2024 REGIONAL SYMPOSIA

September 19-20, 2024

FINAL PROGRAM

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2024 Regional Symposia

Western Symposium: University of Colorado Denver | Anschutz

September 19 - 20, 2024

Program Agenda

Thursday, September 19, 2024

8:00 AM - 8:30 AM	Set-up
8:30 AM - 8:45 AM	Registration / Check-in
8:45 AM - 9:00 AM	Welcome by Site Chairs
9:00 AM - 9:30 AM	*Invited Talk I:* ***Katie Sikes, Colorado State University***
9:30 AM - 10:45 AM	Plenary Session I: ***Shana Kelley, Northwestern University*** ***Joel Collier, Duke University***
10:45 AM - 11:00 AM	Coffee Break
11:00 AM - 11:30 AM	*Invited Talk II:* ***Christopher Snow, Colorado State University***
11:30 AM - 12:00 PM	*Session I: Engineering Cells and Their Microenvironments*
12:00 PM - 1:00 PM	Lunch
1:00 PM - 1:30 PM	*Invited Talk III:* ***Roberta Sabino, University of Wyoming***

Program Agenda

Thursday, September 19, 2024 *(continued)*

1:30 PM - 2:15 PM	Session II: Drug Delivery & Immune Engineering
2:15 PM - 3:30 PM	Plenary Session II: ***Sarah Stabenfeldt, Arizona State Univeristy*** ***Danielle Benoit, University of Oregon***
3:30 PM - 3:50 PM	Break
3:50 PM - 5:00 PM	Session III: Tissue Engineering and Rapid-Fire Poster Presentations I
5:00 PM - 7:00 PM	Poster Session I

Program Agenda

Friday, September 20, 2024

8:00 AM - 8:25 AM	Registration / Check-in
8:25 AM - 8:30 AM	Welcome by Site Chairs
8:30 AM - 9:30 AM	*Invited Talks IV and V:* ***Karin Payne, CU Anschutz*** ***Sayantani Basu, Tolmar***
9:30 AM - 10:45 AM	Plenary Session III: ***Elazer Edelman, Massachusetts Institute of Technology*** ***Cynthia Reinhart-King, Rice University***
10:45 AM - 11:00 AM	Coffee Break
11:00 AM - 12:00 PM	Session IV: Immune Engineering
12:00 PM - 1:00 PM	Lunch
1:00 PM - 2:30 PM	Panel Discussion: Navigating Starting a Career in Academia or Industry - ***Panelists: Wyatt Shields University of Colorado, Boulder; Justin Schaffer, Colorado School of Mines; Vitaly Kheyfets, University of Colorado, Anschutz Medical Campus; Michael Mestek, Medtronic; Micheal Pink, Biodesix***

Program Agenda

Friday, September 20, 2024 *(continued)*

2:30 PM - 2:50 PM	Break
2:50 PM - 3:50 PM	*Invited Talks VI and VII:* ***Wyatt Shields, University of Colorado, Boulder*** ***Jessica Weaver, Arizona State University***
3:50 PM - 5:00 PM	Session V: Engineering Models of Type 2 Diabetes and Rapid-Fire Poster Presentations II
5:00 PM - 5:15 PM	Break / Walk to Poster Session
5:15 PM - 7:00 PM	Poster Session II
7:00 PM - 7:30 PM	Awards Ceremony

2024 Regional Symposia Chair:

Anita Shukla, PhD, Brown University

Western Regional Symposium Co-Chairs:

Nikki Farnsworth, PhD, Colorado School of Mines
Chelsea Magin, PhD, University of Colorado Denver | Anschutz

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Ramya Kumar, PhD, Colorado School of Mines
Kristyn Masters, PhD, University of Colorado Denver | Anschutz
Karin Payne, PhD, University of Colorado Anschutz
Melissa Reynolds, PhD, Colorado State University
Jessica Weaver, PhD, Arizona State University
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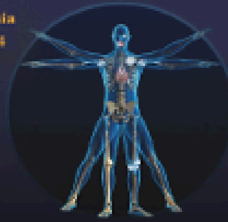
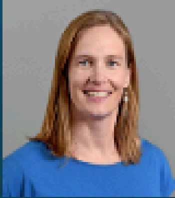
2024 Society for Biomaterials (SFB) Western Symposium, University of Colorado, Anschutz

2024 Regional Symposia
September 19-20, 2024



Plenary Speaker



Dr. Sarah Stabenfeldt, Arizona State University

Invited Speaker

Dr. Katie Sikes, Colorado State University

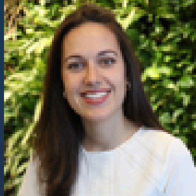
Invited Speaker

Dr. Wyatt Shields University of Colorado, Boulder

Invited Speaker

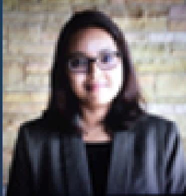
Dr. Karin Payne University of Colorado, Anschutz Medical Campus

Invited Speaker



Dr. Roberta Sabino University of Wyoming

Invited Speaker



Dr. Sayatani Basu Tolmar

Invited Speaker

Dr. Jessica Weaver Arizona State University

Invited Speaker

Dr. Chris Snow Colorado State University

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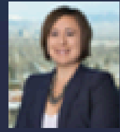

Dr. Chelsea Magin, University of Colorado, Denver | Anschutz

Dr. Nikki Farnsworth, Colorado School of Mines

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Dr. Karin Payne, University of Colorado, Anschutz
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Registration Information

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PLENARY SESSION I Thursday, September 19	PLENARY SESSION I Thursday, September 19	PLENARY SESSION II Thursday, September 19	PLENARY SESSION II Thursday, September 19	PLENARY SESSION III Friday, September 20	PLENARY SESSION III Friday, September 20
Dr. Shana Kelley *Northwestern University*	**Dr. Joel Collier** *Duke University* (2024 Clemson Award for Basic Research)	**Dr. Sarah Stabenfeldt** *Arizona State University*	**Dr. Danielle Benoit** *University of Oregon*	**Dr. Elazer Edelman** *Massachusetts Institute of Technology* (2024 Founders Award)	**Dr. Cynthia Reinhart-King** *Rice University*

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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: ENGINEERING CELLS AND THEIR MICROENVIRONMENT

11:30 AM - 11:45 AM

ENGINEERING PHOTO-ADDRESSABLE HYDROGELS TO INVESTIGATE FIBROBLAST PLASTICITY IN FIBROBLASTIC FOCI IN 3D

Mikala Mueller, University of Colorado Denver Anschutz

Mikala C. Mueller; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; mikala.c.mueller@cuanschutz.edu, Alicia Vaquero Maria; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus, Michael Nott; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; michael.2.nott@ucdenver.edu, Rachel Blomberg; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; rachel.blomberg@cuanschutz.edu

Chelsea Magin; Department of Bioengineering, University of Colorado Denver, Anschutz Medical Campus; Department of Pediatrics, University of Colorado, Anschutz Medical Campus; Division of Pulmonary Sciences & Critical Care Medicine, Department of Medicine, University of Colorado, Anschutz Medical Campus; chelsea.magin@cuanschutz.edu

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease that causes scar tissue formation within the interstitial region of the lung causing a rapid decline in respiratory function. The regions of active in the lung are called fibroblastic foci. Foci are characterized by aberrant, proliferative myofibroblasts that continuously deposit abnormal amounts of extracellular matrix proteins increasing the stiffness of the tissue causing a mechanotransduction feedback loop keeping the myofibroblasts present. Fibroblastic foci are distinct regions with volumes ranging from 1.6 x 104 µm3 to 9.9 x 107 µm3 and focus frequency ranging from 0.9 to 11.1 foci per mm3 with no evidence of interconnectedness between foci. To model the fibroblastic foci in vitro, we have engineered a biomaterial platform that enables dynamic stiffening and softening with precious control to replicate the 3D geometry of a fibroblastic focus. A photodegradable crosslinker was incorporated to study fibroblast plasticity in response to changes in matrix modulus and determine if decreasing the stiffness of the altered region also decreases the fibrotic activation. A 4-arm 5 kg/mol poly(ethylene glycol)-alpha-methacrylate macromer was crosslinked with an MMP9-degradable peptide and an o-nitrobenzyl ether-based photodegradable dithiol crosslinker. The hydrogels were initially measured to be within the range of healthy lung stiffness (1-5 kPa), were dynamically stiffened over 4-fold to mimic fibrosis with spatiotemporal control, and then can be softened back into the healthy range. Due to the arrangement of the hydrophobic groups on the photodegradable crosslinker, the hydrogels exhibit viscoelastic properties with tan(δ) of 0.040 ± 0.005 compared to the non-photodegradable hydrogel with tan(δ) of 0.005 ± 0.003. We have analyzed serial sections of human fibrotic lung tissue to recreate 3D renderings of fibroblastic foci which can be stiffened using two-photon laser scanning lithography into these novel biomaterials. Currently, work is being done to optimize the stiffening and softening of these materials with two-photon laser scanning lithography. Transitioning from a fibrotic state back to the stiffness of healthy lung tissue enables a novel investigation of the fibroblastic response to a mechanical target, potentially returning pathologically activated fibroblasts to homeostasis.

11:45 AM - 12:00 PM

Influence of Cell Seeding Density and ECM Type on Engineered Heart Tissue Behavior

Mackenzie Obenreder, University of Colorado Boulder

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Dr. Jason A. Burdick, University of Colorado Boulder, Jason.Burdick@colorado.edu

Heart disease is the leading cause of death worldwide. In recent years, in vitro cardiac models known as Engineered Heart Tissues (EHTs) have been developed to better understand the etiology and pathology of cardiac diseases using human induced pluripotent stem cell (iPSC) derived cardiomyocytes (CMs). Multiple groups have developed EHT models; however, there is no standard method to generate EHTs and features such as the type of extracellular matrix (ECM) are variable across groups. To fill this gap in knowledge, this work elucidates the influence of cell seeding density and ECM composition on iPSC-CM EHT properties. Our EHT model is generated using iPSC-derived cardiomyocytes in a Digital Light Processing (DLP) printed mold with flexible posts generated using a poly(ethylene glycol) diacrylate resin. The iPSC-CMs are mixed (10 and 60 million cells/mL) within an ECM solution of either type 1 collagen (1 mg/mL) or fibrinogen (4 mg/mL) and then added to the mold for culture. After 10 days of compaction, the EHTs are matured for two weeks using maturation media. The effect of cell seeding density and ECM was compared before (day 22) and after (day 36) maturation. iPSC-CM EHTs with low and high seeding densities (10 and 60 million cells/mL) had comparable functional contractility outcomes of action potential duration (APD), force, and relaxation time before maturation was induced. After maturation, both seeding densities achieved the expected results of increased force and relaxation time; however, only the high seeding density had an increased APD and a decreased time to peak associated with mature iPSC-CMs. To explore the role of ECM, the iPSC-CM EHTs were generated with 1 mg/mL collagen or 4mg/mL fibrinogen, which underwent similar compaction during the 10 days in culture and had comparable results in nearly all functional contractility outcomes before and after maturation. EHTs generated with fibrinogen exhibited a 65.2% greater force of contraction compared to collagen at the day 22 timepoint. By elucidating the effects of cell density and ECM composition over time, we will be able to advance current EHT models and provide the field a platform to study a variety of cardiac diseases and therapies.

SESSION II: DRUG DELIVERY AND IMMUNE ENGINEERING

1:30 PM - 1:45 PM

Particle Shape Modulates Macrophage Phenotype: Insights for Cell-mediated Drug Delivery Systems

Matthew Kwan, University of Colorado Boulder

Matthew M. C. Kwan (University of Colorado Boulder, matthew.kwan@colorado.edu), Nicole B. Day (University of Colorado Boulder, nila1495@colorado.edu), Iain R. Konigsberg (University of Colorado Anschutz Medical Campus, IAIN.KONIGSBERG@cuanschutz.edu), Evan Thoresen (University of Colorado Boulder, evan.thoresen@colorado.edu), Celeste Busch (University of Colorado Boulder, celeste.busch@colorado.edu), Elizabeth J. Davidson (University of Colorado Anschutz Medical Campus, ELIZABETH.DAVIDSON@cuanschutz.edu), Abigail G. Harrell (University of Colorado Boulder, abha3025@colorado.edu), Ivana V. Yang (University of Colorado Anschutz Medical Campus, IVANA.YANG@cuanschutz.edu), C. Wyatt Shields IV (University of Colorado Boulder, Charles.Shields@colorado.edu)

Cell-mediated drug delivery systems are emerging as a new approach to deliver bioactive drugs packaged in micro/nanoparticles to specific, pathologic sites. Circulating immune cells like macrophages have the capacity to enhance particle delivery in vivo through chemotaxis, and once delivered, the cells can participate in the intended therapeutic effect as well. However, particles attached to the surface or internalized are known to exert stresses on macrophages, causing macrophages to polarize between M1-like (proinflammatory) to M2-like (anti-inflammatory) phenotypes. Despite this understanding, little is known about the underlying relationship between particle shape and macrophage phenotype. It has been demonstrated that spherical particle geometries are rapidly phagocytosed in the phagosome within minutes to hours; however, certain non-spherical particle geometries such as discoids are capable of frustrating phagocytosis by macrophages for days, resulting in cell spreading along the particle surface due to inhibited formation of actin structures. Herein, we study the interaction of poly(D,L lactic-co-glycolic acid) discoidal (major axis 7.7 ± 0.5 µm, thickness 276.44 ± 31.8 nm) and spherical (diameter 2.3 ± 0.3 µm) particles on C57BL6-derived primary macrophages to respectively promote or suppress phagocytosis. Discoidal and spherical particles of a fixed volume were fabricated using microcontact printing and homogenization techniques, respectively. Macrophage-disc (MΦ-D) and -sphere (MΦ-S) complexes were manufactured by associating particles in vitro and profiled with RNA-seq, assay for transposase-accessible chromatin with sequencing (ATAC-seq), flow cytometry, and multiplexed ELISA. MΦ-D displayed greater phenotypic shifts compared to MΦ-S. Notably, MΦ-D showed significant differential gene expression [Irf1 (7.93-fold), Il1b (0.80-fold), Vegfa (1.24-fold)], protein biomarkers [Arg1 (31.42-fold), iNOS (3.25-fold), CD40 (0.39-fold)], and secreted cytokines [IL-6 (12.54-fold), TNFα (14.22-fold), CXCL10 (1.08-fold)] compared to unstimulated controls. MΦ-S, on the other hand, displayed significant but less pronounced differential gene expression [Irf1 (1.28-fold), Il1b (0.87-fold), Vegfa (0.76-fold)], protein biomarkers [Arg1 (1.56-fold), iNOS (1.37-fold), CD40 (1.07-fold)], and secreted cytokines [IL-6 (15.91-fold), TNFα (1.52-fold), CXCL10 (0.07-fold)] compared to unstimulated controls. Our work will be useful for designing macrophage-mediated drug delivery systems by enabling the rational selection of cell-particle pairings for the promotion of specific phenotypes that are desirable for certain disease manifestations.

1:45 PM - 2:00 PM

Magnetic biohybrid microrobots for macrophage transport, activation, and imaging

Nicole Day, University of Colorado Boulder

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Background: Adoptive cell transfers (ACTs) are rapidly growing in popularity due to the ability of cells to interact with diseased cells more specifically than small molecule drugs or injected particles. However, ACTs often lack methods to control the localization and function of cells within the body after injection. We have developed a class of non-spherical magnetic microrobots that bind to macrophages to remotely guide cellular phenotypes and transport in physiological environments. For applications involving intravenous delivery, we developed nanodisc “backpacks” that stick to macrophages for spatial control by a gradient magnetic field. For applications involving viscous barriers, we developed helical particles that facilitate cellular transport by swimming in rotating magnetic fields.

Methods: Biodegradable polymer particles were fabricated using standard lithographic techniques: soft lithography (PLGA backpacks) or two-photon lithography (poly(β-amino ester) helices) to encapsulate the small molecule drug resiquimod, which drives the phenotype of macrophages towards an antitumor phenotype. Magnetic field-responsiveness was imparted through entrapping superparamagnetic iron oxide nanoparticles (SPIONs) within the polymer matrix or surface coating by metal evaporation.

Results: Particle aspect ratio and size enables surface binding to macrophages and evasion of phagocytosis. The slow release of resiquimod drives potent M1-like cellular activation by enhanced expression of iNOS and 10-fold increase in IL-12 release on the fifth day compared to free drug. The magnetic properties of the backpacks enabled transport through fluid and tissue environments in vitro in response to a gradient magnetic field. Further, we show that SPIONs within the backpacks serve as tracers for magnetic particle imaging (MPI), a non-invasive imaging modality, with a limit of detection of 5e4 backpacks and accuracy of quantification within 2% for complexes injected into a murine tumor. For macrophage delivery through viscous biological barriers, such as mucus or aqueous humor, we found that the location of macrophage binding to the helical particles gave rise to distinct modes of locomotion, enabling transport as fast as 50 µm/sec through viscous media.

Conclusions: The combination of sustained macrophage polarization, field-responsive transport, and high-resolution monitoring highlights the potential of magnetic cell-based biohybrid robots to improve the performance of ACTs.

2:00 PM - 2:15 PM

Engineering microparticles by coacervation for pulmonary drug delivery

Chima Maduka, University of Colorado, Boulder

Chima V. Maduka1, Fatema Tuj Zohora (fatema.zohora@yale.edu)2, Stephanie Thorn (stephanie.thorn@yale.edu)2, Caroline Zeiss (caroline.zeiss@yale.edu)2, Albert J. Sinusas (albert.sinusas@yale.edu)2, Jason Burdick (Jason.Burdick@colorado.edu)1.

1, University of Colorado, Boulder

2, Yale University

Non-invasive delivery of therapeutics to the lungs can enhance overall safety and efficacy by ensuring the local accumulation of administered drugs, thus minimizing off-target effects. Presenting a unique opportunity, greater than 200 billion capillaries in the human lungs are ≥ 10 microns in diameter. Thus, intravenously administered microparticles passively accumulate in the lung microvasculature, owing to the anatomy of the pulmonary system. To leverage this anatomic feature for drug delivery, we engineered microparticles by charge-driven interaction, also called coacervation, between solutions of chitosan containing small molecules and hyaluronic acid. Dropwise addition of chitosan to hyaluronic acid solutions resulted in the self-assembly of complexes that formed microparticles, which were 32.6 ± 2.7 µm (n=3) in diameter and possessed a Zeta potential of -16.0 ± 0.6 mV (n=3). Using doxycycline as an example of a small molecule drug, we demonstrate an encapsulation efficiency of 31.0 ± 0.5 % (n=3). The loading content present in 10 mg of microparticles was 5.4 ± 0.1 mg (n=3). Further, we demonstrate that doxycycline is steadily released over a 7-day period. To determine the biodistribution of intravenously administered microparticles, we incorporated Cyanine 7 dye (Cy7) in our biomaterial system for in-vivo imaging. Within an hour of intravenous administration, administered microparticles accumulated in the lungs of healthy rats, with minimal Cy7 signals present in the liver, spleen, heart and kidneys. Our strategy is advantageous over inhalational therapeutics that are limited by the epithelial and mucus barriers present in the respiratory tract; as well as therapeutics administered directly into the bloodstream or via nanoparticles, which accumulate in the liver and spleen. Moreover, the biocompatibility of chitosan and hyaluronic acid makes our translatable approach clinically relevant, enabling the local delivery of therapeutics to the lungs of patients.

SESSION III: TISSUE ENGINEERING

3:50 PM - 4:05 PM

Synthetic hydrogel and degradable extracellular matrix supported 3D model for endometriosis

Jinal Mehta, Arizona State University

Jinal M. Mehta1, Rori Hoover1, Rachel E. Young3, Megan Wasson2,Rachel Riley3, Benjamin Bartelle1, Jessica D. Weaver1, 1School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, 2Mayo Clinic, Phoenix, AZ, 3 Rowan University, Glassboro, NJ

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Endometriosis is a chronic condition affecting one in nine women and occurs when endometrial lesions form outside the uterus. Endometriosis is frequently linked to infertility in women, is accompanied by excruciating chronic pain, and can invade and compromise abdominal organs. Laparoscopic surgery is currently the only method to obtain a confirmatory diagnosis of endometriosis, and average time to diagnosis is 7 years after symptom onset. Additionally, treatment methods are limited to hormonal therapies, which have limited efficacy. As such, it is critical to develop methods to improve the diagnosis and treatment of endometriosis.

In this study, we aim to engineer targeted endometriosis imaging and therapeutic approaches using high affinity nanobody-decorated magnetic resonance imaging agents and lipid nanoparticles (LNP). To develop our nanobody candidates, which will be generated using a custom phage display technique, we evaluated human endometriosis single-cell RNA-seq datasets and identified PAEP and IL20RA as proteins with high differential expression in endometriotic lesions. We used immunofluorescence and flow cytometry to identify and quantify these targets in normal endometrial and endometriotic cell lines and are validating our targets in primary patient samples.

We next developed an endometriosis organoid model using an extracellular matrix-mimicking synthetic poly(ethylene glycol) (PEG) hydrogel culture system and normal endometrial and endometriotic epithelial and stromal cell lines. Multicellular organoids were formed by day 3 with an architecture comparable to native endometrium and endometriosis, with a stromal core and an epithelial crust. Endometriotic cells and organoids exhibited higher metabolic activity than normal primary endometrium, and all cells exhibited high viability out to at least 7 days. Preliminary experiments with two untargeted LNP compositions incorporating GFP in the LNP membrane and luciferase mRNA cargo demonstrated enhanced uptake of LNP in endometriotic organoids relative to normal endometrial organoids via quantitative luciferase expression; we confirmed internalization within our organoids via GFP imaging. Future and ongoing studies will establish a humanized endometriosis mouse model in NSG mice to enable in vivo testing of nanobody targeting to implanted organoid lesions

4:05 PM - 4:20 PM

Effects of Hyaluronic Acid and A7R Peptide on Angiogenesis and Osteogenesis in Growth Plate Injury Models in vitro

Elise Collins, Colorado School of Mines

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2Biochemistry, Colorado School of Mines, Golden, CO

3Department of Orthopedics, University of Colorado Anschutz Medical Campus, Aurora, CO

The growth plate is a cartilaginous tissue at the end of long bones that allows bones to elongate over time. Injury to the growth plate is common when children sustain fractures in their long bones, and the damage can create lasting growth complications that require many successive surgeries. Cartilage does not naturally regenerate, so bone tends to grow in the injury site, forming a “bony bar” that prevents the bone from growing normally. A big difference between bone and cartilage is that bone is vascular, which means that angiogenesis, the formation of blood vessels, must occur before osteogenesis. Anti-angiogenesis drugs have been shown to reduce blood vessel formation when encapsulated in a hydrogel matrix, but the effects of insoluble factors attached to the matrix have not been tested. This study aims to evaluate the impact of hyaluronic acid and anti-angiogenic peptides on angiogenesis and “bony bar” formation. Hyaluronic acid (HA) with varying molecular weights (10 kDa, 100 kDa, and 1 MDa) and an anti-angiogenic peptide (A7R with sequence ATWLPPR) with varying concentrations were attached to alginate-chitosan polyelectrolyte complexes (PECs) to assess their impacts on cell behavior and gel properties. These materials were used to separately culture human mesenchymal stems cells (hMSCs), for impact on cell differentiation, and human umbilical vein endothelial cells (HUVECs), for impact on angiogenesis, in vitro. Cells cultured in PECs containing the lowest and highest molecular weight of HA generally showed lowered expression of angiogenic genes compared to the 100 kDa HA PEC after two weeks of culture. However, after three weeks of culture in all PECs containing HA, HUVEC expression of angiogenic genes was significantly lowered. For hMSC cultures, the 10 kDa HA had the greatest effects on chondrogenic gene expression and differentiation. Similarly, the A7R PECs caused downregulation in osteogenic gene expression and dose-dependent upregulation of some chondrogenic genes. The results suggest that A7R peptide can prevent osteogenic gene expression and that fragmented HA (MW 10 kDa) can prevent angiogenic gene expression. In future work, the materials will be tested on growth plate injury rat models in vivo for limb length and bone volume.

SESSION IV: IMMUNE ENGINEERING

11:00 AM - 11:15 AM

Tolerogenic Human Placental Trophoblast Survival and Engraftment in a Xenograft Model

Chishiba Chilimba, Arizona State University

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Co-author: Jessica D. Weaver, School of Biological and Health Systems Engineering, Arizona State University, jdweave5@asu.edu

Cell and tissue transplantation is hindered by graft rejection, which necessitates lifelong chronic systemic immunosuppression. Placental pregnancy is the only natural state of de novo tolerance against allogeneic tissue in adult mammals, and is mediated by placental trophoblast cells, which use diverse mechanisms to induce antigen-specific tolerance. Understanding trophoblast tolerogenic mechanisms holds potential implications for eliminating immunosuppression in allogeneic transplantation.

In this study we aimed to evaluate the potential of human trophoblast model cell line, JAR, to evade graft rejection via tolerogenic soluble factors in an immune competent xenograft model (C57BL/6 recipient). To conduct this, we validated the survival of JAR cells macroencapsulated in spiral devices via live/dead imaging and metabolic activity in vitro over 7 days of culture. To evaluate successful engraftment in vivo in the absence of immune rejection, encapsulated and unencapsulated JAR cells transfected with Nanoluciferase were transplanted into immune deficient NSG mice in the subcutaneous (SUBQ) or epididymal fat pad (EFP) sites with a vasculogenic VEGF-delivering degradable hydrogel to enhance local vascularization. Unencapsulated groups maintained higher luminescent signal than encapsulated groups, increasing gradually over time, and formed palpable tumors SUBQ over 3 weeks. Overall, encapsulated JAR cells exhibited highest signal relative to day 0 in the EFP (~213%) compared to the SUBQ (~74%). Next, we evaluated unencapsulated and encapsulated nanoluciferase JAR survival in an immune competent C57BL/6 xenograft model. JAR were transplanted in SUBQ and EFP sites, with and without vasculogenic degradable hydrogel. The EFP groups experienced rapid rejection, with a reduction to near-baseline signal in all groups by day 10. In the SUBQ groups, rejection was observed between day 14-28, except for the encapsulated with vasculogenic hydrogel group, which demonstrated significantly elevated signal comparable to the NSG group out to day 63. Encapsulated non-tolerogenic control human HEK cell controls were rapidly rejected within 10 days, suggesting that trophoblast-secreted soluble factors contributed to JAR survival.

Overall, trophoblast in vivo imaging survival demonstrated high cell survival in immune compromised mice and comparable survival in immune competent mice in the SUBQ site. Ongoing studies are evaluating mechanisms of trophoblast survival and immune modulation via histology and flow cytometry.

11:15 AM - 11:30 AM

Macroporous Gelatin Scaffolds for Lymph Node Stromal Cell-Mediated Immunomodulation in Type 1 Diabetes

Leonor Teles, University of Miami

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Autoimmune diseases (AD) like Type 1 Diabetes (T1D) are caused by the disruption of T-cell tolerance to self-antigens (Ags), thereby unleashing autoreactive T-cells that attack self-tissues. AD affect 8% of the US population, and their incidence is increasing. Current clinical treatments are limited to systemic T-cell approaches with adverse side effects and restricted efficacy. Therefore, there is a need for localized, Ag-specific therapies that selectively target autoreactive T-cells. A promising strategy involves Fibroblastic Reticular Cells (FRCs). FRCs are lymph node (LN) stromal cells that build the LN reticula and act as non-professional Ag-presenting cells. Upon inflammation, FRCs expand the LNs to facilitate T-cell proliferation and upregulate Ag-presenting machinery and immune checkpoint molecules with limited expression of co-stimulatory molecules. FRCs are thus more likely to regulate rather than activate Ag-specific T-cells. However, their therapeutic potential for Ag-specific immunomodulation for AD remains unexplored. We aim to use biomaterials as templates for the therapeutic application of FRCs in AD. We engineered 3D FRC-based reticula using macroporous gelatin scaffolds with pore diameters that mimic the FRC reticula in normal (>50 µm) and inflamed (>150 µm) LNs to study the effects of FRC reticular pore size on FRC and T-cell interactions and immunomodulation. Our scaffolds are biocompatible and promote FRC viability, reticular formation, extracellular matrix secretion, and local retention for at least 21 days after subcutaneous transplantation in T1D mouse models. In vitro, co-culture of CD8+ and CD4+ T-cells in scaffolds with FRCs and T1D peptide Ags promoted engagement of Ag-specific T cells (proliferation and CD25 and CD44 upregulation) with reduced cytotoxic and increased anergic and regulatory phenotypes. These results suggest an Ag-specific immunomodulation of T cells by our 3D FRC platform. Current studies are unveiling the optimal scaffold pore size for FRC engraftment for in vivo recruitment and immunomodulation. Future work will also evaluate whether incorporating T1D Ags onto scaffolds enables localized and prolonged delivery of Ags for FRC uptake and presentation to T-cells compared to ex vivo Ag pulsing. Our platform could provide a local, retrievable, Ag-specific immunomodulatory therapy to treat AD like T1D by selectively suppressing and/or regulating Ag-specific autoreactive T-cells.

11:30 AM - 11:45 AM

Characterization of Sex-specific Hormone and Gene Expression Alterations in Experimental Model of Traumatic Brain Injury

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Purpose/Objective: Traumatic Brain Injury (TBI) results from a blow or jolt to the head and leads to complex pathologies that can extend years after the initial injury. Unfortunately, current treatments for TBI focus on managing symptoms such as edema and intracranial hemorrhage rather than targeting the pathologies that lead to neurodegeneration. Additionally, little is known about how TBI pathology differs between sexes and how alterations in circulating sex steroid hormones contribute to chronic TBI pathologies. To address these challenges, our group employs both male and female models to assess sex-dependent pathologies of traumatic brain injury. In this project, our goal is to evaluate sex differences in circulating sex steroid hormones alterations, BBB disruption and neuroinflammation after TBI.

Methods: We used a well-established mouse controlled cortical impact (CCI) model to induce a moderate TBI over the primary somatosensory cortex in C57BL/6J mice. Male (n=10) and female (n=12) cohorts were injured at 9-10 weeks of age followed by tissue and blood collection at 24 and 72 hrs post-injury. Plasma samples were analyzed with an LC-MS/MS hormone panel for testosterone, androstenedione, estradiol, estrone, progesterone, DHEA and corticosterone. For the RNA-sequencing, a 4 mm cortical punch was taken directly over the injury penumbra and contralateral hemisphere at sacrifice.

Results: Notably, we observe sex-specific alterations in circulating sex steroid hormones after TBI at both 24 and 72 hours. Females exhibit decreases in progesterone, androstenedione, and testosterone at 24 and 72 hrs compared to naïve females, while males exhibit increases in DHEA and decreases in estradiol at 72 hrs compared to naïve males. Additionally, RNA-sequencing reveals sex- and timepoint-specific gene sets when compared to sex-matched controls. Furthermore, functional enrichment analysis of these gene sets shows the female 24-hour group had significant upregulation in genes associated with innate immune trafficking and activation compared to other groups.

Conclusion/Future Work: Our results indicate there are sex-specific responses to traumatic brain injury, including sex specific alterations in steroid hormone profile and gene expression. We aim to further elucidate hormone-related pathologies by conducting a correlation analysis using gene expression and hormone level.

11:45 AM - 12:00 PM

Extracellular Matrix Regulation of the Neutrophil Infectious Response

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As the body's first line of defense against disease and infection, neutrophils must efficiently navigate to sites of inflammation; however, neutrophil dysregulation contributes to the pathogenesis of numerous diseases, including fibrosis and cancers. While it is known that neutrophils are mechanosensitive cells, our understanding of how the extracellular matrix (ECM) affects their responses during infection is incomplete. This is due, partly, to a lack of experimental systems with the ability to control ECM composition while maintaining a physiologically relevant infectious microenvironment. Specifically, the endothelial cells that line blood vessels are both mechanosensitive and play a critical role in neutrophil activation. Therefore, in addition to directly impacting neutrophil function, changes to the ECM could indirectly influence neutrophils through the blood vessel. To capture these interactions, we used our infection-on-a-chip microfluidic device, which contains a model blood vessel lumen surrounded by collagen, to study how collagen density affects neutrophil function.

Collagen concentration in the ECM varies in healthy and diseased tissues. Additionally, tissues susceptible to infections, such as the lungs, skin, and liver, are more prone to infections when diseased. Therefore, we sought to study how collagen concentration affects neutrophil functionality in response to Pseudomonas aeruginosa. By varying collagen concentration from 2 to 6mg/mL we found neutrophils extravasate more into 4mg/mL collagen than 2 or 6mg/mL collagen in the presence of an endothelium. In the absence of an endothelium no difference in neutrophil extravasation was observed, suggesting an endothelial cell-dependent mechanism by which the neutrophils differentially extravasated. Furthermore, neutrophils migrate further and faster as the collagen concentration in ECM decreased. This could be due to decreased pore size with increasing collagen concentration, restricting neutrophil movement. However, this difference was not observed for neutrophils that had not extravasated through an endothelium, suggesting an endothelial cell-dependent mechanism by which neutrophil migration is regulated. Lastly, no differences in reactive oxygen species production across the collagen concentrations were observed. We are now seeking to investigate how enzymatic crosslinking of collagen, as seen in vivo, affects the ECM and, consequently, the neutrophil response. Together these results demonstrate the importance of studying neutrophil-ECM interactions in microphysiological systems.

SESSION V: ENGINEERING MODELS OF TYPE 2 DIABETES

3:50 PM - 4:05 PM

Extracellular matrix stiffness mediates insulin secretion in pancreatic islets via mechanosensitive Piezo1 channel regulated Ca2+ dynamics

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The pancreatic islet is surrounded by ECM that provides both biochemical and mechanical cues to the islet β-cell to regulate cell survival and insulin secretion. Changes in ECM composition and mechanical properties drive β-cell dysfunction in many pancreatic diseases. While several studies have characterized changes in islet insulin secretion with changes in substrate stiffness, little is known about the mechanotransduction signaling driving altered islet function in response to mechanical cues. We hypothesized that increasing matrix stiffness will lead to insulin secretion dysfunction by opening the mechanosensitive ion channel Piezo1 and disrupting intracellular Ca2+ dynamics in mouse and human islets. To test our hypothesis, mouse and human cadaveric islets were encapsulated in a biomimetic reverse thermal gel (RTG) scaffold with tailorable stiffness that allows formation of islet focal adhesions with the scaffold and activation of Piezo1 in 3D. Our results indicate that increased scaffold stiffness causes insulin secretion dysfunction mediated by increases in Ca2+ influx and altered Ca2+ dynamics via opening of the mechanosensitive Piezo1 channel. Additionally, inhibition of Piezo1 rescued GSIS in islets in stiff scaffolds. Overall, our results emphasize the role mechanical properties of the islet microenvironment plays in regulating function. It also supports further investigation into the modulation of Piezo1 channel activity to restore islet function in diseases like type 2 diabetes (T2D) and pancreatic cancer where fibrosis of the peri-islet ECM leads to increased tissue stiffness and islet dysfunction.

4:05 PM - 4:20 PM

Changes in extracellular matrix stiffness mediate pancreatic islet function: insights into glucose metabolism and mitochondrial dynamics

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In the pancreas, the islet is surrounded by a specialized extracellular matrix (ECM) which regulates cell survival and insulin secretion from islet β-cells. Little is known about how mechanical properties, like stiffness, of the ECM regulate islet function in health and disease. Previous research conducted on islets from a rat model of type 2 diabetes (T2D), characterized by increased ECM stiffness and reduced insulin secretion, revealed changes in mitochondrial morphology marked by increased mitochondrial fragmentation. Mitochondrial morphology is regulated by membrane potential (ΔΨ), with alterations in ΔΨ influencing fusion-fission dynamics crucial for maintaining mitochondrial function. Insulin secretion in the β-cell is tightly controlled by metabolism and mitochondrial function; however, the mechanisms underlying mechanotransduction regulation of glucose metabolism and mitochondrial dynamics have not been well studied in intact islets. We hypothesize that increasing matrix stiffness will yield a depolarized ΔΨ, an increase in mitochondrial fragmentation, and impaired insulin secretion. To test our hypothesis, mouse and human cadaveric islets were encapsulated in PEG-maleimide with an RGD-cysteine binding ligand crosslinked via Michael-type addition to PEG-dithiol. This 3D PEG hydrogel mimics the islet microenvironment, where 3wt% PEG gels (0.204±0.007 kPa) represent healthy pancreas stiffness and 7.5wt% (2.25±0.153 kPa) represents stiffness seen in T2D ECM. Our results indicate that increased scaffold stiffness causes a 57% reduction in insulin secretion stimulation index (p=0.029), a 220-240% decrease in TMRE fluorescence intensity (p=0.0043) indicating depolarization of the ΔΨ at 2mM and 20mM glucose respectively, and mitochondrial fragmentation. Overall, our results emphasize the role mechanical properties of the islet microenvironment plays in regulating insulin secretion and mitochondrial function. It also supports further investigation into the modulation of glucose metabolism and mitochondrial dynamics to restore islet function in diseases like T2D where fibrosis of the peri-islet ECM leads to increased tissue stiffness and islet dysfunction.

RAPID FIRES

Drug Delivery

4:20 PM - 4:26 PM

Designing polycaprolactone nanocapsules to target and promote pancreatic β-cell survival during the onset of type 1 diabetes

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The current gold standard for the treatment of T1D does not provide a cure but relies on continuous administration of exogenous insulin to regulate hyperglycemia and manage associated symptoms. Clinical interventions to prevent pancreatic β-cell stress or death during T1D onset are promising curative strategies that could halt or reverse the disease progression. Systemic administration of peptide therapeutics such as δV1-1 with excellent potential to promote β-cell survival often leads to severe off-target effects and reduced efficacy due to non-specific targeting and poor cellular uptake. We hypothesize that encapsulating δV1-1 in polycaprolactone nanocapsules coated with targeting peptides can lead to the preferential targeting of pancreatic β-cells, thereby enhancing the therapeutic efficacy of δV1-1. To test our hypothesis, Cy5, δV1-1, or δV1-1 conjugated to cell-penetrating TAT peptide were encapsulated in polycaprolactone nanocapsules using emulsification solvent evaporation method. The nanocapsules were coated with either glucagon-like peptide-1 receptor agonist Exendin-4 conjugated to hyaluronic acid (HA-Ex4) or an antibody against the human-specific β-cell marker, ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3 AB). The coated nanocapsules were characterized and evaluated in vitro using isolated mouse and human cadaveric islets treated with or without cytokines and in vivo with NOD mice. Our results showed that coatings of nanocapsules with targeting peptides facilitate selective uptake of the Cy5 by the pancreatic β-cells in both in vitro and in vivo models. A significant proportion of Cy5 nanocapsules coated with ENTPD3 AB were trafficked into the pancreatic islets with more than 70% delivered to the insulin-positive β-cells. Targeted delivery of δV1-1 by the nanocapsules improves the effectiveness of δV1-1 to protect pancreatic cells against cytokine-induced death when compared to the action of free δV1-1. Also, the viability percent of cytokine-treated islets in the presence of HA-Ex4 coated δV1-1 nanocapsules was not statistically different from the untreated islets. Conclusively, these data showed that targeted delivery of δV1-1 to the β-cells by the polycaprolactone nanocapsules enhanced its therapeutic efficacy. It also supports further preclinical investigation of these nanocapsules in promoting pancreatic β-cell survival in mouse models of T1D onset.

4:26 PM - 4:32 PM

Co-assembly of block and statistical copolymers into mixed micelles augment pDNA delivery performance and polyplex serum stability

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Gene therapy has demonstrated tremendous therapeutic promise for inherited diseases as well as highly prevalent diseases such as cancer, diabetes, or age-related macular degeneration. To mediate efficient gene delivery into target cells, numerous synthetic nanocarriers have been engineered. Cationic polymers have been extensively studied due to their low cost, availability, and the ability to readily condense bulky anionic payloads such as plasmids (pDNA) but their therapeutic applicability is limited by polymer toxicity and interactions with serum proteins. Earlier, we synthesized block (B), gradient (G), and statistical (S) copolymers to elucidate the role of polymer microstructure on polyplex stability and gene delivery efficiency. We hypothesized that mixed micelles, co-assembled of block copolymers with statistical/gradient copolymers, will outperform conventional micelles as well as linear polymers in maintaining colloidal stability, promoting efficient pDNA delivery, and minimizing toxicity. Block copolymers were co-assembled with either gradient or statistical copolymers using different mixing ratios (B/G and B/S) to form mixed micelles, which were then complexed with pDNA via electrostatic interactions, forming mixed micelleplexes. We compared the toxicity, serum stability, and pDNA delivery efficiency as a function of B/G and B/S mixing ratios. Morphological analysis using transmission electron microscope indicated that the size distribution and aspect ratio of mixed micelleplexes were highly sensitive to the mixing ratios used (B/G and B/S). Dynamic light scattering verified that all mixed micelleplexes were sized between 150 to 200 nm, which favors high cellular uptake. Mixed micelleplexes were less toxic than polyplexes and conventional micelleplexes. Mixed micelleplexes formed from block and statistical copolymers performed best among all the combinations tested. Interestingly, while the B/G mixing ratio governed the pDNA delivery efficiency of block: gradient mixed micelles, the delivery efficiency of block statistical mixed micelles was insensitive to B/S mixing ratio. To explain this trend, we performed static light scattering measurements and calculated pDNA loading per micelleplexes as a function of mixing ratio. Our study reveals design rules for rationally co-assembling copolymers with varied monomer spatial distribution (block/gradient/statistical) into mixed micelles that exhibit gene delivery performance superior to that of conventional micelles (formed by block copolymers only).

4:32 PM - 4:38 PM

Using Triazole Crosslinks and a Monofunctional Hydrazide to Engineer an Injectable Hydrazone Crosslinked Hydrogel for Cell Delivery

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Covalent Adaptable Networks (CANs) can enable self-healing and injectable properties in hydrogels for noninvasive drug and cell delivery. The alkyl-hydrazone crosslink is one such chemistry used in hydrogel CAN systems as it is biocompatible and biorthogonal compared to other clinically used amine-involved reactions such as imine ligation. Due to its adaptable nature and the reactivity of aldehydes with primary amines, the alkyl-hydrazone crosslinked hydrogels lack stability in protein rich environments. Additionally, hydrogels utilizing hydrazone crosslinks and biopolymers such as Hyaluronic Acid (HA) typically require low polymer content and low molecular weight polymers to enable injectability due to polymer entanglements. To achieve enhanced stability, a high molecular weight (>60 kDa) hyaluronic acid alkyl-hydrazone crosslinked hydrogel, comprised of HA functionalized with hydrazides (HA-Hyd) and HA functionalized with aldehydes (HA-Ald), was modified by introducing slow reacting triazole crosslinks. By reacting HA-Hyd with a small amount of benzaldehyde-poly(ethylene glycol)3-azide, HA-Hyd effectively becomes dual functionalized with hydrazides and azides which enables the formation of the triazole bond via a biorthogonal and slow reacting strain-promoted azide-alkyne cycloaddition (SPAAC) reaction with an 8 arm poly(ethylene glycol) (PEG) functionalized with bicyclononyne. With increasing percent of bonds formed through SPAAC, the stability of the dual crosslinked hydrogel in media was increased, as observed through reduced visual changes in shape and by measuring the relative HA erosion over a 14 day period. In situ shear rheology and force extrusion studies demonstrated the slow crosslink formation of SPAAC bonds enabling a temporary state of injectability that was similar to an alkyl-hydrazone crosslinked hydrogel. To improve the injectability of the hydrazone and triazole crosslinked hydrogel, a monofunctionalized hydrazide, methyl-PEG4-hydrazide (m-PEG4-Hyd), was reacted with HA-Ald to selectively slow down the alkyl-hydrazone crosslinks. By selecting concentrations of m-PEG4-Hyd that significantly slowed network evolution, tunable extrusion force was achieved. Decreasing extrusion forces with m-PEG4-Hyd enabled improved viability of post-extruded rat mesenchymal stem cells (rMSCs) inside the dual crosslinked hydrogel. Additionally, rMSCs encapsulated inside the hydrogel with high post-extrusion viability secreted relatively more VEGF and relatively less MCP-1 and TIMP-1 than rMSCs encapsulated inside a hydrogel with low post-extrusion viability.

4:38 PM - 4:44 PM

Ionic liquid-embedded adhesive hydrogel system for tunable transdermal drug delivery

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Background: Hydrogels are an effective platform for administering drugs to cells in the skin due to their biocompatibility, flexible chemistry, and mechanical similarity to living tissues. However, mechanisms governing drug release are often inextricably linked to bulk properties of the hydrogel, and drug transport across the stratum corneum is limited. There remains a need for a simple transdermal delivery system that enables multimodal release of immunomodulatory drugs for a variety of skin diseases.

Methods: We describe a tissue-adhesive hydrogel embedded with drug-loaded microparticles and ionic liquid to enable tunable release of drugs in a manner independent from the properties of the hydrogel. This hydrogel was designed to sustainably deliver localized treatments to skin while adherent, after which it can be removed by peeling. The hydrogel was synthesized using a one-pot free radical polymerization to create a biocompatible and nondegradable polyacrylamide gel containing adhesive galloyl groups, capable of encasing multiple populations of particles to release drugs in a multimodal manner. Embedded silicone particles were synthesized using a nucleation and growth technique. Ionic liquids, comprising an anion and a bulky, asymmetric cation, were incorporated to mediate drug transport across the stratum corneum for applications where the skin barrier is not compromised, such as by surgical resection or a wound site.

Results: We demonstrate multimodal drug release by designing particles that can release resiquimod (a macrophage-stimulating drug) over several hours and palbociclib (a T-cell-stimulating drug) over several days. We show cell activation by promoting polarization of macrophages in vitro and ex vivo toward anti-tumor phenotypes, with significantly higher expression of macrophage inflammatory markers (iNOS, CD86, and MHCII) compared to free drug over five days. Furthermore, we show that that ionic liquids embedded in the hydrogel can facilitate the transport of the small molecule drug ruxolitinib (a JAK1/2 inhibitor) across the stratum corneum, resulting in higher drug efficacy than clinical creams and enabling the treatment of skin disease with intact or hyperkeratinized stratum corneum.

Conclusions: The multimodal nature of this hydrogel system has implications in treating a variety of skin disorders and delivering vaccines at well-defined rates.

Immune Engineering

4:44 PM - 4:50 PM

Dissolved Gases from Elevated Pressure in the Lungs Elicit Innate Immune Cell Responses

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Conventional dogma suggests that decompression sickness (DCS) is caused by nitrogen bubble nucleation in the blood vessels; however, recent studies indicate that the abundance of bubbles is not indicative of DCS severity. Since immune cells are known to respond to chemical and environmental cues, we hypothesized that dissolved gasses from elevated hydrostatic pressures drive aberrant immune cell phenotypes in the vasculature while diving. We describe a system to determine this response using a custom hyperbaric chamber and human lung-on-a-chip devices. The hyperbaric chamber was designed with a compressor connected to an ASME-code pressure tank equipped to generate pressures up to 7.5 atm, corresponding to dive depths of 67.2 m (220 ft). Two-channel lung-on-a-chip devices were seeded with primary human alveolar and microvascular cells in the top and bottom channels, respectively. Human blood samples extracted from healthy donors were infused in the lung-on-a-chip devices, exposed to alveolar gas mixtures, and pressurized to either 1.0 atm or 3.5 atm for one hour followed by decompression at a fixed rate. Phenotypes of neutrophils, dendritic cells, and monocytes were determined by flow cytometry and multiplexed ELISA. Results indicate an immune response occurs at 3.5 atm compared to 1.0 atm controls. From the flow cytometry data, dissolved gases activated several phenotypic markers of innate immune cells (e.g., as indicated by an elevated expression of CD41a and MPO in neutrophils and CD80 in monocytes and dendritic cells). The cytokine secretion data showed distinct differences between effects from increased oxygen vs. nitrogen partial pressures, corresponding to compressed alveolar air vs. compressed oxygen-reduced air commonly used in diving (e.g., significant differences were observed in the secretion of IL-1α, IL-1β, GM-CSF, IFNα, and IFNγ). This work suggests innate immune reactions may play a role in DCS, which has implications in identifying high-risk individuals and may allow for new means of mitigating DCS. In future work, we will perform epigenetics and transcriptomics on cells in devices to determine the pathways of gene expression that mediate the cellular responses observed.

4:50 PM - 4:56 PM

Engineering trophoblast extracellular vesicle-delivering hydrogels for localized tolerance induction

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Cell therapies are a promising method to treat diseases such as type 1 diabetes; however, this approach necessitates systemic immunosuppression to prevent graft rejection, which imparts severe risks such as cancer and infection. Strategies such as cell encapsulation and localized delivery of tolerogenic factors within the cell graft site targeting the direct and indirect antigen presentation pathway, respectively, have been explored to prevent graft rejection. In pregnancy, the placental trophoblast uses a combined approach of inert surface to block direct antigen recognition and secreted soluble factors, such as extracellular vesicles (EVs), to mitigate indirect antigen recognition of the allogeneic fetus and induce tolerance. Trophoblast EVs contain a multitude of tolerogenic factors which influence the behavior of immune cells. In this work, we engineered a synthetic tunable hydrogel-based trophoblast EV delivery system for sustained delivery of EVs within an encapsulated cell transplant site to induce localized graft tolerance in a similar manner to placental pregnancy.

We first characterized EV delivery kinetics from degradable and nondegradable synthetic poly(ethylene glycol) (PEG)-based hydrogels, where EVs are chemically linked directly to the hydrogel matrix with or without a hydrolytic linker; tethered EV release was compared against passive hydrogel entrapment. EV delivery was evaluated in vitro and in vivo using longitudinal IVIS imaging, where matrix-tethered EV delivery was extended relative to untethered and passively entrapped EVs. In vitro immune cell studies demonstrate that hydrogel-delivered trophoblast EVs reduced natural killer cell (NK-92) activation in vitro, and upregulated TNFalpha secretion in THP-1 M0 macrophages. Imaging studies demonstrated significant EV uptake by M0 macrophages. Ongoing studies are evaluating hydrogel-delivered EV influence on THP-1 M1 macrophages, and primary human PBMCs via luminex cytokine panels. Histological analysis of in vivo EV delivery and localization to the graft site and draining lymph nodes are ongoing. Future studies will investigate the localized immune response at graft site through flow cytometry and histology.

Engineering Cells and Their Microenvironments

4:20 PM - 4:26 PM

Photo-responsive hydrogel to study 4D mechano-transduction during intestinal tissue homeostasis

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Background: Mammalian gut epithelial lining has a crypt-villi architecture and serves many crucial functions. The epithelium experiences rapid cellular turnover and regenerates continuously from multipotent intestinal stem cells (ISCs) at the crypt base. During homeostasis, the ISC niche exists together with differentiated cells. Anomalies in this homeostatic regulation can cause diseases like colorectal cancer (CRC). While molecules secreted by the surrounding mesenchymal cells constitute vital biochemical signals in guiding cell fate and organization, the role of mechanical cues during ISC differentiation has only been recently identified. However, the spatiotemporal role of mechano-transduction in the crypt formation process is not fully deciphered due to lack of appropriate in vitro models. More importantly, the role of cell nuclei in integrating the extracellular mechanical cues with genetic transformation during differentiation is unknown.

Methods: We employed an interdisciplinary approach integrating murine ISC derived primary organoids, novel photo-responsive hydrogels, sophisticated microscopic techniques like light-sheet imaging and in vivo tissue sections. The hydrogel system consists of poly(ethylene glycol) (PEG) chains functionalized with nitrobenzyl-azide and dibenzylcyclooctyne (DBCO). Upon mixing of the two PEG macromers, the azide reacts with DBCO via a strain promoted alkyne-azide cycloaddition (SPAAC) bio-click reaction that can be used to encapsulate ISCs and grow organoids. Uniquely, the ortho-nitrobenzyl (oNB) crosslinks can be cleaved with a 405nm confocal laser, softening the hydrogel at predefined regions, thus directing crypt formation of controlled dimensions at specified points in space and time allowing high resolution time-lapse microscopy.

Conclusion: Our spatio-temporally controllable crypt-growth approach revealed noticeable changes in the nuclear envelope architecture and composition as ISCs differentiate. We identified changes in the chromatin accessibility of the cells due to altered histone methylation levels. This is accompanied by changes in localization of cell nuclei with respect to the epithelium which is also observed in vivo. These observations indicate that the nuclei might play an active role in the process of crypt formation and differentiation. Cumulatively, we showcase the design and utilization of a novel photo-responsive biomaterial platform for studying intestinal crypt homeostasis and pathology.

4:26 PM - 4:32 PM

Inducing synthetic cryptobiosis in mammalian cells via reversible intracellular hydrogelation

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Some organisms can withstand extreme environmental conditions by undergoing transient vitrification of their cellular contents, providing protection against irreversible aggregation of intracellular proteins and other biomolecules. Typically, this “cryptobiotic” process relies on molecular crowding by polysaccharides or disordered proteins, but human and other mammalian cells lack this capability. In this work, we transfect mammalian cells with reactive poly(ethylene glycol) macromers to form degradable intracellular hydrogels as a synthetic mimic of cryptobiosis, which we term “biostasis.” Using fluorescence correlation spectroscopy, cells containing hydrogels are shown to have restricted intracellular mobility on the nanoscale, resulting in decreased cell cycle activity and cytoskeletal remodeling while retaining high viability in both conventional 2D cell culture and 3D culture of multicellular epithelial spheroids. By forming intracellular hydrogels with photodegradable crosslinks, we show that on-demand degelation via light exposure reverses biostasis. The reversal process is monitored by label-free multiphoton fluorescence lifetime imaging to probe NADH mobility and binding as a proxy for bioenergetics. Finally, we show that biostasis treatment results in increased cellular viability in the presence of harsh stimuli, including peroxides, hypo-osmolar media, and an apoptosis-inducing peptide ligand. Taken together, these results demonstrate that our approach for inducing synthetic cryptobiosis offers a novel and potentially transformative method for controlling cell cycle activity and protecting mammalian cells from a variety of stressors. This technology holds significant promise for applications in multiple fields, including cellular preservation, biophysics, and regenerative medicine.

4:32 PM - 4:38 PM

FUNCTIONALIZING HUMAN DECM FOR INCORPORATION INTO 3D PULMONARY FIBROSIS MODELS

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Idiopathic pulmonary fibrosis (IPF) is an incurable lung disease characterized by dysregulated fibroblast activation and excessive extracellular matrix (ECM) deposition, culminating in critical lung dysfunction. Existing preclinical models inadequately simulate the nuanced pathogenesis of IPF, primarily due to their reductive representation of pulmonary architecture, thereby hindering advancements in disease comprehension and therapeutic intervention. To overcome this limitation, we have engineered an advanced 3D lung model incorporating induced pluripotent stem cell (iPSC)-derived human epithelial cells and fibroblasts within hybrid-hydrogels containing decellularized ECM (dECM) from control and fibrotic lung tissues. These models aim to emulate the intricate cellular interactions and ECM dynamics underlying IPF pathogenesis, providing a biomaterial-centric and physiologically relevant framework for elucidating disease mechanisms and evaluating potential treatments.

Protocols for dECM preparation from control and fibrotic lung tissues were first optimized for incorporation into dynamically tunable poly(ethylene glycol) alpha-methacrylate (PEGαMA) hydrogels. This process entailed systematic decellularization, pepsin-mediated digestion (10 mg dECM/mL Pepsin-HCl for up to 96 hours), and chemical functionalization to enable a thiol-ene Michael addition reaction with PEGαMA. Analytical assessments were performed at 12-hour intervals throughout the digestion phase. A bicinchoninic acid (BCA) assay measured total protein quantification, ninhydrin assays evaluated primary amine concentrations, and SDS-PAGE quantified protein fragmentation. Results showed a 48-hour digestion period for both healthy and fibrotic lung tissues led to the highest levels of soluble protein and free primary amines. Subsequently, naturally occurring primary amines on the 48-hour-digested dECM were converted to thiols via 2-iminothiolane (Traut's reagent) at 25-, 50-, 75-, or 100-molar excess. Conversion was quantified through ninhydrin and Ellman's assays and functionalization at a 75-molar excess resulted in high levels of thiolation that did not significantly increase with increasing molar excess. Current work is focused on characterizing dynamic stiffening of human control and fibrotic hybrid-hydrogels with the goal of recapitulating healthy (1-5 kPa) and fibrotic (>10 kPa) lung tissue mechanics. Future directives will concentrate on characterizing cellular responses to biochemical and biophysical cues within these hybrid-hydrogels, further elucidating IPF pathophysiology and facilitating the development of innovative treatment modalities.

Tissue Engineering

4:38 PM - 4:44 PM

Aberrant Tissue Mechanics and Mechanotransduction During Heart Development in Down Syndrome

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Individuals with Down syndrome (DS, trisomy 21) account for 70% of all cases of patients diagnosed with an atrioventricular septal defect (AVSD), the most serious of structural heart defects that arise from endocardial cushion defects1

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underlying phenomena driving septation in Down syndrome, we proposed two goals: (1) to determine extracellular matrix (ECM) composition and tissue stiffness of the endocardial cushions using the Dp16 mouse model of Down syndrome; and (2) to use these findings to interrogate cell mechanotransduction of trisomy 21 induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) in response to substrate stiffness and cyclic mechanical stretch. We hypothesized that in DS, upregulation of type VI collagen and hyaluronic acid in the endocardial cushion increases cushion stiffness, altering cellular mechanotransduction and ultimately leading to differences in cell proliferation and gene expression that perturb heart development. Results found that endocardial cushions of the Dp16 mouse model of DS have an increased stiffness compared to WT. Furthermore, iPSC-CM with trisomy 21 exhibited decreased proliferation following culture on substrates of increasing stiffness, and following cyclic mechanical stretch, DS

iPSC-CM developed stress fibers, disorganized sarcomeres, and a decreased expression of mature cardiac markers. Yet cyclic mechanical stretch of control iPSC-CM induced sarcomere alignment and increased mature cardiac gene expression compared to static conditions These data argue that tissue mechanics, driven by upregulation of ECM proteins, lead to increased endocardial cushion stiffness in the Dp16 mouse, and that iPSC-CM with trisomy 21 aberrantly respond to changes to stiffness and stretch, ultimately proposing a novel avenue to investigate congenital heart defects in the Down syndrome population.

4:44 PM - 4:50 PM

4D Granular Composites to Program the Shaping of Cartilage Tissue

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Background and purpose: Cell-cell interactions and extracellular matrix (ECM) remodeling drives tissue shape transformations and is essential for the functional evolution of tissues. 4D biomaterials (providing shape transformations) have been coupled with cells to mimic tissue morphogenesis processes; however, hydrogels often restrict cell-cell interactions and lack synchronous degradation with tissue evolution that result in improper tissue formation. Granular composites (spheroids and microgels) have recently been demonstrated to intrinsically possess cell-cell interactions, allow contraction via fusion, and easily deposit and remodel ECM. Here, we demonstrate a programmable, 4D approach that layers granular composites of various remodeling rates to elicit tissue shape transformations for engineering complex topological tissues.

Methods: Norbornene-modified hyaluronic acid (degradable or non-degradable) microgels were fabricated via batch emulsion, crosslinked with UV, and washed. MSCs were seeded into microarrays and allowed to form spheroids over 2 days. Spheroid and microgels were mixed at a 35:65 volume ratio where remodeling rate and ECM deposition was monitored. Composite remodeling rate was varied by modulating individual microgel degradation or microgel population degradation via microgel mixtures. Granular composites of different remodeling rates were cast layered and allowed to culture. Bend angle, tissue mechanics, ECM deposition have been evaluated at the terminal timepoint.

Results and conclusions: Degradable and non-degradable NorHA microgels were fabricated and demonstrated to fully degrade by day 3, 14 or never. Swelling 60% by end coincided with degradable microgel formulations. Granular composites (pure microgel populations or mixed microgel populations) demonstrated varying rates of remodeling. Generally, the higher concentration of NorHA in the system leads to reduced remodeling and heterogeneous ECM deposition. Layered granular composites of varying remodeling resulted in rapid tissue shape transformations (i.e. bending >300°) over a 28-day period compared to controls (<100°). Together, a programmable 4D granular composite approach has been developed to elicit tissue shape transformations for engineering complex topological tissues.

POSTERS

Poster Number: 1

Exploring Human Mesenchymal Stromal Cell-Matrix Interactions through Nascent Protein Deposition in Bottlebrush Polymer Hydrogels

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In native extracellular microenvironments, cells synthesize and deposit various proteins to create and remodel the surrounding extracellular matrix (ECM) leading to eventual tissue creation. Hydrogels provide a platform to study cell behavior in three dimensions (3D). Prior studies in the field have investigated how hydrogel stiffness, composition, and stress-relaxation influences cell protein deposition. A key mechanical property of native cell microenvironments is the balance between strain-stiffening and stress-relaxing mechanical properties which are inherent to fibrous extracellular matrix components. Although stress-relaxation has been extensively studied, the impact of strain-stiffening biomechanical cues on cellular ECM deposition has yet to be fully understood. This work utilizes a bottlebrush polymer hydrogel system that the lab has previously developed to vary strain-stiffening behavior across a range of critical stresses (2-75 Pa). Human mesenchymal stromal cells (hMSCs) were encapsulated within the bottlebrush polymer hydrogels to determine the influence of strain-stiffening properties on protein secretion. Fluorescent noncanonical amino acid tagging (FUNCAT) was used to visualize the amount, morphology, and location of the nascent proteins secreted by cells within the bottlebrush polymer hydrogels, as a function of critical stress. The encapsulated hMSCs were cultured in methionine-free media supplemented with a methionine analog, L-Azidohomoalanine (AHA), for three and six days. After fixing, copper-free azide-alkyne cyclo-addition was used to click a fluorophore onto the proteins containing AHA for visualization. Nascent protein organization varied depending on the critical stress. The deposited protein was pericellular to the spherical cell body outside the biologically relevant regime (critical stress >25 Pa). Interestingly, inside the biologically relevant regime (critical stress <25 Pa), a more fibrous/punctate protein morphology was observed that was deposited further from the cell body and protrusions. Therefore, cells cultured in high-critical stress hydrogels are hypothesized to interact with their own deposited matrix rather than the surrounding hydrogel network. In contrast, the cells cultured in low critical stress hydrogels are hypothesized to interact equally with the deposited proteins and surrounding hydrogel matrix. This work highlights how the organization of deposited proteins can be altered by modulating the biomechanical feedback of the surrounding microenvironment.

Poster Number: 2

Development and Evaluation of Targeted Polycaprolactone Nanocapsules for Selective Delivery to Pancreatic β-Cells in Type 1 Diabetes

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Type 1 diabetes (T1D) is a chronic autoimmune condition marked by immune-mediated destruction of pancreatic β-cells. While research into the protection and proliferation of -cells has advanced, the lack of therapies that selectively target -cells has hindered therapeutic efficacy and progress in developing new treatments for T1D. To circumvent this problem, we have developed a novel polycaprolactone (PCL) nanocapsule (NC) that can be coated with interchangeable targeting ligands for β-cell targeted drug delivery. We hypothesize that NCs coated with a targeting ligand will selectively target human islet -cells. Initially focusing on Exendin-4 (Ex4), a GLP1 agonist, conjugated to hyaluronic acid (HA) for β-cell targeting, we subsequently explored ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3) antibody targeting due to its specificity for human β-cells. Our NCs averaged 258.3 nm +/- 5.46 nm in size. Coating NCs with 0.2 mg/mL fluorescently labeled HA and washing via centrifugation revealed 0.0022 mg/mL HA remained on the NCs, demonstrating stability of NC coating with peptides. NCs were loaded with Cy5 fluorophore and coated with ENTPD3 or HA-Ex4 and HA alone or guinea pig IgG antibody as negative controls. These NCs were used to treat cadaveric human islets for 24- 48-hours and islets were subsequently stained with NucBlue for cell nucleus identification and FluoZin-3 for -cell identification. NC+ cells were manually counted in ImageJ by locating FluoZin-3 positive cells with co-localized Cy5 signal, indicating NC internalization. We found HA-Ex4 and guinea pig IgG coated NC uptake in 5% and 7% of insulin+ -cells respectively, while ENTPD3-coated NCs showed uptake in 17% of imaged -cells . Additionally, NCs injected via tail vein into NOD-SCID mice were shown to target the pancreas in vivo, where NCs coated in HA-Ex4 were enriched in -cells by ~400% compared to HA only coated NCs 24h post injection (p=0.014). Pancreas specific uptake of NCs was confirmed by IVIS imaging. Our results indicate that NCs can be coated with targeting peptide for specific delivery to -cells in vitro and in vivo. The results from this study present a novel strategy for selective delivery of therapeutic cargo to β-cells, that may lead to novel treatments for T1D.

Poster Number: 3

Reversible Intracellular Gelation of MCF10A Cells Enables Programmable Control Over 3D Spheroid Growth

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In nature, some organisms can survive in extreme environments by inducing a biostatic state within the organism's molecular structure. Synthetic biostatic states in adherent mammalian cells have been previously achieved via intracellular network formation. This is attained by using bio-orthogonal strain-promoted azide-alkyne cycloaddition (SPAAC) reactions between functionalized poly(ethylene glycol) (PEG) macromers introduced to the cell by a lipofectamine aided transfection. These macromers are able to spontaneously crosslink within the cytosol. In this work, the effects of intracellular network formation in a complex 3D epithelial MCF10A spheroid model are explored. Transfected cells are encapsulated in a proteinous 3D matrix, Matrigel, and overall spheroid area is reduced by ~50% compared to controls. Interestingly, intracellular network formation also induced a change in cell cycle state. Network formation results in a higher quiescent cell population indicated by the loss of phospho-Rb and a gain in p21 expression within single cell analysis of spheroids. After lipofectamine-aided transfection with SPAAC macromers, the formed network reduces overall bioenergetic (ATP/ADP) levels and functional metabolic rates, while also inducing quiescence -like effect in the mitochondrial electron transport chain. These effects are confirmed by Fluorescence Lifetime Imaging Microscopy (FLIM) and Seahorse Cell Metabolic Analysis. To enable reversibility of the observed biostasis effect within the model, a photodegradable nitrobenzyl moiety is incorporated into an azide containing macromer. This allows the PEG network to experience photoinduced degradation. The degraded network allows for continued proliferation and a return to normal spheroid growth. Following light exposure at day 12, growth and metabolic rates return to control levels, while SPAAC treated spheroids that did not receive light exposure (i.e., spheroids containing intact intracellular networks) remain smaller and less metabolically active through this same period. These results demonstrate that photodegradable intracellular polymer networks in 3D spheroid culture is a novel method that can control metabolic states and induce a reversible quiescent state.

Poster Number: 4

Characterization of low-cost nanoprecipitation system for nanoparticle fabrication

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Statement of Purpose:

Nanoparticles (NPs) have a variety of tunable characteristics that make them efficient drug delivery tools. Diameter is an important characteristic that directly impacts blood clearance, biodistribution, and biological barrier penetration. NPs within the range of 10-200 nm are preferred as this enhances extravasation and bioavailability. Nanoprecipitation is a popular fabrication technique effective for small drug encapsulation. It involves dissolving a biomaterial and drug in an organic solvent that is mixed in a stirring aqueous solution. Under adequate parameters, NPs will spontaneously nucleate and form a colloidal suspension.

In this study we developed and characterized a low-cost, open-source syringe pump that can fabricate sub-100nm lipid nanoparticles (LNPs) while encapsulating a small drug (diclofenac). The LNPs are made of a 10:48:40:2 blend of 1,2-distearoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-3-trimethylammonium-propane, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-2000], respectively.

Methods:

An open-source syringe pump was adapted for this application; the pump was assembled with 3D-printed components. The stepper motor was controlled by an Arduino board and a compatible driver. For the biomaterial injection, a gastight glass syringe was coupled with a 20cm section of fused silica tubing (100µm inner diameter). The organic solution was prepared by dissolving 4mg of lipids with 1.2-2mg of diclofenac in ethanol. The syringe injected the organic solution at 1.5-3 ml/min into deionized water stirring at 400-1200rpms. The aqueous to organic ratio ranged between 45:1 to 5:1. A quarter factorial design of experiments (DOE) was conducted to assess the variables and their effects on the LNP physical properties. The LNP size, polydispersity, and zeta potential was assessed via dynamic light scattering. The combination of parameters generating the smallest LNP were subsequently employed for drug encapsulation (diclofenac). The encapsulation efficiency (EE) was evaluated via ultraviolet-visible spectroscopy.

Results:

The DOE demonstrated that all variable combinations yielded sub-100nm LNPs. The formulation of A:O of 5:1, injection rate of 3ml/min and stirring rate of 400rpms yielded the smallest LNPs (55.58nm). We observed an increase in LNP size between 98.5 to 110.5nm when encapsulating diclofenac at an EE of 33.5% to 26.85%, respectively. This study demonstrated the feasibility of a cost-effective method for LNPs synthesis and drug encapsulation.

Poster Number: 5

Engineering 3D Lung Models with Magnetically Labeled Fibroblasts

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Idiopathic pulmonary fibrosis (IPF) is a chronic disease that progressively affects the lung, leading to respiratory failure. The extracellular matrix (ECM), the proteins and other molecules supporting the cells in our tissues, plays an important role in lung development, repair, and disease. Biophysical and biochemical signals produced by the ECM regulate the function of various cells including fibroblasts in the lungs. Fibroblasts are important lung structural cells providing production and repair of the ECM. There is an increased ECM deposition during IPF that results in increased tissue stiffness and a positive feedback loop of fibroblast activation. The Magin lab has advanced technologies for engineering models of lung tissue and created hybrid-hydrogels to mimic, decouple, and study biochemical and biophysical changes in the cellular microenvironments. In my project, I decellularized human lung from healthy and fibrotic donors and incorporated the resulting proteins and other molecules into 3D hybrid hydrogel models. In these models, we recreated lung geometry by magentically aggregating a layer of fibroblasts around stem cell-derived epithelial cell spheroids. We confirmed the decellularizaion process by analyzing the residual DNA that was below 50 ng/mg in both healthy and fibrotic samples. We selected fibroblast densities and aggregation time to create the structure that best replicates distal lung architecture. By embedding these structures in hybrid-hydrogels containing either healthy or fibrotic dECM, we will investigate the role of both cellular and extracellular components in the progression of IPF. This model will allow for patient-centered research by using human cells and dECM to study fibrotic phenotypes in a system that mimics the geometry, composition, and biomechanical properties of lung tissue.

Poster Number: 6

Tailoring Cationic Polymer Microstructures for Improved Nucleic Acid Delivery

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Viral vectors have proven safe and efficacious in clinical gene therapy, but their exorbitant costs motivate the development of synthetic nanocarriers, such as polymers. Thanks to their manufacturability, cationic polymers are promising for nucleic acid delivery, but their efficiency and safety profiles are highly sensitive to their physiochemical properties and interactions with nucleic acids, plasma proteins, and cellular targets. In this study, we explore how polymer microstructure engineering – synthesizing statistical, block, and gradient copolymers to vary the spatial distribution of cationic and neutral monomers –tunes plasmid (pDNA) delivery efficiency, hemocompatibility, and cytotoxicity. Careful physiochemical characterization revealed distinct behaviors among polymer microstructures. For instance, we compared polyplex sizes under different environmental conditions (water and 10% serum) and established that block copolymers granted the most serum-stable polyplexes. Similarly, block polyplexes are cytocompatibility but fall short in cellular internalization and transfection efficiency. In contrast, statistical polyplexes excel in cellular internalization and transfection at the cost of cell viability, whereas gradient polyplexes strike an optimal balance between transfection efficiency and cytotoxicity. Overall, our work highlights the importance of tailoring polymer microstructure to optimize multiple competing objectives such as serum stability, transfection efficiency, and cytocompatibility.

Poster Number: 7

Photopolymerizable dual-crosslinked boronate ester hydrogels to study cellular responses to rapid stress relaxation

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Viscoelasticity is an important characteristic of biomaterials, influencing many cellular responses such as morphology, proliferation, migration, and extracellular matrix (ECM) deposition. As a result, interest has grown in designing viscoelastic biomaterials as synthetic matrices for more physiologically relevant in vitro tissue models. While current synthetic ECMs cover a wide range of stiffnesses and rates of stress relaxation, fast-relaxing materials (half times on the order of seconds, resembling the native properties of

brain or adipose tissue) are relatively under-explored, partly due to a limited number of chemical and physical crosslinking methods that can achieve such fast relaxation. In some applications, covalent adaptable networks utilizing boronate ester crosslinks have been developed to access these material properties. While these systems enable fast-relaxing synthetic ECMs, most modern viscoelastic biomaterials have two fundamental limitations: lack of homogeneity due to fast crosslink formation and difficulty tuning the degree of stress relaxation. In this work, we design and characterize a fast-relaxing dual-crosslinked PEG hydrogel that addresses both limitations and apply this system to cell and organoid culture. Uniquely, our system utilizes a combined competitor/crosslinker molecule which produces highly homogeneous photopolymerizable hydrogels, affording spatiotemporal control over network formation and facile manipulation of material (visco)elasticity by altering the ratio of elastic to viscoelastic crosslinks. Cells encapsulated in this material display remarkable cellular remodeling and contraction of the matrix when cell-adhesive peptides are incorporated into the hydrogel network. By developing improved fast-relaxing biomaterials, we can investigate how mechanobiological cues such as the degree of stress relaxation affect cell behavior in hydrogels with varied stiffness. Finally, the photopolymerizable nature of this system is compatible with light-based additive manufacturing techniques, potentially improving not only the physiological relevance of fast-relaxing tissue models, but also allowing for biofabrication-based approaches to manufacturing tissue-scale cellularized architectures.

Poster Number: 8

Synthesis and in vitro applications of polymeric CO-releasing molecules

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Although known for its toxicity, carbon monoxide (CO) also exhibits therapeutic potential against a variety of diseases including cancer, autoimmune disorders, and sepsis. Nevertheless, the precise mechanism underlying CO's therapeutic action remains elusive due to the lack of non-toxic CO delivery methods with controlled and triggerable CO release. A novel platform of CO-releasing polymers (CORPs) has been designed and synthesized leveraging the CO releasing moiety diphenyl cyclopropenone (DPCP). DPCP and DPCP-derived polymers release CO gas via photodecarbonylation upon exposure to light. The DPCP analogue has been polymerized with hydrophilic moieties to improve water solubility and allow for biological applications. This presentation will discuss the synthesis and application of these new polymers in-vitro.

Poster Number: 9

Engineering Hydrogel Biomaterials to Study the Role of Integrin Binding and Environmental Stiffness in AT2-to-AT1 Lung Cell Differentiation

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Chronic respiratory diseases such as emphysema and pulmonary fibrosis are characterized by changes in extracellular matrix (ECM) composition and stiffness. These biochemical and biophysical alterations may influence differentiation of alveolar type 2 (AT2) lung progenitor cells into alveolar type 1 (AT1) cells. AT1 cells are responsible for gas exchange in the lung. AT2 cells have been shown to express mainly laminin-binding integrins, such as α6β1 and α3β1 in vivo. Integrins such as αvβ6, αvβ3, and α2β1 have been implicated in the differentiation of AT2 to AT1 cells and αvβ3 has been described in the fully differentiated AT1 cells. Our understanding of how these cues influence differentiation is limited by current alveolar models that rely on Matrigel, limiting physiological relevance and our ability to assess the impact of both ECM signaling and stiffness on AT2 cell stemness and differentiation. We have designed new peptide-functionalized hydrogel biomaterials to evaluate the influence of microenvironmental stiffness and ECM composition on AT2-to-AT1 differentiation. Induced pluripotent stem cell (iPSC)-derived AT2 cells (iAT2s) were seeded onto poly(ethylene glycol) alpha methacrylate (PEGαMA) hydrogels of three distinct stiffnesses, ranging from healthy (E = 3.96 kPa ± 0.196) to fibrotic (E = 20.27 kPa ± 0.546) as well as tissue culture polystyrene (TCPS). The effect of these cues on differentiation was analyzed through qPCR and immunofluorescent staining for AT2 (LAMP3, ABCA3, STFPA2, SFTPC), AT2-to-AT1 transitional (KRT8, KRT17, AREG, CLDN4), and AT1 markers (AGER, PDPN, SPOCK2, GPCR5A). Preliminary experiments show that iAT2s seeded onto TCPS with exposure to serum expressed greater KRT17. Furthermore, in a preliminary run of iAT2s on hydrogels with stiffnesses of 4 kPa, 12 kPa, and 20 kPa, data suggests that higher stiffness generally leads to greater AT1 gene expression (AGER, PDPN, SPOCK2, GPRC5A) compared to lower stiffnesses through day 7 and SFTPC expression, denoting AT2 identity, is greater in lower stiffnesses through day 7. Using this hydrogel model, we can separately study the effects of stiffness and integrin binding on both AT2 differentiation and stemness. Future studies could use this model to construct environments capable of supporting alveolar cell populations for drug testing and more accurate lung tissue engineering.

Poster Number: 10

Photocatalytic Targeted Release of CO as a Therapeutic Agent

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In recent years, there has been a growing interest surrounding the use of carbon monoxide (CO) in therapeutic settings. Methods of delivery include direct CO inhalation, which inherently lacks dosing control. The newer delivery approach is to use chemically bound CO which can be isolated in solids improving control, however current systems have toxicity issues. Hence, a need has arisen to create a system which allows for targeted and quick release of CO in the body at controlled locations with little to no adverse side effects. This poster will look at the synthetic approach and optimization of new polymeric CO releasing molecules with photocatalytic capabilities and near IR light triggered CO release.

Poster Number: 11

Nonlinear Elastic Microenvironments During Osteogenic Differentiation Promote Osteocytogenesis and Dendritic Network Formation

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The unmineralized collagen I-rich osteoid supports the transition of osteoblasts into osteocytes during bone formation as osteoprogenitor cells become encased and buried within this nonlinear elastic, fibrous matrix. Inspired by how strain-stiffening extracellular microenvironments play a critical role in cellular transformations in vivo, poly(ethylene glycol)-based bottlebrush polymer hydrogels were engineered to mimic the strain-stiffening mechanical properties found in the unmineralized osteoid. Previous work has shown that these covalently crosslinked bottlebrush polymer hydrogels can recapitulate strain-stiffening biomechanical microenvironments at biologically relevant stresses. By tailoring the bottlebrush polymer length in the hydrogel network, the critical stress associated with the onset of stiffening can be systematically varied to occur outside and within a biologically relevant stress regime (BRSR). When the critical stress of the material is within the BRSR, encapsulated human mesenchymal stromal cells (hMSCs) adopted a unique protrusion-rich morphology. This morphology was elucidated to be driven by cell-matrix interactions and regulated through actomyosin dynamics. In this work, the bottlebrush polymer hydrogels are used as a model culture platform probe how nonlinear elastic mechanical properties regulate cell fate. To date, there have been few successful attempts to monitor and support the hMSC-to-osteoblast-to-osteocyte transition using a three-dimensional culture platform in vitro. Using the bottlebrush polymer hydrogels as an osteoid-mimetic synthetic culture platform, a 6-week osteogenic differentiation protocol was used to pre-differentiate hMSCs into osteoblast-like cells for 2 weeks on 2D followed by 4 weeks of continued differentiation encapsulated within 3D bottlebrush hydrogel networks or collagen I controls. In the bottlebrush hydrogels, the pre-differentiated osteoblasts are observed to form dendritic protrusions which fuse into a functional dendritic network over the course of 4 weeks. These protrusion-rich morphologies coincide with an upregulation of E11 and DMP-1 (pre-osteocyte markers) and the formation of connexin 43 positive gap junctions. Fluorescence recovery after photobleaching experiments reveal the real-time recovery of calcein in photobleached cells from surrounding cells through functional gap junctions. The nonlinear elastic mechanical properties of the bottlebrush polymer hydrogels drive the up-regulation of a protrusion-rich morphotype promoting osteocytogenesis, and these engineered synthetic extracellular microenvironments hold great potential as a in vitro 3D culture model for osteocytes.

Poster Number: 12

Biocompatible, photocurable poly(β-amino ester) nanoparticles with programmable shape, degradation and mechanical stiffness for drug delivery

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Over the past several decades, numerous synthetic polymer systems have been considered to enhance the delivery of drugs for treating disease. Such polymer systems have been established to control cross linking, encapsulate drugs of various hydrophilicities, and degrade at prescribed rates. However, few of these polymer systems also enable the manufacturing of nanoparticles with well-defined shapes. This critical gap in capability is a major impediment to the needs of next-generation drug-delivery vehicles, as particle shape has been shown to play an important role in nanoparticle delivery and fate. To address these needs, we developed methods to fabricate micro/nanoparticles from UV-curable polymers with tunable shape and mechanical properties. Our research has explored poly(beta-amino ester) (PBAE) synthesis from 1,4-butanediol with either isobutylamine or benzhydrazide at various molar ratios of diacrylate:amine, ranging from 1.2:1 to 10:1. The addition of diphenyl (2,4,6-trimethylbenzoyl)phosphine oxide (TPO) allows the pendant diacrylate groups to crosslink when exposed to UV light, enabling a myriad of unique nanoparticle shapes through photolithography (e.g., discoids, hexagonal prisms) using a mask aligner. Particle height is tunable by using different spin speeds when spin coating the polymer onto a silicon wafer. The various molar ratios achieve different molecular weights, which results in varying crosslink densities and a range of elastic moduli (i.e., ~3 to 750 MPa). We manipulated PBAE chemistry to temporally control polymer degradation in an accelerated degradation study. In 1M NaOH at 37°C, some compositions showed full degradation in just a few days, suggesting the material is degradable at physiological timescales, while other compositions showed <10% degradation after seven weeks. To find the degradation time of microparticles in physiological conditions, we investigated the degradation of 8 unique PBAE compositions in PBS at 37°C. We observed similar programmable degradation. Further, we used an MTT assay to assess the effect of different diacrylate:amine molar ratios on biocompatibility. We observed that none of the studied PBAEs were cytotoxic. Altogether, our work establishes photocurable PBAEs as a biocompatible material that enables the formation of nanoparticles with tunable shape and mechanical properties, readily available for drug delivery and related applications.

Poster Number: 13

Anisotropic shape remolding of dithiolane crosslinked microgels

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Widespread deployment of granular biomaterials in tissue engineering has resulted from their unique properties, including porosity, tunability, injectability, and 3D printability. However, granular hydrogels have typically been restricted to spherical particles, with limited deployment of more intricate particle morphologies, typically requiring the individual templating of microgels through microfluidics or molds. Here, we use a dithiolane crosslinked hydrogel system for the fabrication of spherical microgels through a batch emulsion photopolymerization. Owing to the dithiolane crosslinking, these materials have inherent dynamic disulfides present at the crosslinks, which we harness to reconfigure the particle shape. Utilizing unconfined compression between parallel plates, we transform these particles into anisotropic disks through photoinitiated radical-mediated disulfide exchange. Analysis of this procedure indicates regions of effectively zero curvature of the regions of the microgel surface in contact with the parallel plates, while the curvature at the boundary regions is increased. When cultured in the presence of C2C12 myoblasts, the cells were found to localize to areas of higher curvature on the disk-shaped microgel surface. This preferential localization impacts the cell-driven assembly of large supraparticle scaffolds, with spherical particles assembling without specific junction structures, while disk microgels assemble interfaces preferentially on their curved faces. These findings present a distinctive spatiotemporal process for the rapid transformation of microgels into anisotropic shapes, offering new avenues to explore shape-driven mechanobiological cues during and after granular hydrogel assembly.

Poster Number: 14

Fabrication of a printable PLGA nanoparticle-loaded hydrogel for in vitro glioblastoma drug delivery models

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Bioprinting has enabled tissue engineers to control the spatiotemporal deposition of cells in an extracellular matrix-like material with increasingly sophisticated precision. The Crosby Lab at Southwestern University and the Clegg Lab at the University of Oklahoma seek to use these developments in polymer modification, nanoparticle synthesis, and open-source hardware to fabricate a novel hydrogel for treating neurological diseases. Specifically, we aim to partly recapitulate the brain parenchyma's physical and chemical characteristics for deployment in in vitro drug delivery models with a focus on models of glioblastoma. We incorporated gelatin methacryloyl (GelMA), methacrylated hyaluronic acid (HAMA), PEGDA, and blank PLGA nanoparticles to achieve this goal and mimic local therapeutic delivery. To ensure that our in vitro tissue scaffold captures the viscoelastic properties of brain tissue, we conducted compression and swelling tests on hydrogel samples. We performed these tests on five 8 mm diameter cylindrical samples consisting of GelMA, GelMA-HAMA (GH), GelMA-HAMA-PEGDA (GHP), and GelMA-HAMA-PLGA (GHPP). GelMA was synthesized by adding methacrylic anhydride (MAAnh) in a buffered solution according to the one-pot method. HAMA was synthesized by dissolving sodium hyaluronate in ultrapure water, adding MAAnh, and maintaining a basic pH with NaOH over two days. PEGDA was purchased from a commercial supplier. The PLGA nanoparticles were synthesized by dissolving PLGA in acetone and adding the mixture dropwise to a poly(vinyl alcohol) solution. The compression testing was performed on a Univert CellScale. The swelling tests were conducted in separate DI water baths over several days. Weight measurements were taken for the first few hours, and diameter measurements were recorded over the total duration. Results from compression testing indicated that GHP exhibited the highest compressive modulus, and that the addition of nanoparticles did not influence the final modulus. However, adding the nanoparticles enhanced the extrudability of the hydrogel while significantly improving printability. Adding HAMA reduced the swelling of the pristine GelMA hydrogel, and the additions of PEGDA and PLGA had little effect on the swelling ratio. This data and future rheological testing will further inform the encapsulation of primary glioblastoma cells alongside doxorubicin-loaded nanoparticles in a drug delivery microenvironment.

Poster Number: 15

Islet survival under cytokine stress is mediated by laminin interactions in a 3D reverse thermal gel scaffold

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Pancreatic islets are directly surrounded by a dense network of proteins and polysaccharides known as the extracellular matrix (ECM). Type 1 diabetes (T1D) is characterized as an autoimmune-mediated destruction of insulin-producing β cells within the islet. Infiltrating immune cells in the pancreas secrete high levels of pro-inflammatory cytokines that disrupt islet function and lead to β-cell death. The peri-islet ECM provides a barrier against infiltrating immune cells and is crucial for islet survival. Specifically, laminin is an ECM component that has been shown to protect β cells from cytokine-induced apoptosis via specific transmembrane integrins. To investigate this, we have employed a 3D biomimetic hydrogel functionalized with full-length laminin. This reverse thermal gel (RTG) spontaneously forms a 3D scaffold at body temperature and can easily be reverse gelled to extract the cells for biochemical analysis. We have encapsulated islets in an RTG polymer alone or functionalized with laminin (RTG-LAM) and treated with a pro-inflammatory cytokine cocktail of TNF-α, IL-1β and IFN-γ to show that islet interactions with laminin promote islet survival.

Poster Number: 16

Islet survival under cytokine stress is mediated by laminin interactions in a 3D reverse thermal gel scaffold

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Pancreatic islets are directly surrounded by a dense network of proteins and polysaccharides known as the extracellular matrix (ECM). Type 1 diabetes (T1D) is characterized as an autoimmune-mediated destruction of insulin-producing β cells within the islet. Infiltrating immune cells in the pancreas secrete high levels of pro-inflammatory cytokines that disrupt islet function and lead to β-cell death. The peri-islet ECM provides a barrier against infiltrating immune cells and is crucial for islet survival. Specifically, laminin is an ECM component that has been shown to protect β cells from cytokine-induced apoptosis via specific transmembrane integrins. To investigate this, we have employed a 3D biomimetic hydrogel functionalized with full-length laminin. This reverse thermal gel (RTG) spontaneously forms a 3D scaffold at body temperature and can easily be reverse gelled to extract the cells for biochemical analysis. We have encapsulated islets in an RTG polymer alone or functionalized with laminin (RTG-LAM) and treated with a pro-inflammatory cytokine cocktail of TNF-α, IL-1β and IFN-γ to show that islet interactions with laminin promote islet survival.

Poster Number: 17

Synovial fibroblasts support vascular function in both health and inflammatory disease

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The synovium is a vascularized, joint-lining tissue present in the knee, as well as in other articulating joints. After an acute trauma, the synovium undergoes marked changes in cellular composition. While advances in single cell sequencing have elucidated these cellular changes, the distinct roles of such cells have yet to be identified in both health and disease (inflammation). In this study, we aimed to develop a microphysiologic model of the human synovium to determine the specific role of synovial fibroblasts in maintaining vascular health. And so, endothelialized vessels were engineered within collagen gels (one of the primary matrix components of the synovium), exposed to IL-1B (inflammation/injury), and functional outcomes (permeability, collagen remodeling, synoviocyte-vessel colocalization) were assessed over 8 days in culture. We first discovered that the vessels matured rapidly with the addition of synoviocytes. Next, we introduced the pro-inflammatory cytokine IL1B daily to the cell culture media, and reassessed permeability at Day 8. Interestingly, the lumens without synoviocytes demonstrated a strong increase in permeability in response to IL1B treatment. However, the lumens surrounded by synoviocytes maintained their permeability. To probe the mechanisms behind this finding, we fabricated vessels using FITC-labeled collagen, enabling tracking of matrix remodeling. On Day 8, we imaged the bottom half of the vessels, and binarized the images to quantify collagen accumulation around the vessel periphery. These images highlight how endothelial cells alone remodel their environment; however, the synoviocytes elevate this endothelial cell-mediated response. In comparison to the -IL1B baseline, +IL1B appears to diminish the layer of collagen around the vessels. As mentioned, we believe that synoviocytes instruct the endothelial cells to remodel their surrounding environment, creating a ‘basement membrane,’ as we see no remodeling in the absence of endothelial cells. Lastly, we observed significantly more synovial fibroblasts surrounding our healthy vessels, in comparison to the +IL1B group. Future work will identify cell-secreted factors from both synoviocytes and endothelial cells that contribute to matrix remodeling. Overall, this model is the first-of-its-kind to investigate the role of synovial fibroblasts in health and inflammatory disease, focusing on synovial-vascular interactions.

Poster Number: 18

Immunomodulatory vaccine for traumatic brain injury

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Traumatic brain injury (TBI) affects over 2.5 million people each year in the US. Moderate-severe TBI is linked to increased risk of cognitive decline after injury, yet therapeutic interventions are limited. Therefore, we examined the efficacy of immunomodulatory polyesters of alpha-ketoglutarate (paKG) particles dosed prophylactically prior to TBI in a mouse to diminish chronic inflammation. This formulation aimed to generate antigen-specific memory T cells to myelin proteolipid protein (PLP) and modulate the immune metabolism resulting in altered phenotype.

paKG microparticles (MPs) encapsulated the glycolytic inhibitor PFK15 alone or in combination with the peptide antigen PLP 139-151. C57BL/6J male mice received subcutaneous injections of particles or saline at 17 and 3 days prior to controlled cortical impact (CCI). Day 7 and 28 post-injury, the brain, spleen, and lymph nodes were processed to assess immune cell profiles by flow cytometry. Brain tissue was analyzed with Nanostring.

Day 7 post-injury, flow cytometry revealed alternatively activated antigen presenting cells at the injury site and in secondary lymphoid organs. paKG(PFK15+PLP) increased cells or frequency of immunosuppressive PLP-specific central memory Th2 and Treg in the brain versus no treatment. Moreover, at day 28 post-injury paKG(PFK15+PLP) and paKG(PFK15) decreased expression of PLP specific T-cell receptors on Th1 and Th17 cells in cervical lymph nodes versus no treatment. Nanostring proteomic analysis at day 7 post-injury revealed that paKG(PFK15+PLP) exhibited upregulation in proteins associated with autophagy, potentially indicating neuroprotective effects. Rotarod motor behavior assay indicated functional impact of paKG(PF15+PLP) by increase in motor learning acutely post-TBI observed when compared to paKG(PFK15) and saline control. The open field test on day 27 showed significantly greater distance traveled with paKG(PFK15+PLP) in mice treated with paKG(PFK15+PLP) compared to paKG(PFK15) and naïve control, which may indicate lower anxiety.

This study was the first to our knowledge that assessed a vaccine-based approach to prime the immune system prior to TBI. This revealed that the circulating immune profile, infiltrating immune cells after TBI, and local neuroinflammatory process can be modulated with the vaccine, shifting away from a pro-inflammatory state. This resulted in modest behavioral outcomes, underscoring the critical importance of the neural-immune crosstalk in neurotrauma.

Poster Number: 19

Combinatorial Macrophage Modulation Using Cavitating Mesoporous Silica Nanoparticles

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Solid tumors avoid immune recognition by recruiting macrophages and driving their polarization toward anti-inflammatory phenotypes, which protect the tumor from killer T cells and promote tumor growth. Therefore, reprogramming tumor-associated macrophages toward a pro-inflammatory phenotype proposes a promising method of treating so-called “cold” tumors. To realize this strategy, we developed a mesoporous silica nanoparticle system that can (1) deliver the immunomodulatory drug resiquimod (R848) to macrophages and (2) act as seeds for cavitating microbubbles, which results in damage to cancer cells and release of damage-associated molecular patterns (DAMPs). Mesoporous silica nanoparticles were synthesized using a modified Stöber process and were coated with an alkyl moiety, creating a hydrophobic surface. Under high intensity focused ultrasound (HIFU), the surface facilitates the formation of cavitation bubbles. The violent collapse of these bubbles has been shown to lyse cancer cells and release DAMPs. Using this system, we studied the combined effects of resiquimod and DAMPs on the phenotypes of macrophages cultured in tumor-like conditions (tumor-conditioned media and hypoxia). We tested our drug delivery system on 4T1 murine mammary carcinoma cells in vitro due to its reputation for being highly nonimmunogenic. Our results show that CD86, MHC II, and iNOS were all upregulated in macrophages treated with R848-loaded particles actuated under HIFU. However, IL-12 secretion was severely dampened compared to cells treated with R848 or DAMPs separately. We also found our material control of particles without R848 or HIFU stimulation induced a slight inflammatory phenotypic shift in macrophages, mainly due to upregulation of CD86 and iNOS and downregulation of CD206. Future work will entail understanding how this material can prime the immune system for relapsing tumors, which could give rise to an innovative method for cancer vaccination. Overall, this work shows that hydrophobically modified mesoporous silica nanoparticles, under the influence of HIFU, can enhance macrophage effector functions like T cell activation through the upregulation of CD86 and MHC II and macrophage-mediated cytotoxicity from secretion of nitric oxide through iNOS. Together, these effector functions offer a new route of malignant cell clearance in cold tumors.

Poster Number: 20

Modulating Immune Responses in Vaccine Immunotherapy: The Impact of Adjuvant Crystallinity

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Biomaterials can act as pro- or anti-inflammatory agents; however, the effect of the degree of crystallinity of biomaterials on immune responses is poorly understood. Herein, we demonstrate that the adjuvant-like behavior of covalent organic framework (COF) biomaterials depends on its crystallinity, which controls anti-tumor immunity in mouse melanoma models. An inverse relationship was observed between COF crystallinity and activation of mice and human dendritic cells (DCs) and antigen-presentation by mice DCs. Also, amorphous COFs upregulated NFkB, TNFa, and RIG-I signaling pathways compared to crystalline COFs, and chemotaxis-associated gene unc5c was found to be inversely correlated with crystallinity. Furthermore, it was determined that COFs with the lowest crystallinity admixed with OVA antigen were able to prevent B16F10 expressing chicken ovalbumin (OVA) tumor growth in 60% of mice, and the lowest crystalline COFs admixed with TRP2 antigen were able to prevent YUMM1.1 tumor growth in 50% of mice. The lowest crystalline COFs also induced antigen-specific pro-inflammatory T-cell responses in B16F10-OVA tumors. These results demonstrate that the crystallinity of biomaterials is an essential factor to consider when designing immunotherapy for pro- or anti-inflammatory applications.

Poster Number: 21

Biodistribution Assessment of Macrophage-Nanoparticle Complexes in B16-F10 Melanoma Cancers

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Systemic administration of nano- and microparticles offers limited control of their site-specific accumulation. As a result, adoptive cell therapy (ACT) is an attractive alternative. Among the types of cells used in ACT, macrophages are a potential therapeutic platform because they can navigate biological barriers and respond to inflammatory signals throughout the body unlike synthetic particles. Macrophages can adopt multiple phenotypes that are beneficial in homeostatic contexts, but this plasticity can pose challenges for engineering consistent therapeutic behaviors when reprogramming macrophages ex vivo and readministering for ACT applications. In particular, we have shown that macrophages associated with microparticles of discrete geometries shape impact their phenotype through differential changes in chromatin accessibility, gene expression, biomarkers, and cytokine secretion. Therefore, characterizing their biodistribution profiles is essential to evaluate their potential as an ACT modality. Here, we assessed the in vivo biodistribution of macrophages associated with particles of two shapes, discoidal and spherical, in B16-F10-tumor-bearing C57BL/6 mice. This is accomplished by associating discoidal or spherical particles to macrophages in vitro, staining cells with VivoTrack 680 (a lipophilic near-infrared dye), and administering complexes via a single tail vein injection. We used a homogenization method to track the biodistribution of the stained cells into native tissue types including the lungs, liver, spleen, kidneys, brain, heart, and B16-F10 tumor. We developed a quantitative fluorescence-based biodistribution method by determining VivoTrack 680 fluorescence saturation in bone marrow-derived macrophages, generating fluorescent standard curves of VivoTrack 680 in each organ homogenate extract, and calculating extraction efficiencies for each whole organ. Fluorescence of the homogenate extract was then measured and the percentage of cell infiltration was reported based on respective standard curves. This quantitative method was complemented with spatiotemporal analysis using an in vivo imaging system (IVIS). Our work reveals that the shape of nanoparticles, when bound to macrophages, affects their accumulation in B16-F10 tumors, which aligns with our multi-omics analyses of macrophage activation states induced by particle association. Our work observes that particle shape can modulate macrophage phenotype and downstream trafficking of macrophages to tumors, thus having implications for controlling biodistribution profiles in macrophage-mediated ACT.

Poster Number: 22

Scalable, high throughput biomanufacturing of hydrogel encapsulated cell products

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Clinical islet transplantation, a cell therapy-based treatment for insulin-dependent diabetic patients, would eliminate the pain and cost burden of daily insulin injections, as well as the risk of long-term secondary complications, by restoring native insulin signaling. While clinical successes have demonstrated the feasibility of achieving insulin independence via this therapy, the necessity of long-term immunosuppressive drugs limits the widespread applicability of this procedure. As a result, researchers have investigated devices to isolate transplanted cells from the recipient immune system, including hydrogel macroencapsulation devices which prioritize whole graft containment, retrievability, and safety. To generate macroencapsulation devices with complex geometries in a high-throughput and scalable manner, we have developed a hydrogel injection molding technique. This technique can be automated to minimize labor costs and enhance sterility of the procedure, and be fully integrated into a biomanufacturing workflow. A critical component of this system is the requirement of hydrogel component mixing prior to injection within the molding system. To achieve this, we have engineered two prototype microfluidic mixing systems, a 'paddle' and 'channel' mixer. Here, we (1) use fluid dynamic modeling to predict the efficacy of hydrogel mixing and turbulent flow within the devices and minimize laminar flow, and (2) evaluate the mixers in vitro for mixing efficiency.

Solidworks Computational Fluid Dynamics software was used to simulate Newtonian and Non-Newtonian fluid flow for PEG and alginate hydrogels, respectively. A range of flow rates at varying ratios within the inlets were evaluated to achieve homogenous mixing at the outlet within the simulation. To validate in silico observations, we 3D printed our "paddle" and "channel" microfluidic mixer designs and used food coloring to label first water, then hydrogel components in the inlets. Images of fluid flow at the outlet are used to quantify the degree of mixing in each of the tested solutions. We are currently evaluating whether our mixer designs generate laminar or turbulent flow in vitro and under what conditions. Finally, future studies will incorporate a cellular channel within our microfluidic mixer flow and evaluate cell viability and function post-encapsulation.

Poster Number: 23

FAP-Responsive Hydrogel for Cardiac Repair Post-Myocardial Infarction

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Despite recent advances in pharmacological and reperfusion treatments, many patients will experience heart failure after myocardial infarction (MI), making cardiovascular disease the leading cause of death worldwide. Hydrogels are being explored for post-MI treatment due to their mechanical support of the tissue, as well as their ability to deliver a variety of therapies (e.g., cells, drugs) for local and sustained presentation. Hydrogels can also be customized for disease-specific applications, such as the introduction of stimulus-responsive features that trigger the release of an encapsulated therapeutic. With this in mind, fibroblast activation protein α (FAP), a membrane-bound protease, is specifically upregulated in the heart post-MI, and provides an opportunity to deliver therapeutics on demand based on local FAP activity. To create an FAP-responsive hydrogel, hyaluronic acid (HA) was modified with maleimide (MA) groups, which selectively react with thiols at the appropriate pH. MAHA was reacted with an FAP-responsive peptide (GCNSGPSNCG) containing thiols on both ends for crosslinking or dithiothreitol as a control. To evaluate hydrogel degradation, recombinant FAP was added in two concentrations (0.1 ug/ml, 0.001 ug/ml) and compared to phosphate buffer saline, and the solutions were collected over time (1, 3, 5, 24, 72, 120, 168 hrs) and measured for HA release via uronic acid assay. The results indicated that FAP-responsive gels degraded in an FAP concentration dependent manner, with less degradation observed in buffer over the time period measured. Further, the introduction of a non-degradable crosslinker also limited hydrogel degradation. These findings indicate the tunability of hydrogel degradation based on local enzyme concentration, which could be used to release encapsulated therapeutics on demand, which is important due to the heterogeneity of expression of FAP within MI patients. Future studies will adapt the hydrogel design to introduce injectability for easy tissue delivery and explore FAP-dependent release of therapeutics.

Poster Number: 24

Additive Manufacturing of Highly Entangled Polymer Network Tissue Adhesives

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Tough hydrogels are a broadly useful class of materials for biomedical applications due to their robust mechanical properties and ability to form strong adhesions with wet tissues. Toughness is important for adhesives to dissipate stress under loading and to maintain contact with tissues. Load dissipation is typically introduced into soft materials through double network hydrogel design; however, double network hydrogels suffer from high hysteresis and require multiple steps to fabricate, limiting their utility in biomedical applications with repeated loading and 3D printing techniques. Further, most hydrogels designed as tissue adhesives are presented as uniform materials, which can limit adhesion on non-planar interfaces. To address these concerns, we looked towards the additive manufacturing of single-network hydrogels that are highly entangled to improve toughness. Others have introduced polymer chain entanglements within single network hydrogels to synergistically improve stiffness and toughness, yet attaining such dense entanglements through lithography-based additive manufacturing (e.g., digital light processing, DLP) remains elusive. Here, we introduce a facile strategy that combines light and dark polymerization through controlled initiator and light presentation to allow constituent polymer chains to densely entangle as they form within 3D printed structures. This generalizable approach occurs at room temperature and avoids the need for additional post-processing steps with light or heat to increase network conversion, and allows the additive manufacturing of highly entangled hydrogels and elastomers that exhibit 4 to 7-fold higher extension energies in comparison to traditional DLP alone. Towards the engineering of advanced tissue adhesives with controlled structures due to additive manufacturing, we printed adhesives with ridges, microfluidic networks, and programmed meta-material designs to develop adhesives with controlled levels of adhesion, depots for drug delivery, and directional adhesion, respectively.

Poster Number: 25

Engineering Large Anisotropic Meniscal Microtissues via Digital Light Processing Printed Molds

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Introduction

The menisci are highly organized fibrocartilaginous tissues that provide load distribution, joint lubrication, and shock absorption in the knee. Meniscal injuries are among the most common musculoskeletal injuries and may lead to pain and early onset of osteoarthritis if left untreated. Although meniscectomies are often used for treatment, meniscal tissue removal has long term biomechanical consequences, motivating the development of new therapies. Our approach leverages advances in 3D printing and the fabrication of microtissues to produce implantable meniscal constructs that recapitulate the anisotropic structure of healthy tissues.

Methods

To accomplish this, digital light processing was used to fabricate molds with posts spaced at a 4:1 aspect ratio and varied dimensions (e.g., rectangular versus curved) using a poly(ethylene glycol) resin. Suspensions of meniscal fibrochondrocytes (MFCs) in collagen were added to the molds and cultured for up to two weeks. Images were acquired during culture to quantify construct width via ImageJ and select constructs were fixed and stained for nuclei (DAPI) and actin (phalloidin) and analyzed for nuclei/actin alignment (Directionality plugin in ImageJ). Cytochalasin D was investigated to alter MFC actin polymerization and limit construct contraction and necking.

Results

All constructs cultured in rectangular molds of various lengths (6, 12, 24 mm) underwent visible contraction and collagen reorganization, with rapid increases in nuclei and actin alignment. However, over time the constructs underwent undesired necking, where contraction continued and altered construct shape. To address this, curvature was incorporated into molds to increase initial construct volume while maintaining post spacing. This approach decreased necking during 7 days of culture relative to constructs cultured in rectangular molds. Further, cytochalasin D was potent in also limiting construct contraction and necking. With these tools now available, ongoing work is to culture larger constructs for longer periods (e.g., 8 weeks) in chondrogenic media and assess extracellular matrix deposition, construct mechanical properties, and integration with meniscal tissue.

Poster Number: 26

Quantifying Thrombogenicity: A Bioanalytical Protocol for the Absorbance-Based Assessment of Vascular Implants with Human Plasma

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Statement of purpose: Assessing thrombogenicity is critical for the evaluation of various biomaterials, especially those that interface with whole, flowing blood. Cardiovascular diseases are the leading cause of death globally. Coronary artery disease, cerebrovascular disease, and peripheral vascular diseases are the most common and are often treated with a vascular stent or graft. This protocol aims to standardize the assessment of such biomaterials using human plasma by quantifying the light absorbance of the biomaterial in the plasma to determine its thrombogenicity.

Methods: To evaluate the thrombogenicity of a vascular implant biomaterial, we longitudinally section the tubular vascular implant (stent or graft) into small pieces and place them in a low-adhesion 96 well plate. Using human plasma rich in or depleted of platelets, we measure the absorbance of light passing through the plate over an hour and plot the resulting curve. This procedure is used to quantify the thrombogenicity of a biomaterial using platelet-rich plasma (PRP) or platelet-poor plasma (PPP). Included in our tests are vascular implants from top brands, such as vascular stents, including bare metal stents and drug-eluting stents, vascular grafts, and stent-grafts. We investigate the impact of a multitude of parameters, including relative efficacy of various anticoagulants, antagonists, and surface coating molecules, on the thrombogenicity of these vascular implants.

Results: This procedure provides a facile, fast, high-throughput means to quantify the thrombogenicity of a vascular implant using the absorbance readings of separated plasma. Higher absorbance values correspond with more thrombogenesis.

Conclusions: This protocol establishes a standardized way to compare the thrombogenicity of different biomaterials using human plasma and get quantitative results.

Poster Number: 27

A 3D in vitro fibrin model to evaluate how strain heterogeneity alters ECM deposition

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Dermal fibroblasts responses to mechanical stimuli in the context of dermal wound healing has been the focus of both in vitro and in vivo studies. Still, how ECM deposition and dermal fibroblast-generated mechanical forces are influenced by the heterogenous mechanical environment in the wound have not been adequately described. To create more accurate computational models of cell behavior and dermal wound healing outcomes and ultimately inform better treatments, we developed an in vitro 3D dermal wound model using heterogeneous fibrin gels and primary normal human dermal fibroblasts (nHDFs). By creating fibrin gels with regions of differing fibrin density, we are emulating the heterogenous mechanical environment in the wound and surrounding tissue during dermal wound healing.

We previously published the mechanical and microstructural properties of 2 and 4 mg/mL fibrin gels and incorporated them into a multiscale fibrin mechanics model. Using these results, we created a 3D in vitro model to evaluate nHDF behaviors in 2 and 4 mg/mL fibrin gels and at their interface. Using a biopsy punch to remove an inner region of a larger fibrin gel and filling it with a different concentration fibrin, we created a repeatable heterogenous model. Using fluorescently labeled fibrinogen, we evaluated the microarchitecture of the fibrin interface. We incorporated nHDFs in the fibrinogen solution to create cellular models. Over 2 weeks, nHDFs fully infiltrated an initially acellular inner region. Additionally, we tested the feasibility of quantifying fibroblast-synthesized ECM in fibrin gels using liquid chromatography – tandem mass spectrometry. We resolved the deposition of major ECM components such as type I collagen and fibronectin by NIH 3T3 cells despite abundant fibrin.

By confocal imaging fluorescent bead-embedded gels and using digital image correlation, future studies will quantify local strains in bulk and interface regions to quantify changes in local stiffness in these regions and correlate this data with proteomics to evaluate the distribution and identities of nHDF deposited ECM proteins. Additionally, we will expand this approach to heterogenous collagen-fibrin gels to further evaluate how ECM composition and strain heterogeneities alter cell behavior to inform computational models and improve understanding of dermal wound healing processes.

Poster Number: 28

Optimizing Regenerative Cell Infiltration in Vascular Grafts: Enhanced strategies to engineer pore microstructures during fabrication

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Introduction: In tissue engineering, the goal is to create scaffolds that seamlessly integrate into the human body, guiding native tissue regeneration while slowly degrading. A crucial aspect of this process is achieving scaffold infiltration of functional cells. The initial phase centered on the material aspect, particularly different polymer behaviors, including degradation rates. Production methods represent another modifiable parameter to fine-tune the device structure, especially its porosity. Additionally, post-fabrication techniques can refine the microstructure and enhance interaction with the human body. The investigation originated with a control group, PCL+PEG-NB coaxially electrospun and air-dried. Ultimately, four groups were created by systematically altering one or more parameters and examined to assess their impact.

Methods: Coaxial electrospinning was used to achieve a strong core (PCL/PLCL) surrounded by a functionalized sheath (PEG-NB). The mixed condition was fabricated by directly blending sheath and core solutions before electrospinning. Samples were soaked in PBS for 24 hours prior air-drying or freeze-drying. Surface topography, including fiber structure and porosity, was investigated by SEM. Uniaxial tensile testing was adopted to determine the impacts of different parameters on the mechanical properties of the graft. The groups were implanted subcutaneously and explanted at various time points (1, 4, and 16 weeks). Histological and fluorescent analyses were adopted to visualize tissue morphology and cellular penetration.

Results: Freeze-dried samples demonstrate higher Young's Modulus, and, more importantly, higher porosity, subsequently, increased cell infiltration than their air-dried counterparts. PLCL shows significantly higher degradation than PCL, PLCL was overall weaker and had a less rigid structure than the PCL as shown by mechanical tests and SEM images. Mixed fibers also displayed increased degradation compared to the control and were shown to be slightly weaker in tensile tests.

Conclusions: Through systematic experimentation, we have uncovered the benefits of freeze-drying in enhancing scaffold porosity and cell infiltration, while also highlighting the importance of selecting polymers with suitable degradation rates. Work is still ongoing to determine optimal fabrication parameters. Moving forward, these insights can guide the development of advanced vascular grafts with improved regenerative capabilities, paving the way for more effective clinical applications in tissue engineering.

Poster Number: 29

Engineering entanglement of microfiber hydrogels

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Gelatin fibers have shown promise in the fabrication of tissue engineering scaffolds due to their general biocompatibility and cell adhesion properties. However, such scaffolds often fail to mimic the toughness of natural tissues, and rather exhibit high extensibility and low stiffness due to insufficient crosslinking, or brittleness due to over crosslinking. Motivated by molecularly entangled hydrogels where entangled chains act as crosslinks that can slide past each other under strain to dissipate stress, we investigated the entanglement of gelatin microfibers to improve scaffold mechanical properties, namely stiffness and extensibility. Wet spinning was used to fabricate gelatin fibers, which allows for micrometer control of fiber size and alignment, and has the potential for industrial scale capacity. Hyaluronic acid (HA) was used as a carrier polymer during wet spinning, to template reactive gelatin precursors and improve microfiber production. Gelatin was modified with lipoic acid, which allows crosslinking with light without a photoinitiator and enables the single step fabrication of stable wet spun microfiber hydrogels. Stretching the fiber and photocrosslinking while still in the coagulation bath aligned and stabilized the structures during collection. Taking inspiration from living highly entangled structures, we are currently developing methods to entangle gelatin fibers, where the tuning of features of gelatin fibers such as length, diameter, and level of entanglement can alter mechanical properties. This engineered physical entanglement of microfiber gelatin fibers is an innovative approach to (i) improve our understanding of entanglement, where gaps in knowledge of how entanglement affects fracture, fatigue, and friction exists and (ii) develop translatable scaffolds to improve tissue repair. Further, the incorporation of multi-fibers (more than one type of fiber) and the introduction of inter-fiber chemical crosslinking could expand on the available properties possible to better mimic features of natural tissues.

Poster Number: 30

Spatiotemporal hydrogel swelling induces basal curvature within intestinal monolayers to drive villi formation

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Intestinal stem cell derived tissues have been utilized to investigate morphogenesis and tissue mechanosensing in both 2D and 3D context, however in vitro tissue morphologies that mimic the native intestine (containing both crypt and villi structures) have to date only been formed utilizing scaffold guided monolayer growth. Here we utilize spatiotemporally defined hydrogel surface swelling to initiate villi morphogenesis within initially planar intestinal stem cell derived monolayers. First, poly(ethylene glycol) (PEG) based synthetic hydrogels of 1.6 kPa shear storage modulus, containing peptides to facilitate cell adhesion (GFOGER) and cell mediated protein remodeling (BM), were formed to contain allyl sulfide crosslinks to enable exchange based photoresponsive behavior (1.6 kPa-G-BM). Hydrogels were formed through a strain promoted azide alkyne cycloaddition reaction between an 8 arm 40 kDa PEG functionalized with dibenzocyclooctyne (Peg8DBCO) and an allyl sulfide bis (Peg11 Azide) crosslinker. Hydrogel crosslink degradation was afforded by equilibrating hydrogels in solution containing a monofunctional thiol (glutathione) and a photoinitiator, lithium phenyl-2,4,6trimethylbenzoylphosphinate (LAP), and subsequently irradiating with light (365 nm, 5 mW cm-2). Spatiotemporal hydrogel irradiation using lithographic based techniques with circular mask features of 50, 75, 100, and 200 m diameter and irradiation times of 40, 60, 80, and 100 sec resulted in spatially controlled hydrogel swelling that increased with pattern size and irradiation time with values ranging from 0.025 – 0.1 um-1. Intestinal monolayers grown on 1.6 kPa-G-BM hydrogels were irradiated at confluence to induce spatiotemporal defined hydrogel swelling. Cells within the monolayer overlaying irradiated regions were found to have increased basal curvature leading to the formation of protrusions of defined size. Lastly, the differentiation of these monolayers was investigated under static and bulk flow conditions, and the height of protrusions was found to increase when cultured flow conditions. Importantly, monolayers were observed to compartmentalize in response to differentiation following spatiotemporal hydrogel swelling, with enterocytes expressing Aldolase B/C accounting for a majority of the cells within the protrusions, while LGR5eGFP expressing stem cells were found to localize within inter-pattern regions.

Poster Number: 31

Hyaluronic Acid Attenuates Collagen Fiber Strain Recovery in Tendon

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Tendons perform a critical function by transmitting forces from muscle to bone. The extracellular matrix (ECM) provides tendons with the mechanical structure necessary to bear loads. While fibrillar type I collagen serves as the primary load-bearing element, proteoglycans and glycosaminoglycans (GAGs) surround collagen fibers, aiding in cellular communication and biochemical regulation, and are hypothesized to affect fiber sliding and the tendon mechanical response. Hyaluronic acid (HA) is widely used as a tendon therapeutic and biocompatible scaffold despite little being known on its role in tendon mechanics. The objective of this study was to measure the effect of HA on multiscale tendon mechanics to improve understanding of HA interactions with collagen fibers and surrounding ECM.

ECRL tendons from adult mice were harvested and incubated in either buffer (control) or hyaluronidase (Hyal) to digest endogenous HA. Tendons were mechanically tested using new methodology I developed that leverages laser ablation of collagen fibers in situ to perform fiber-scale mechanical characterization within intact tissue. It includes our custom soft-tissue testing system integrated with a multiphoton microscope to enable tendon loading to a prescribed force, holding for macroscale stress relaxation, then ablating two cuts across an individual collagen fiber within the tendon and imaging, via second harmonic generation, the contraction of the center fiber segment as the load is removed. Macroscopically, Hyal and control tendons showed no differences in cross-sectional area or stress relaxation (N=5/group). At the fiber scale, Hyal tendons demonstrated significantly less elastic strain recovery, the normalized change in fiber length from loaded to immediately after ablation ($p<0.01$), as well as total strain recovery after 5 minutes ($p<0.05$). Viscoelastic strain recovery, the normalized change in fiber length from after ablation to after 5 minutes, was lower in Hyal tendons, however the results were not significant ($p=0.06$). These results indicate that Hyal treatment decreased collagen fiber sliding but did not alter bulk tissue viscoelasticity.

Ongoing work in this study aims to confirm the effect of HA on collagen fiber mechanics by reintroducing exogenous HA to control and Hyal treated tendons, which can inform how current HA-based treatments can affect tissue mechanics and biocompatible scaffolds.

Poster Number: 32

Highly entangled photodegradable poly(acrylamide) networks enable spatiotemporal control over tough and low-hysteresis hydrogels

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Hydrogels consist of a hydrophilic network of crosslinked polymer chains. Typically, covalent crosslinks are used to mechanically stabilize the topology of network-forming polymers, but this results in embrittlement of the resulting material. Tanglemers are an emerging class of polymer networks that are characterized by dense entanglements between long polymer chains with sparse covalent crosslinks, leading to relatively high stiffness while maintaining toughness, extensibility, and elasticity. Although tanglemers have been previously prepared with degradable crosslinks, it is unclear what effect spatial variation of covalent crosslinks may have on these materials. Here we show the synthesis and characterization of a highly entangled polyacrylamide hydrogel system containing photodegradable covalent crosslinker, enabling tunability and spatiotemporal patterning of swelling, entanglement mobility, and modulus. Originally, both degradable and nondegradable gels have a comparable modulus and work of fracture, low hysteresis, and strain rate independent properties. We also observe birefringence through the strained hydrogel which is attenuated as covalent crosslinks are photodegraded, suggesting that covalent crosslinks facilitate alignment between long polymer chains under strain. Further, using photomasks or laser patterning to precisely vary the density of covalent crosslinks allows for the fabrication of materials with spatially graded properties. Overall, our results expand our understanding of highly entangled polymer systems and outline a method by which they can be made to accommodate spatial manipulation of covalent crosslink density. By interfacing known techniques to modulate standard hydrogels with properties unique to tanglemers, we allow for expanded use of the material properties space and foresee the development of new tissue mimetics or biomedical devices.

Poster Number: 33

WITHDRAWN

Poster Number: 34

Hyaluronic Acid Hydrogels: Mechanics at the Macro and Micro Scale

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Introduction: Bulk hydrogels are commonly employed in tissue engineering and drug delivery applications due to the tunability of their biophysical and biochemical properties; however, their uniform structure and nano-scale porosity often limit cell invasion, soluble factor diffusion, and ECM deposition. As an alternative, hydrogel microparticles, known as "microgels," have emerged as a promising approach to overcome these limitations when packed into granular hydrogels. Granular hydrogels exhibit inherent porosity due to microgel packing and are injectable due to microgel flow under loading. Despite this interest and the importance of local mechanics in mechanobiology studies, there has been little work to compare the mechanical properties of hydrogels when processed as either bulk hydrogels or microgels. To address this and using norbornene-modified hyaluronic acid (NorHA) as an exemplary hydrogel, we have engineered an active-feedback micropipette aspiration device to quantify the mechanics of individual microgels, which is then compared to traditional methods to quantify bulk hydrogel mechanical properties (e.g., uniaxial compression testing).

Methods and Results: Hyaluronic acid (HA) was chemically modified with reactive norbornenes via an aqueous esterification route with an organic triazine coupling agent (4-(4,6-dimethoxy-1,3,5-traizin-2-yl)-4-methyl-morpholinium chloride (DMTMM)). NorHA modification extents were controlled via time of reaction, and ~30% modification was obtainable, as characterized by 1H NMR. NorHA underwent thiol-ene crosslinking either as (1) bulk hydrogels or (2) microgels fabricated via oil-in-water batch emulsion (350RPM) in the presence of a di-thiol crosslinker (dithiothreitol (DTT)), photoinitiatior, and UV light. Polymer concentration was varied (1, 3, 5 wt%), and bulk mechanics demonstrated typical increases in compressive moduli (~3, 33, and 82 kPa) and decreased swelling ratios (39, 26, 19%), respectively. To test microgel mechanics, individual microgels were aspirated at varying pressures, and deformation was recorded to calculate the elastic modulus. Preliminary data demonstrates that average bulk mechanical properties were conserved in the microgel form; however, more testing is required to make this conclusion. Additionally, microgel size was found to be proportional to microgel elastic modulus. Together, this project enables the investigation of biomaterial mechanical properties in both the bulk and micro scale, empowering future microgel design.

Poster Number: 35

Degradation of Chlorothalonil by Catalytic Biomaterials

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Chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile) is a halogenated fungicide originally introduced in the United States in 1966, and currently applied to a variety of crops, most notably potatoes, turfgrass, and cherries. In recent years, it was banned in the European Union due to its carcinogenicity, embryo lethality, and high chronic oral toxicity in mammals, among other effects. However, chlorothalonil is still widely used in other parts of the world, including the United States, and has been found in countless drinking water sources around the globe. While previous studies have investigated its degradation in drinking water, this has proved a difficult problem to solve. Chlorothalonil dehalogenase (Chd), originally isolated from the bacterium Pseudomonas sp. CTN-3 in 2010, offers a potential solution. Chd performs the first step in the degradation of chlorothalonil, forming the product 4-OH-chlorothalonil. This study shows that Chd is active when encapsulated in tetramethylorthosilicate (TMOS) gels using a sol-gel method. Encapsulation has been confirmed after incubation with the endopeptidase trypsin. When encapsulated in sol- gels, Chd is stable over a larger temperature range (5 to 80 C) and has shown a potential for storage and reuse over a month-long period. Activity of these encapsulated enzymes has also been observed over a broad range of pH. These data show that encapsulated Chd may have applications for chlorothalonil degradation in drinking water.

Poster Number: 36

Investigation of Shear and Extensional Rheology of Silk Fibroin in Applications of Tissue Engineering

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Many tissues of the body rely on innate elastic properties to convey the proper function of the tissue. Loss of elasticity, especially in blood vessels, often leads to the onset of pathology. The tissue composition of blood vessels contain a high percentage of a protein that is called elastin. Elastin is largely responsible for the expansion and recoil of the vessel wall throughout the cardiac cycle. Elastin is notoriously challenging to process ex vivo while still retaining the same level of mechanical properties. Silk fibroin (SF) is an elastic protein, similar to elastin, that has impressive mechanical properties and biocompatibility, making it an attractive material in the development of tissue engineered blood vessels. Silk fibroin can be easily isolated from Bombyx mori silkworm cocoons however, the regenerated SF in water is an unstable solution delivering inhomogeneous results. The goal of this research is to control and characterize the SF at each step of the extraction-solubilization process to produce regenerated SF (rSF) in water. The resulting rSF solution is characterized via small amplitude oscillatory shear measurements and extensional rheology. The rSF is then processed using electrospinning or dip-coating to form a tube that is mechanically characterized via burst pressure and compliance measurements. Relationships can be formed between the rheological measurements and resulting mechanics of the tube. Furthermore, based on the rheological characterization of the solution, we can predict the successful application of a processing method and mechanical output of the final product.

Poster Number: 37

Design of Injectable and Adhesive Hydrogel Cardiac Patches

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Myocardial infarction (MI), more commonly known as a heart attack, is one of the leading medical issues globally. The delivery of therapeutics such as drugs, cells, and biomaterials is being explored to mitigate the damage caused by MI. One delivery method of great interest is the application of a patch to the surface of the heart. Patches can be made of a multitude of materials and are typically implanted surgically and applied with an adhesive or sutures [1]. Unfortunately, this approach is not minimally-invasive and can cause trauma to the patient and tissue during the surgical procedure [2].

To address this concern, our goal is to develop a patch that can be injected into the pericardial space around the heart and that presents an adhesive on one-side for application to the heart. This is so that once injected, the patch only attaches to the heart muscle and not the pericardium lining. Our approach is to use hydrogel foams that are formed as a cryogel (reaction once frozen), that can be compressed for loading into a syringe and then expands once injected. Specifically, we are creating hydrogel foams from hydrogel precursors such as methacrylated-hyaluronic acid (MeHA) that crosslinks in the presence of a radical initiator once frozen, to effectively introduce macropores into the structure during crosslinking. This allows for dehydration, rehydration, and injectability of the patch. Various fabrication methods are currently being explored to spatially control adhesion and future work includes investigation of the therapeutic activity of the material once implanted.

[1] Mei et al., Recent developments in Therapeutic Cardiac Patches, Frontiers, 2020.

[2] Adu-Berchie et al., Adoptive T cell transfer and host antigen-presenting cell recruitment with cryogel scaffolds promotes long-term protection against solid tumors, Nat. Comm., 2023.