C, structural fidelity, and long-term mechanical stability upon dual photocrosslinking. FTIR microscopic images confirmed the crosslinking of the trihybrid component. The rheological analysis of the scaffold revealed that PMCAT, which contains penta-allylated catechin, has the highest final storage modulus (G') of 2.1 × 105. SEM images reveal an open porous structure with a smooth surface of a successful printed multilayered hydrogel with a grid-like pattern. All scaffolds were fully rehydrated after 15 min, with an average swelling ratio of 113.4 %, indicating that the PMANTOX scaffolds can carry over twice their dry weight in water without disintegrating for 21 days. The 3D scaffolds displayed a narrow average size distribution of pores and strand diameters, with an average pore size of 0.993 + 0.04 mm and strand diameters average of 1.29 + 0.03 mm. In the 1-1-Diphenyl picrylhydrazyl (DPPH) assay, antioxidant activity was increased under 20 % O2, with PMATP exhibiting the highest antioxidant activity (24.48%). Further studies include long-term maintenance of 3D scaffold with proliferation of extracellular matrix proteins. This study introduces a novel direct ink write printing concept for 3D scaffolds, providing practical insights for developing 3D scaffolds for clinical applications.

Poster #: 90

Understanding tissue functionality and cell types formed during cardiac differentiation of hydrogel supported microspheres and scaffold-free aggregates

Shireen Singh, Auburn University

Shireen Singh, szs0302@auburn.edu, Department of Chemical Engineering, Auburn University
B. Justin Harvell, bjh0076@auburn.edu, Department of Chemical Engineering, Auburn University
Mohammadjafar Hashemi, mzh0111@auburn.edu, Department of Chemical Engineering, Auburn University
Francisco X. Galdos, fxgaldos@stanford.edu, Stanford Cardiovascular Institute
Carissa Lee, carislee@stanford.edu, Stanford Cardiovascular Institute
Sidra Xu, sidraxu@stanford.edu, Stanford Cardiovascular Institute
Shenbageshwaran Rajendiran, szr0102@auburn.edu, Department of Chemical Engineering, Auburn University
Selen Cremaschi, szc0113@auburn.edu, Department of Chemical Engineering, Auburn University
Sean M. Wu, smwu@stanford.edu, Stanford Cardiovascular Institute
Elizabeth A. Lipke, eal0003@auburn.edu, Auburn University

One of the major roadblocks in disease modeling is the inability to produce chamber specific cardiomyocytes (CMs). To generate clinically relevant CMs, there is a need to better understand the different cell types produced when hiPSCs undergo cardiac differentiation. This work investigates the cell types produced when scaffold-free and hydrogel supported hiPSC tissues undergo the standard B27 cardiac differentiation protocol, as well as assess and compare the resulting functionality and CM yield of these tissues. Robust cardiac differentiation protocols have been around for a decade and 2D cardiac differentiation was found to yield almost exclusively left ventricular (LV) CMs; however, cardiomyocyte sub-type populations generated in 3D from this protocol are yet to be investigated. We have previously generated engineered cardiac microsphere tissues (MS) by encapsulating and differentiating hiPSCs within poly(ethylene glycol) fibrinogen (PF) 3D hydrogels. Additionally, self-aggregated hiPSC tissues have been successfully differentiated to yield CMs; however, detailed analysis of the cell types formed is needed. Following hiPSC encapsulation (MS) or shaker flask initiation of forced aggregation (aggregates), tissues were cultured for 3 days prior to initiation of differentiation. On differentiation day (DD) 10, a higher CM yield in MS compared to aggregates was observed by flow cytometry analysis for the cardiac troponin T (cTnT) marker. Contraction and relaxation velocities for MS were 40.1±9.7 μm/sec and 46.8±15.5 μm/sec respectively, compared to aggregates with 12.6±7.0 μm/sec and 23.1±6.2 μm/sec respectively. DD 15 tissue cryosections showed positive staining for cTnT and α-smooth muscle actinin. Tissues were optically mapped on DD 45; MS were able to be paced up to 3 Hz. Higher calcium transition velocities and lower calcium transition durations were observed in MS compared to aggregates. Preliminary single-cell RNA sequencing (scRNA-seq) data identified presence of cardiomyocytes, fibroblasts, and endodermal cells. To further investigate the different cell types formed during differentiation, DD 5 and DD 15 tissues were dissociated for scRNA-seq and samples are currently being sequenced. Overall, MS show higher functionality than aggregates and, therefore, are more advantageous for disease modeling and use in further studies to generate specific chamber-specific CMs.

Poster #: 91

Human stem cell-derived β-cells delivered with vasculogenic hydrogels reverse hyperglycemia in diabetic immunodeficient mice

Sophia Kioulaphides, Georgia Institute of Technology

Angelica Torres, Georgia Institute of Technology, alt7@gatech.edu

Michael Hunckler, Georgia Institute of Technology, mdhunck@gmail.com

Graham Barber, Georgia Institute of Technology, gfbarber@gatech.edu

Nathaniel Hogrebe, Washington University St. Louis, nhogrebe@wustl.edu

Jeffrey Millman, Washington University St. Louis, jmillman@wustl.edu

Esma Yolcu, University of Missouri, esma.yolcu@health.missouri.edu

Haval Shirwan, University of Missouri, haval.shirwan@health.missouri.edu

Andres Garcia, Georgia Institute of Technology, andres.garcia@me.gatech.edu

Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreas. The most promising therapy thus far is the transplantation of cadaveric islets, but this approach is severely limited by the lack of donors, poor engraftment, and the need for chronic immunosuppression. To address the lack of insulin-producing cells, human stem cell-derived β (SC-β) cells have become a promising scalable source of insulin-producing cells. This study focuses on hydrogel delivery vehicles for to promote engraftment and function of SC-β cells. SC-β cells were transplanted to the gonadal fat pad (gFP) of non-diabetic SCID-beige mice either with a polyethylene glycol (PEG) hydrogel delivering vascular endothelial growth factor (VEGF) (PEG-VEGF) or PEG gel without VEGF. Immunohistochemistry (IHC) images showed higher signal of C-peptide when SC-β cells were delivered with the PEG-VEGF carrier, indicating that the PEG-VEGF gel promoted engraftment and survival of the SC-β cells. We next transplanted 5.5•106 SC-β cells to the gFP of streptozotocin (STZ)-induced diabetic SCID-beige mice, both with and without PEG-VEGF hydrogels. At 21 days post-transplant, normoglycemia was restored in both treatment groups and persisted for 90 days. At 6 weeks post-transplant, an intraperitoneal glucose tolerance test (IPGTT) confirmed that the grafts responded to glucose stimuli in real-time, and blood serum samples showed the presence of circulating C-peptide. These results motivate current dosing studies to determine the marginal graft that can correct diabetes, and whether the PEG-VEGF gel carrier can promote vascularization of the marginal graft and reverse hyperglycemia. Success in these studies will be a step forward to future T1D correction studies incorporating immunomodulatory strategies in the omentum of immune competent and larger animal models.