Vanderbilt University Biomaterials Day
October 28, 2022

The Vanderbilt Student Chapter of the Society for Biomaterials was proud to host Biomaterials Day in the Student Life Center on Vanderbilt's campus on October 28, 2022. This one-day event consisted of several research sessions featuring early-career faculty from various regional institutions, rapid-fire trainee presentations, a poster session, a racial disparities in healthcare workshop, and a keynote address.

Participants: Vanderbilt's Biomaterials day consisted of 123 registered participants. Undergraduates, graduate students, postdocs, and faculty came from 11 distinct regional universities including Vanderbilt, University of Kentucky, University of Memphis, University of Mississippi, University of Cincinnati, Washington University in St. Louis, University of Arkansas, Duke University, and Tennessee Technological University. A full list of registered participants can be found in the Appendix of this report.

Sponsors and Funding: Funding sources and funding amounts are outlined as follows:
- Society for Biomaterials: $2,500
- Vanderbilt Student Services Fees: $2,000
- Vanderbilt School of Engineering: $5,000
- Vanderbilt Institute of Nanoscale Science and Engineering: $3,000
- Chan-Zuckerberg Initiative: $5,000
- Rollover Organization Funds: $72.27

Total Funding: **$17,572.27**

Expenses: Major expenses for Biomaterials Day are outlined below:
- Breakfast: $1,215.00
- Lunch: $2,370.69
- Reception: $6,739.87
- Thursday Night Social: $1069.67
- Shirts: $1,317.66
- Transportation (flights, hotels, parking): $2347.72
- Awards and honorariums: $1,556.84
- Other: $860.00

Total Expenses: $**17,477.45**

Speakers: Vanderbilt's Biomaterials Day was proud to have 11 faculty research presentations, 6 trainee rapid-fire talks, and 1 workshop presenter. Speaker names and presentation titles are outlined below:

Keynote Presentation:

- **Dr. Tatiana Segura**, Duke University - “Microgel size in Microporous Annealed Particle Scaffolds (MAPS) impacts macrophage polarity”

Faculty Presentations:

- **Dr. Chris Nelson**, University of Arkansas - “Expanding applications of in vivo genome engineering to rare disease and regenerative medicine”
- **Dr. Richard d’Arcy**, Vanderbilt University - “Ultrahigh Drug-Loaded Micelles for the Treatment of a Triple-Negative Breast Cancer”
- **Dr. Ramkumar Annamalai**, University of Kentucky - “Immunomodulatory Therapy for Bone Regeneration”
- **Dr. Vincent Venditto**, University of Kentucky - “Antibiotics to Heal a Broken Heart”
- **Dr. Thomas Werfel**, University of Mississippi - “Polymeric Drug Delivery Systems for Interval Delivery of Psychedelics”
- **Dr. Eden Tanner**, University of Mississippi - “Bio-inspired Ionic Liquids for Drug Delivery”
- **Dr. John Martin**, University of Cincinnati - “Healing-Responsive Biomaterials for Engineering Tissue Regeneration”
- **Dr. Nate Huebsch**, Washington University in St. Louis - “Biomaterial platforms to study role of biomechanical stress in maturation and hypertrophic cardiomyopathy phenotypes in iPSC-derived heart muscle”
- **Dr. Alexandra Rutz**, Washington University in St. Louis - “3D Printed Conducting Polymers as Bioelectronic Scaffolds”
- **Dr. Stacey Schutte**, University of Cincinnati - “Bilayered Scaffold Designed for Innervation”

Trainee Rapid Fire Presentations:

- **Harshini Suresh Kumar**, University of Kentucky - “Biomechanical Stimulation of Muscle Impacts the Adaptive Response of Bone by Modulating Myostatin (GDF8) Secretion”
- **Elizabeth Curvino**, Duke University - “Inspired by Natural Antibodies: An Anti-Phosphorylcholine Nanofiber Immunotherapy for Inflammatory Bowel Disease”
- **Dr. Brian O’Grady**, Vanderbilt University - “A Three-Dimensional Model of the Neurovascular Unit Using Human Ex Vivo Brain Tissue”
- **Emily Montgomery**, University of Memphis - “Hydroxyapatite in combination with 2-heptylcyclopropane-1-carboxylic acid counteracts S. aureus planktonic growth and biofilm formation”
- **Jessalyn Baljon**, Vanderbilt University - “A Nanocarrier Vaccine Platform for Co-Delivery of Peptide Neoantigens and Synergistic Adjuvants”
- **Joanne Lee**, Vanderbilt University - “Co-Engineering Synthetically Programmed Cells and Biomaterials for Regenerative Medicine”

Workshop Presentation:

- **Dr. Katie Young**, Vanderbilt University - “Racial Health Disparities: Learning through the Lens of a Cancer Researcher”

Poster Session: Vanderbilt's Biomaterials Day poster session consisted of 72 posters. Poster presenters represented all the regional universities that were present and consisted of undergraduate, graduate, and postdoctoral trainees. Seven prizes were awarded including a People's Choice award, 3 third place prizes, 2 second place prizes, and 1 first place prize. A full list of poster abstracts can be found in the appendix to the report.

Feedback: After the conclusion of the event, a survey was sent out to all the participants asking for their feedback about the event. The survey responses were overwhelmingly positive, with the diversity of speakers and research areas, the workshop, and the band at the reception being noteworthy areas of praise from most of those who responded. Some of the criticisms of the event that will be taken into account for the next time were the length of time for the poster session and the densely packed schedule. With the large number of speakers we had, the schedule allowed for little flexibility with regards to breaks, lunch, and the poster session. A few breaks were integrated and the poster session overlapped with lunch. This led to a long program that didn't allow for much networking among the attendees. In the future we will definitely give more priority to networking and sufficient time to explore the poster session.

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APPENDIX

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Poster Abstracts:

Juan Colazo, Albumin-Hitchhiking MMP13 siRNA Conjugate (siMMP13<(EG18L)2) for the Treatment of Rheumatic Disease

Background: Osteoarthritis (OA) and rheumatoid arthritis (RA) decrease quality of life due to joint destruction, pain, and decreased function. Multiple joint osteoarthritis (MJOA) occurs in over 50% of OA cases. The high incidence of MJOA motivates the development of systemic therapies. Although there are successful therapies for RA treatment, many patients do not benefit, and they can cause systemic side effects. In OA/RA, matrix metalloproteinases (MMPs) drive joint degeneration. Small molecule MMP inhibitors were tested clinically, but were dose limited by toxicities caused by lack of MMP selectivity. Short-interfering RNA (siRNA) therapies can be designed to silence “undruggable targets” but are limited due to significant biological barriers. Albumin-based drug delivery is promising due to its high half-life, recycling ability, and its accumulation in diseased joints. Herein, we characterize a chemically stabilized MMP13 siRNA molecule, siMMP13<(EG18L)2, which spontaneously binds albumin 'in situ'.

Methods: The siRNA was synthesized with alternating 2′-OMe and 2′-F ribosugar modifications to stabilize against endonucleases and phosphorothiate linkages on the backbone to block exonucleases. For albumin-binding abilities, a splitter phosphoramidite (<) (to allow for diacyl addition i.e., 2 lipid tails) at the 5' end of the sense strand was added followed by 3 repeats of a hexa-ethylene glycol (EG6) phosphoramidite. Thus, 18 EGs were added prior to each eighteen-carbon (C18) acyl (2x, L2

nomenclature). An overload-induced osteoarthritis mouse model (9N, 250 cycles, 3x/week) and the K/BxN serum transfer arthritis (STA) mouse model were used for OA and RA studies, respectively.

Results: RNA stabilization chemistries increased serum/synovial fluid stability, while maintaining potent MMP13 silencing. Albumin-binding dye Evan's Blue and fluorescent albumin accumulated more in OA knees/K/BxN hindpaws than healthy knees/hindpaws. siMMP13<(EG18L)2 showed preferential delivery to OA knees/K/BxN hindpaws over healthy knees/hindpaws and demonstrated greater pharmacokinetics than non-end-modified siRNA and cholesterol-conjugated siRNA. In the K/BxN model, siMMP13<(EG18L)2 reached/treated multiple joints including forepaws, knees, and hindpaws. Overall, siMMP13<(EG18L)2 treatment was safe, provided potent MMP13 mRNA/protein knockdown, provided joint pain benefits, reduced arthritis clinical score, reduced arthritis-related genes, provided joint protection, and performed better than or equal to gold standard clinical controls.

Conclusions: Albumin-hitchhiking siRNA shows promise as a platform technology that can be readily adapted for targeting of many rheumatic disease-driver genes.

Mariah Bezold, Shear-thinning, Nanoparticle-based Hydrogels as an Injectable Dual Delivery Platform for Repair of Chronic Diabetic Skin Wounds

Nanocomposite hydrogels demonstrating shear-thinning/self-healing behavior through reversible crosslinking of natural biopolymers possessing extracellular matrix (ECM) with synthetic polymers are highly desirable in tissue engineering applications. In this work, a nanoparticle-based, shear-thinning hydrogel was assembled by guest-host chemistry to mediate physical crosslinking between hyaluronic acid (HA) and self-assembled ROS-responsive polymer nanoparticles. A library of adamantane (AD) functional linear ABC triblock polymer nanoparticles composed of poly(propylene sulfide)-b-poly(N,N-dimethylacrylamide)-b-poly(N,N-dimethylacrylamide-co-adamantane acrylamide) [PPS-b-PDMA-b-P(DMA-co-ADA)] with varied hydrophilic middle DMA block lengths and grafting density of AD in the third block was synthesized as the guest macromer and was then mixed with the host macromer, β-cyclodextrin grafted hyaluronic acid (HA-CD), to assemble the final nanocomposite hydrogels. The four lead hydrogels were screened from a full library based on their variable mechanics, the stoichiometric ratio of synthetic/biological macromers, crosslinking density of guest/host molecules, and superior shear-thinning/self-healing properties compared to HA shear-thinning hydrogels. The degradation kinetics of hydrogels were assessed under oxidative, enzymatic, and combined oxidative and enzymatic environments, and nanocomposite hydrogels demonstrated protection of HA compared to HA shear-thinning hydrogels. Additional in vitro characterization studies demonstrated cytocompatibility and protection of cells against cytotoxic ROS and mechanical shear stress when encapsulated in nanocomposite hydrogels. When subcutaneously injected in mice, cellular infiltration along with degradation of nanocomposite hydrogels was found to depend on hydrogel mechanical strength, crosslinking density, and stoichiometric ratio between guest/host macromers. This work provides insight into the development of a synergetic hybrid hydrogel with the ability to mimic natural ECM while incorporating ROS-responsive polymer chemistry as a promising platform for therapeutic cell/drug delivery, wound healing, and other tissue engineering applications.

Shrusti Patel, Expanding the Therapeutic Index of siRNA Nanoparticles Toward Rictor/mTORC2 Treatment of Triple Negative Breast Cancer

Triple negative breast cancer (TNBC), an aggressive clinical subtype, currently lacks druggable molecular targets, making neoadjuvant chemotherapy (NAC) the standard of care. Nearly 70% of NAC-treated TNBC patients harbor residual disease, with phosphatidyl inositol-3 kinase/ mechanistic target of rapamycin (PI3K/mTOR) mutations found in nearly 50% of these, correlating it to disease. Inhibition of mTORC1 is ineffective in TNBCs, but less is known regarding mTORC2. siRNA technology can be

leveraged to block expression of the mTORC2 obligate cofactor, Rictor, and test the therapeutic efficacy of selective mTORC2 inhibition in TNBC.
The overall objective of our work is to develop siRNA-carrying nanoparticles (si-NPs) that overcome systemic delivery challenges and promote potent siRNA delivery to tumors with a reasonable therapeutic index. Here, we developed ternary si-NPs composed of a core-forming poly[(2-(dimethylamino)ethyl methacrylate)-co-(butyl methacrylate)] (DMAEMA-co-BMA, 50B) polymer and corona-forming 20kDa poly(ethylene glycol)-block-50B (20kPEG-50B) polymer. We evaluated the impact of the amount (ratio of 50B:20kPEG-50B) and the molecular weight of the core-forming 50B polymer on ternary si-NP function. Through concomitant optimization of 50B ratio and size, we identified lead ternary si-NPs that balanced activity and toxicity in vivo. A single 1 mg/kg injection of 50B8-DP100 si-NPs resulted in 80% tumor gene knockdown in an orthotopic MDA-MB-231 model. Efficacy of our lead 50B8-DP100 formulation was then tested for delivery of siRNA against Rictor (siRictor-NPs) in TNBC. In an orthotopic HCC70 TNBC model, tumors treated with siRictor-NPs displayed decreased tumor growth compared to tumors treated with siControl-NPs, which doubled in the 7-day period. Together, this work identifies a novel nanotechnology and strategy for the treatment of PI3K-active TNBC.

Diana Popa, Towards the development of liquid state promazine drugs
The phenothiazine drugs promazine, chlorpromazine, and triflupromazine are solid-state pharmaceuticals with multiple biological properties but no analgesic effects. Combining these phenothiazine cations with various anions such as NSAIDs and docusate anions results in the formation of new liquid state drugs that retain the biological properties of the constituent ions. This will not only add analgesic properties but also aid in the development of new delivery methods for these phenothiazine drugs. This presentation focuses on the replacement of the inorganic counter ions with ibuprofenate and docusate anions in various molar ratios to synthesize new double salt ionic liquid phenothiazine drugs. The identity and purity of the new compounds were determined using known spectroscopic techniques.

Hayden Pagendarm, Flash nanoprecipitation for the production of STING-activating nanoparticles
The delivery of biomacromolecular drugs to cytosolic targets has been a long-standing engineering challenge due to the presence of multiple biological barriers including cellular and endosomal membranes. Although many promising carriers designed to facilitate endosomal escape have been developed, the clinical translation of these carriers is often limited by complex production processes that are not amenable to scaled-up manufacturing. In this study, we employed flash nanoprecipitation (FNP) for the rapid, scalable, and reproducible assembly of nanocarriers composed of the pH-responsive, endosomolytic diblock copolymer poly[(ethylene glycol)x-block-[((2-diethylamino) ethyl methacrylate)0.6-co-(butyl methacrylate)0.4]y (PEG-b-DEAEMA-co-BMA). We found that varying the second block molecular weight, while holding the first block molecular weight constant, significantly influenced nanoparticle self-assembly and hence nanocarrier properties and function – including drug encapsulation, endosomolytic capacity, cytotoxicity, and in vitro activity of a cytosolically-active drug cargo, a cyclic dinucleotide (CDN) stimulator of interferon genes (STING) agonist. We found that while increasing second block molecular weight enhanced the capacity of nanocarriers to induce endosomal destabilization, larger second block molecular weights also lead to increased cytotoxicity, increased particle size and heterogeneity, increased the encapsulation efficiency of small (<0.5 kDa) hydrophilic drugs, and decreased long-term particle stability. Collectively, these results demonstrate the utility of FNP for the rapid and scalable production of uniform PEG-b-DEAEMA-co-BMA nanocarriers and implicate an optimal hydrophilic mass fraction for balancing desirable nanoparticle properties with cytosolic cargo delivery efficiency.

Jordan Hill, Molecular Weight Effects on siRNA-Polymer Conjugates Pharmacokinetics and Biodistribution

Short interfering RNA (siRNA) are an effective tool for gene knockdown. The biodistribution and PK affects on on the RNA can be improved by increasing the molecular weight of the polymer. We employ a open-to-air, blue light RATF polymerization technique to graft a polymer from an siRNA macro chain transfer agent.

Morgan Struthers, Non-Viral Delivery of CRISPR/Cas9 Ribonucleoprotein for the Treatment of Osteoarthritis

OA is characterized by excessive production of MMP13, an enzyme that degrades critical ECM components such as type II collagen, resulting in cartilage erosion and progression of OA. We are seeking to non-virally deliver the CRISPR/Cas9 system [ribonucleoprotein (RNP) complex of Cas9 protein and guide RNA (gRNA)] to make targeted cuts in the MMP13 gene to trigger non-homologous end joining (NHEJ) and resultant indels and MMP13 gene knock-out (KO). The RNPs were delivered using a porous silicon nanoparticle (PSNP) aminated by APTES (AP) to create a cationic surface to improve loading of anionic RNPs. RNP-loaded PSNPs were further surface coated with varying ratios of the endosomolytic polymer poly(dimethyl aminoethyl methacrylate -co- butyl methacrylate) or “DB”.

In MTMG cells, the lower PSNP:DB ratio showed the best editing, which also correlated with increased endosome escape activity of the formulations with higher DB content. The 10:1 formulation also created up to 95% indel% in chondrocytes in vitro. Using Ai9 reporter mice, histological analysis on knee joints following induction of OA and intra-articular PSNP-RNP treatment showed that a single injection resulted in sparse tdtomato reporter turn-on in the chondrocytes and synovium.

The PSNPs with optimized DB polymer coating are a promising system for non-viral delivery of CRISPR/Cas9 RNPs. These data prove that this system can be used to efficiently knock out disease relevant genes in vitro and that the system is effective in vivo at generating targeted double-stranded breaks in the genome of joint cells following local, intra-articular delivery. Because gene editing is permanent, it is conceptually feasible that one injection or small series of injections could be given to permanently block OA. Ongoing studies are focusing on optimizing this system for knock-in (homology directed repair) strategies.

Jenna Mosier, Confinement in 3D Collagen Microtracks Drives Mechanical Memory in Migratory Breast Cancer Cells

While many advances have been made in targeting early-stage breast cancer, metastasis remains a clinical challenge due to a limited understanding of cancer cell behavior and secondary tumor formation. Migration, occurring early in metastasis, is dependent in part on the mechanical environment in the tumor that can change cell behavior. Confinement, imparted on cells by dense collagen in tumor tissue, significantly changes cell behavior, with previous work revealing cell speed and energetics increase. It is still unknown whether these changes are temporary, occurring only when the cell is actively confined, or permanent, affecting future cell motility. Long-term 'mechanical memory' has been observed in response to substrate stiffness, with mechanical priming on stiff surfaces having lasting effects. Determining the factors driving this memory in response to mechanical cues may highlight crucial therapeutic targets for metastasis. To determine if cancer cells maintain memory of confinement, we utilized microfabrication and micromolding to develop an engineered, collagen microtrack platform that mimics the geometric confinement found in tumor tissue without compromising material composition or stiffness. We observed that cells in high confinement increase mitochondrial localization, with mitochondria moving ahead of the nucleus. By designing microtracks that force cells to first navigate a region high confinement followed by a region of low confinement, we can assess mechanical memory of confinement. We have shown that cells maintain speeds attained in high confinement after their exit, indicating that memory may be dependent on the duration of confinement. Further, cells maintain mitochondrial localization after high confinement,

suggesting that increased organization of metabolic machinery, like mitochondria, may allow cells to continue fast migration even when mechanical cues have disappeared. By understanding what drives mitochondrial localization, we can better understand the relationship between confinement and mechanical memory and identify potential targets to inhibit breast cancer metastasis.

Matthew Patrick, Elucidating Immune Dysregulation in Destabilized Femoral Fracture Mouse Model

Introduction: Nonunion of long bones is a debilitating injury, and there is an urgent clinical need for the treatment of such recalcitrant wounds. Treatment remains difficult because of the insufficient mechanistic understanding of nonunion development. The clinically predominant cause of nonunion is a lack of mechanical stability, which increases local tissue strain leading to poor healing. We hypothesized that increased strain in fracture callus causes aberrant macrophage phenotypes and subsequent immune dysfunction that disrupts proper healing.

Methods: To validate our hypothesis, we analyzed the macrophage phenotypic response to different strain levels in 3D fibrin matrices in vitro with high and low cyclic loading and in a murine femoral fracture stabilized with high and low stiffness intramedullary NiTi rods.

Results and Discussion: In 3D fibrin matrices, macrophages exhibited a prohealing response to low strain (<5%), with significant upregulation of M2 markers. While in the high strain (>15%) condition, we saw a significant downregulation of prohealing markers and upregulation of the proinflammatory markers. The rise of these aberrant macrophage phenotypes in higher strain conditions can cause bone resorption and osteolysis. In murine fractures, the high strain condition induced by the low stiffness rod resulted in a larger callus volume, while minimal callus was formed in the high stiffness group. Flow cytometric analysis of callus tissue showed a strong positive correlation between callus volume and macrophage populations.

Conclusions: Overall, our in vivo data show that higher tissue strains caused larger callus volume, and higher macrophage infiltration. Further, our in vitro analysis shows that larger tissue strain resulted in aberrant macrophage phenotypes, which can cause bone resorption and osteolysis. Our long-term goal is to develop biomaterial interventions to pacify higher strains to provide a therapeutic avenue to promoting healthy bone regeneration.

Harshini Suresh Kumar, Biomechanical Stimulation of Muscle Impacts the Adaptive Response of Bone by Modulating Myostatin (GDF8) Secretion

Introduction: Diabetes is a chronic metabolic disorder that can lead to diabetic myopathy and bone disease. The pathophysiology of these musculoskeletal complications is not fully understood. However, exercise training has shown promise in preventing diabetic myopathy and bone disease. Here we have developed an in vitro model to elucidate the effects of mechanical strain on muscle secretome (myokines) and its impact on bone metabolism isolated from physical stimuli.

Methods: We fabricated bone constructs by seeding preosteoblasts on gelatin microgels and muscle constructs by seeding myoblasts in fibrin hydrogel. Muscle constructs were linearly stretched to mimic exercise training and myokines secretion were evaluated. Further, muscle constructs were co-cultured with bone constructs to study the effects of myokines on bone phenotype. The role of myostatin on bone functional properties was also evaluated using 3-point bending and microCT analyses on femurs from adult GDF8-/- mice.

Results and Discussion: The preosteoblast seeded on gelatin microgels exhibited osteocyte phenotype confirming the osteoinductivity of the microgels and the muscle constructs formed myotubes within few days. When the muscle constructs were subjected to exercise training under hyperglycemia, we observed a down-regulation of the myostatin (Mst) a critical suppressor of skeletal muscle and bone metabolism. Then we co-cultured them and subjected the muscle constructs to exercise training facilitating only

biochemical interaction between the constructs. The co-culture resulted in downregulation of Mst in muscle constructs and upregulation of osteogenic genes (Col1a1 and Spp1) in the bone constructs. When bone constructs were treated with recombinant myostatin, we saw significant downregulation of osteogenic genes (Osx, Bglap, Col1a1, Runx2, and Spp1), confirming its inhibitory effects on osteoblastogenesis. Further, we saw a significant increase in the trabecular thickness, separation and pattern, and a superior trabecular microarchitecture as well as increased young's modulus in GDF8-/- mice compared to wildtypes indicating improved bone strength.

Conclusions: We found that under hyperglycemic conditions, exercise training generally favored the secretion of anabolic myokines and inhibited the catabolic myokine, myostatin. Further, we found that myostatin significantly constrains osteoblastogenesis in vitro and trabecular microarchitecture and bone mechanical properties in vivo. Overall, our study shows the protective effects of exercise training on bone phenotype through modulation of muscle secretome.

Acknowledgments: GDF8-/- mice were a kind donated from Regeneron Pharmaceuticals Inc.

Joshua McCune, ROS Degradable Polythioketal Urethane Foam Dressings Promote Porcine Ischemic Wound Repair

We have previously developed a class of reactive oxygen species (ROS) responsive polythioketal urethane (PTK-UR) foam dressings that promote wound healing. These foams overcome limitations associated with hydrolytically-degradable wound dressings, which are susceptible to autocatalytic degradation and biomaterial-associated inflammation. We hypothesize that treating ischemic excisional wounds with PTK-UR dressings will promote scaffold resorption by scavenging ROS while minimizing material-associated inflammation to promote improved tissue repair in a challenged wound model.

EG7 PTK-UR scaffolds were evaluated in vivo in an ischemic full-thickness porcine wound model compared to NovoSorb BTM, an FDA-approved dermal substitute. Wound area was tracked throughout the study, and the quality of wound healing was evaluated blindly by a histopathologist. Additionally, immunohistochemistry was conducted to evaluate re-epithelialization and the biomaterial-associated immune response 17 days post-implantation.

Within 17 days, ischemic wounds treated with EG7 PTK-UR achieved 76.1% closure, while NovoSorb treated wounds achieved 8.3% closure. EG7 PTK-UR treated wounds also achieved nearly complete re-epithelialization, while NovoSorb treated wounds achieved less than 40% re-epithelialization. Histological analysis of the treated wounds illustrated decreased inflammation and improved collagen deposition associated with EG7 PTK-UR, resulting in a greater wound healing score. Analysis of the inflammatory response associated with EG7 and NovoSorb via IHC indicated an increase of both macrophages and neutrophils associated with NovoSorb compared to EG7 PTK-UR.

Ultimately, EG7 PTK-UR foam dressings have demonstrated improved wound healing in comparison to NovoSorb in a challenged wound model, decreasing the material-associated inflammatory response while promoting re-epithelialization and improved overall wound repair.

Sydney Henriques, Localized Repolarization of Tumor-Associated Macrophages via Cytokine-Loaded, Injectable Cryogels

Tumor-associated macrophages (TAMs) are the most abundant immune cell in most types of solid, epithelial tumors, including breast cancer. M2-like TAMs promote tumor progression and create an immunosuppressive tumor microenvironment (TME). High levels of M2-like TAMs correlate with poor prognosis and reduced patient survival. Macrophages, however, display phenotypic plasticity: TAMs can

be reprogrammed to an inflammatory, M1-like phenotype to stimulate antitumor immunity. Exposure to inflammatory cytokines induces M1-like functions in macrophages, but systemic delivery of these cell-signaling molecules results in undesirable off-target effects, including toxicity. To alleviate these concerns, we have developed an injectable alginate cryogel (hydrogel fabricated at -20°C) which can be loaded with inflammatory cytokines and macrophage-specific chemokines to create a localized macrophage repolarization depot. Fabrication at sub-freezing temperatures generates a macroporous scaffold, allowing for cell infiltration into the gel. Peritumoral injection of the cryogel system into FVB female mice with mammary tumors resulted in significantly slowed tumor growth, an increase in T cell infiltration, and an increase in the M1:M2 ratio of macrophages. This activated immune response and higher presence of T cells suggests that localized chemokine/cytokine treatment slows tumor growth and 'primes' the TME, potentially making it more susceptible to immunotherapies that rely on T cells, such as immune checkpoint blockade. Currently, a sequential release approach utilizing poly(lactic-co-glycolic acid) (PLGA) nanoparticles co-formulated with the macroporous cryogel is being developed. It is hypothesized the nano-in-cryo system will induce a more pronounced repolarization effect by allowing the chemoattractant to induce M2 migration prior to the release of the inflammatory cytokines from the PLGA nanoparticles.

Elizabeth Curvino, Inspired by Natural Antibodies: An Anti-Phosphorylcholine Nanofiber Immunotherapy for Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic autoimmune disease for which a long-lasting and broadly effective therapy has yet to be achieved. Preexisting natural antibodies against the small molecule epitope phosphorylcholine (PC) have been shown to be beneficial for both fighting infections and ameliorating inflammatory diseases. Natural antibodies are produced by B1a cells; however, these cells are reduced in IBD patients, with this decrease being indicative of more advanced disease. It has also been demonstrated that the adoptive transfer of B1a cells in a murine IBD model results in increased anti-PC antibody production and lessens disease severity. Here, we strove to develop an immunotherapy consisting of PC as an epitope displayed on self-assembling peptide nanofibers to produce therapeutic anti-PC antibodies.

We began by synthesizing two PC-peptide conjugates with either single or multiple PC copies per self-assembling peptide termed PC-Q11 and PCM-Q11, respectfully. Both conjugates formed nanofibers and induced anti-PC antibody responses after i.p. vaccination. Nanofiber uptake into peritoneal B1a cells, was determined in cells isolated from i.p. lavage fluid after i.p. nanofiber injection. PCM-Q11 showed increased selectivity for B1a cells, with greater uptake in B1a cells compared to all other B cells and enhanced recruitment of B1a cells into the peritoneal cavity. Additionally, control over the immune phenotype elicited was achieved via the inclusion of a T-cell epitope and/or CpG adjuvant. Immunizations with PCM-Q11/T-cell epitope with or without CpG were then evaluated for efficacy in a DSS-induced colitis model. Interestingly, immunization with both formulations was protective, significantly improving disease severity over unimmunized controls. Furthermore, we explored the effects of anti-PC immunization on microbiome diversity. Finally, passive transfer of PCM-Q11 immunized sera before DSS-induced colitis showed that the induced anti-PC antibodies offer some protection against severe colitis. These findings indicate the promising potential of PCM-Q11 immunization as a novel, durable therapy for IBD.

Brian O'Grady, A Three-Dimensional Model of the Neurovascular Unit Using Human Ex Vivo Brain Tissue

The neurovascular unit (NVU) is a multicellular, complex structure that facilitates molecular transport of required nutrients and excludes potentially harmful components from entering brain tissue. Recent novel methods and newly developed stem cell differentiation strategies have improved in vitro modeling of the

NVU. However, there are currently no engineering techniques or strategies to develop an anatomically correct NVU that fully recapitulates the complex multi-cellular hierarchy and cellular interactions. Thus, these models are often oversimplified and limited in their capabilities to accurately mimic physiological and pathological mechanisms of the human brain. To address this limitation, we developed a new biomaterial functionalized with a peptide from the extracellular epitope of N-Cadherin that promotes maturation of single-cell suspensions of iPSC-derived glutamatergic neurons into synaptically connected networks. In addition, he found that this hydrogel supports robust and continuous growth of primary human ex vivo arterial- and capillary-specific brain vasculature. The newly formed vasculature consisted of the correct anatomical hierarchy for capillaries and arteries similar to what is seen in vivo. We embedded the ex vivo human brain tissue in microfluidic devices generated from high-resolution 3D printed molds, and induced anastomosis to form a lumen-perfusable NVU-on-a-chip. We believe this technology will provide an exceptional opportunity for investigating new therapeutic options in vitro that could ultimately impact clinical care for neurological diseases.

Andrew Kjar, N-cadherin functionalized gelatin as a fully defined matrix for cerebral organoid culture

Cerebral organoid are self-patterned tissue culture models which recapitulate key early neural developmental milestones. Although these models have become ubiquitous, typical cerebral organoid culture relies on poorly defined, mouse-derived matrix materials to provide mechanical and chemical cues for neural differentiation. To overcome this challenge, two novel hydrogel materials were developed and validated for their ability to support functional organoid maturation. A peptide derived from the extracellular epitope of neural cadherin (N-cadherin) was conjugated to a gelatin backbone, which was subsequently crosslinked into a hydrogel using either photo-initiator based or enzyme-based methods. The measured Young's modulus of the resulting hydrogel was 4 kPa, near stiffness of native brain tissue, thus providing relevant mechanical cues. Organoids grown within the N-cadherin functionalized hydrogel were highly consistent, retaining a high degree of circularity over long-term culture. In contrast, control organoids grown in Matrigel were highly heterogenous in shape as early as day 30. When investigated via immunofluorescent staining and whole mount imaging, populations of organized neural progenitors and differentiated neurons were observed in hydrogel-embedded organoids, comparable to conventionally generated organoids. After two months of culture, populations of cortical layer-specific neurons emerged (i.e. SATB2+ and CTIP2+), indicating the hydrogel was capable of supporting long-term differentiation. This method is a fully defined alternative to conventional organoid generation protocols and results in reproducible, mature organoid tissue. Organoids generated within the N-cadherin functionalized hydrogel will be amenable for future studies including chemical screening, disease modeling, and investigations of early development.

Kate Nevils, Spin Coating Multilayer Polymer Films to Improve the Consistency and Longevity of Interval Drug Release

Treatment-resistant depression (TRD) refers to depression that does not respond to standard treatment regimens and accounts for approximately 50% of depression patients. Evidence suggests patients with TRD can be treated by microdosing classical psychedelics such as lysergic acid diethylamide (LSD). However, issues of patient compliance to the complex regimen and abuse/diversion of the schedule I controlled substances remain unresolved. The goal of this project is to create an implantable, surface eroding drug delivery device that releases microdoses of LSD into the bloodstream for treatment of depression. The technique of spin coating is used to create the thin, stackable films to construct the device. Using this approach will allow for the formation of a multilayer device that alternates between blank and active (drug loaded) layers. Determining a spin curve is the most important step in this process, as it will create the standard for thickness of the films at varying spin conditions. The data among the spin curves provides film thickness at specific time and RPM for the varying concentrations of solvent. From

the curves produced, any film thickness can be obtained, ranging from less than 10 micrometers to 500 micrometers. Using these curves will provide effective results in the fabrication of a multi-
layer device. Implementing a release study on the spin coated films allows for discovery of how much time it takes each layer to fully erode, with the goal being a release of drug once every twenty-four hours.

Maria Lopez Cavestany, Fabrication of Superhydrophobic Microwell Arrays for High-Throughput Culture of 3D Cancer Models

3D cancer models offer higher physiological relevance than 2D cancer models. Products to produce cancer spheroids, such as the Aggrewell800, are available commercially but have difficulty forming tight spheroids in some cell lines and provide only two microwell sizes. The goal of our study was to utilize microfabrication techniques to design a chip with tunable microwell array and a superhydrophobic surface at the bottom to achieve a superior low-attachment device that can produce a wide range of 3D cancer models. Superhydrophobic surfaces were fabricated by growing a nanorod layer onto a silicon wafer and then coating with a non-sticky polymer in the VINSE cleanroom. Ultrathick SU8 lithography was performed to obtain a microwell wall height of about 350μm. Each line was spaced 800μm apart resulting in square sections that are 650x650μm. Throughout the fabrication process, the thin film and nanorod layers were surveyed by SEM imaging. The ZnO nanoparticle deposition method was confirmed to be highly uniform and reproducible. The average nanorod length was measured to be 500μm. Nanorods were also confirmed to be randomly oriented on the wafer surface. Device coating with the polymer layer was confirmed via SEM and water contact angle (WCA) measurements. A WCA of 115° was measured for blank wafer coated in the non-stick polymer. When the ZnO nanorods were coated with the non-sticky polymer, a WCA of 167° was achieved. Confocal images showed uniform spheroid formation of 90μm in diameter using the colorectal cancer cell line HCT116.

Olivia Wright, Production of a Tunable CTC Cluster in vitro Model via a Superhydrophobic Microwell Array Device

High levels of circulating tumor cells (CTCs) present in the blood stream are associated with poor patient survival. However, only 0.01% of CTCs form distant metastases after surviving high levels of fluid shear stress and interactions with immune cells. Clustering with other CTCs, immune cells, or stromal cells, has been shown to confer metastatic advantage. Current methods of in vitro CTC cluster formation do not form tight clusters and size cannot be controlled. The goal of this project was to develop a novel method to reproducibly grow size-controlled CTC clusters in vitro to better understand the advantages cancer cell clustering in the circulation confers to the metastatic cascade. To reproducibly grow size-controlled CTC clusters in vitro, a superhydrophobic microwell array device produced in the King Lab was adapted. In short, a silicon wafer was coated in a ZnO nanorod layer. SU8 lithography was performed to deposit a 75μm thick grid, creating the 100 x 100μm2 square microwells. The devices were then coated in a non-sticky Teflon-like polymer, diced, placed into a 24-well plate, and assessed using SEM. CTC cluster size typically ranges from 2-19 cells. Therefore, 4 cell concentrations of PC3 cells were plated so that clusters of 3, 5, 7, and 10 cells would form after centrifugation into the device. Clusters were incubated for 72 hrs, then observed via brightfield. Current CTC cluster formation methods were used for comparison. We demonstrated a novel method of CTC cluster formation via a superhydrophobic microwell array device.

Christopher J Hansen, Fabrication and Purification of Size-Discrete Poly(lactic-co-glycolic) Acid Microparticles for Tocolytic Application

Preterm labor (PTL) is the leading cause of infant mortality and morbidity worldwide, occurring in 10% of all live births in the U.S. Current drugs used off-label have short-term tocolytic efficacy arresting uterine contractility for only 48-72hrs. Yet, their long-term tocolytic efficacy is unknown due to the adverse maternal and fetal effects. Recent advancements in nanomedicine provide a platform to reduce off-target

effects via targeted delivery systems. Poly(lactic-co-glycolic) acid (PLGA) has gained popularity due to its biocompatibility, FDA-approval, and capacity for sustained release. Our goal was to fabricate low polydispersity (PDI) PLGA microparticles loaded with current tocolytics at 800-1200nm in size, in consideration of the placental barrier, a major obstacle in obstetric medicine. The formulations use either dichloromethane (DCM) with polyvinyl alcohol (PVA) or ethyl acetate (EtAc) with D-α-tocopheryl polyethylene glycol succinate (TPGS) purified with serial centrifugation to isolate subpopulations by diameter. We found that the EtAc/TPGS emulsion could produce particles at 1350 (+/- 64.50)nm (PDI: 0.30). However, this formulation was more polydisperse when compared to the DCM/PVA emulsion with average particle diameters of 1251 (+/- 300.4)nm (PDI: 0.20) with low day-to-day variability (range: 1137 – 1734 nm). Drug encapsulation with two common tocolytics (indomethacin and nifedipine) had minimal effect on particle size (1267 +/- 82.72nm and 1142 +/- 1.155 nm, respectively). Moreover, our formulation presents the reactive surface chemistry necessary to conjugate oxytocin receptor antibodies capable of selectively binding the particles to uterine muscle. In summary, our work provides the preliminary optimization needed to develop a viable tocolytic delivery system.

Taylor Scott, Rapid prototyping of a bone marrow-on-a-chip model with in situ longitudinal imaging of the endosteal niche

In vitro models of the bone marrow (BM) microenvironment can be powerful tools to study BM pathophysiology and test new therapeutics. Realization of these models is difficult due to the complex cellular and structural components of the BM. Furthermore, conventional trabecular bone-mimicking scaffolds are difficult to image due to the tortuous pores that are impermeable to light. We have designed new scaffolds that are permeable to both light and fluid flow. Microfluidic polystyrene (PS) devices have been fabricated for cell imaging by injection molding. However, machining the aluminum molds is costly and time intensive. Our hybrid injection molding approach comprises a machined aluminum mold fitted with ejector pins to demold the part and a scaffold insert mold 3D printed from a high deflection temperature stereolithography (SLA) resin. Light-permeable PS scaffolds fabricated by injection molding were perfused with human mesenchymal stem cells (hMSCs, 10^6 cells/mL) labeled with red fluorescent CM-DiL dye (553/570 nm) for 24 h. hMSCs were perfused with osteogenic medium for 21 days to promote their differentiation to osteoblasts. The scaffolds were then perfused with CD34+ HSPCs (83,000 cells/mL) for 24 h. Engraftment of HSPCs in the osteoid matrix deposited by osteoblasts was observed by confocal microscopy. These PS scaffolds are amenable to longitudinal imaging of hematopoiesis and can be rapidly manufactured (<2 min cycle time) by injection molding to support high-throughput drug screening experiments. Furthermore, the hybrid injection molding approach allows for rapid prototyping of scaffold design to optimize HSPC engraftment.

Joanne Lee, Co-Engineering Synthetically Programmed Cells and Biomaterials for Regenerative Medicine

Millions of people are affected by the inflammatory and damaged tissues produced from aging, autoimmune diseases, cancer, and injuries. To address these problems, the field of regenerative medicine leverages cell therapies and biomaterials. Although current biomaterials technologies are becoming ever more sophisticated and responsive to the environment, there is still a lack of precise control over the activities of transplanted cells. Here, we design a system with an engineered cells:biomaterials interface, where our engineered cells are modified to respond precisely to inputs displayed by the biomaterial. With this engineered interface, we can directly couple specific cell behaviors to selected inputs to establish a privileged channel of communication with our engineered cells. We utilize the tools of synthetic biology to engineer our cells with synthetic Notch receptors that can respond to bioinert soluble inputs in a spatially regulated manner determined by programmable cell:material interactions. In these studies, we demonstrate tunable responsiveness to ligands in a concentration- and substratum-dependent manner. We also show that GFP present in the bulk culture medium can activate engineered cells only in regions

functionalized to interact with synNotch ligand. We illustrate that this cells:biomaterials interface can regulate diverse cell activities including CRISPR-mediated transcriptomic effects, attenuation of inflammation, and stem cell differentiation. With our platform, we hope to develop advanced regenerative medicine therapeutics that overcome limitations of current cell-based therapies and tissue engineering approaches.

Karina A. Bruce, Engineering of Degradable Linkers to Improve Oxidative Sensitivity of Thioketal-Based Biomaterials

Introduction: Bioresponsive materials that leverage biological stimuli like reactive oxygen species (ROS) have been of increasing interest in localized drug delivery systems. Newly designed biomaterials containing oxidation-sensitive thioketal (TK) linkers have shown promise in localized drug delivery. However, some TK-based systems demonstrate insufficient sensitivity to physiological doses of ROS. As the conventional TK bond is relatively hydrophobic, we hypothesize that engineering TK linkers to contain more hydrophilic pendant groups will yield formulations that are more sensitive to degradation via ROS. In short, increasing the oxidative sensitivity of TK-based materials would improve and expand the potential for localized drug delivery systems.

Materials and Methods: TK linkers were synthesized according to protocols outlined in previous literature.1 Protected cysteamine and TK-forming groups (2,2 dimethoxy propane, levulinic acid, and pyruvic acid) were reacted form respective TK linkers. Products were isolated via column chromatography and confirmed using 1H nuclear magnetic resonance (NMR).

To evaluate TK linker degradation, samples were incubated in varying concentrations of hydrogen peroxide (H_2O_2) and evaluated using NMR. The characteristic TK linker peak was integrated in reference to an internal standard and compared over time.

Results and Discussion: NMR has confirmed the formation and isolation of the DMPTK, LATK, and PATK linkers with predicted hydrophilicity values (Log P) of 2.61, 2.20, and 2.15, respectively. Preliminary degradation kinetics of DMPTK, LATK, and PATK incubated in 10 mM H_2O_2 demonstrated that the more hydrophilic linkers LATK and PATK degraded at an accelerated rate in comparison to the base DMPTK linker. The difference in degradation rates supports the hypothesis that hydrophilic TK linkers are more sensitive to oxidation via ROS.

Conclusions: NMR data indicates that multiple TK linkers can be synthesized and isolated using methods from previous literature. Preliminary degradation studies show promising increases in oxidative sensitivity with more hydrophilic linkers. To further support the hypothesis, additional TK linkers will be synthesized, and degradation kinetics will be evaluated.

Lauren Drake, An Engineered Human Brain Microdevice to Study Propagation of Tau Pathology

Many neurodegenerative conditions, including Alzheimer's Disease (AD) and Frontotemporal Dementia (FTD), are characterized by dense neurofibrillary tangles, which are formed by the microtubule-associated protein tau. Each disease is believed to be caused by a different conformation of neurofibrillary tangle that spreads among neurons and contributes to cell death [2]. However, after decades of research, basic questions about the underlying mechanisms of tauopathies remain unanswered. Most studies of tauopathies have been performed in mice, which are limited in only providing information about endpoint pathology, have limited relevance to human physiology and are of limited utility due to time and cost. Hence, a high-throughput in vitro model, particularly in a human genetic background, would allow for broad exploration and characterization of tauopathies and a better understanding of disease progression. To address this challenge, our lab has developed a triple-chamber microfluidic device that consists of two perfusion channels flanking a central cell culture zone, which contains n-cadherin hydrogel-embedded iPSC-derived neurons. The device inoculates the central cell culture zone with tau on one side through one of the perfusion channels, allowing observation of tau uptake and spread through the neurons. We have observed higher neuron death in aggregation-prone mutant tau monomer (tauP301L)-inoculated

devices compared to PBS control. We analyzed the presence of phosphorylated tau (pTau), a marker of tau aggregation and precursor to tau pathology, in neurons on the opposite side of the device and observed high pTau accumulation in central bodies of neurons. Because this tauopathy phenotype was observed distal to the site of tauP301L exposure, our preliminary conclusion is that the aggregated tau was propagated through synaptically connected neurons in the hydrogel. The tau propagation human engineered microdevice offers an in vitro alternative to in vivo modeling for studying tau propagation through neurons.

Hannah Brien, Engineered Substrata and Cells for Synthetic Morphogenesis of Gastruloids

Gastrulation is the process whereby the epiblast establishes the three germ layers, a process orchestrated by the morphogens WNT, BMP, and NODAL. in vitro models for studying gastrulation leverage pluripotent stem cell (PSC) technology to investigate the interplay between these signaling pathways and the spatial establishment of the germ layers. However, efforts to recapitulate gastrulation in PSC models are hampered by the complexity of a system reliant on temporally gated, locally tuned exchanges. Many current gastruloid protocols rely on media supplementation to recreate morphogen gradients and the use of a basement membrane substratum to ensure PSC adhesion. Here we present our synthetic morphogenesis approach to reconstitute human PSC gastrulation using bioinert cues. Our approach takes advantage of two innovations: (1) a synthetic signaling platform, synNotch, which responds to immobilized ligands to implement spatially constrained gene expression programs and (2) a defined biomaterial substratum that supports PSC maintenance and differentiation with the ability to convert soluble orthogonal ligands into localized inputs that activate morphogenetic programs via synNotch signaling. We engineered three separate H9 hPSC lines to express a GFP-responsive synNotch receptor. In one line, GFP-induced synNotch activation renders overexpression of the reporter transgene mCherry, whereas the remaining two lines express either BMP4 or WNT3A. We also engineered a substratum composed of defined peptides from vitronectin, fibronectin, and N-cadherin to support PSC differentiation potential. Crucially, our substratum incorporates a GFP-capturing motif, GFP-TRAP. Results demonstrate highly efficient synNotch activation (>95%) of PSCs cultured on our defined substrate in multiple media conditions, an outcome not attained with Matrigel or laminin surfaces pre-treated with GFP-TRAP. Furthermore, this activation is sufficient to encourage engineered PSCs to inducibly express Wnt3a and BMP4 transgenes to differentiate into a brachyury and/or kinase insert domain receptor (KDR) positive population, indicative of a transition to an early peri-gastrulation cell state. Ongoing work extends these results onto micropatterned surfaces to deploy synNotch as a platform to coordinate 2D gastrulation.

Ben Hacker, The Wound Healing Response Following Normal Tissue Radiation Drives Triple Negative Breast Cancer Invasion via Macrophage Mediated IL-6 Signaling

The majority of breast cancer patients receive radiation therapy. Although this treatment improves outcomes overall, patients with triple negative breast cancer (TNBC) have a significantly higher risk of distant and locoregional recurrence, especially if they are immunocompromised. Of the many cytokines associated with a radiation response, high serum levels of IL-6 are associated with lower overall survival rates and a poorer prognosis in breast cancer patients. Preclinical models have shown that radiation damage facilitates macrophage recruitment that leads to unresolved inflammation and ultimately recurrence. While it is known that macrophages display a diverse and plastic set of functions in injured tissue, much remains unknown about the influence of macrophage-stromal interactions on the wound healing process and subsequent tumor cell recruitment. We hypothesized that higher infiltration of macrophages with an anti-inflammatory M2 phenotype facilitates tumor cell recruitment through IL-6 signaling after irradiation. In this study, we developed a 3D biomimetic spheroid model of normal tissue injury to investigate the role that macrophages play in dysfunctional healing of radiation-damaged tissue.

First, we analyzed relevant populations and phenotypes of infiltrating macrophages following irradiation of mammary glands from immunocompromised Nu/Nu mice in vivo via flow cytometry. Next, we applied this knowledge to engineer a biologically relevant spheroid model of radiation damage using co-cultures of primary stromal cells isolated from Nu/Nu mouse mammary glands, primary bone marrow derived macrophages, and GFP-labelled murine 4T1 TNBC (4T1GFP+) cells. We used this model to visualize macrophage-stromal interactions by incorporating polarized macrophages in controlled M2/M1 ratios. We evaluated 4T1GFP+ infiltration via live cell imaging over 3 days of co-culture. We quantified IL-6 secretion in conditioned media (CM) from spheroid co-cultures via ELISA, and we investigated 4T1 cell invasiveness in a transwell assay using the CM.

We observed a significant increase in anti-inflammatory M2 macrophages in mouse mammary glands (***$p<0.001$) while the infiltration of pro-inflammatory M1 macrophages remained the same 10d post-20 Gy irradiation, a timepoint that corresponds to tumor cell recruitment following radiation damage. IL-6 secretion in mouse tissues was also enhanced after normal tissue irradiation (**$p<0.01$). In our co-culture model, 4T1s increased infiltration into spheroids with a 2:1 M2:M1 macrophage ratio compared to spheroids with no macrophages present (***$p<0.001$). Finally, we determined that the combination of 2:1 M2:M1 macrophages and irradiation resulted in increased secretion of IL-6 in CM from spheroid co-cultures (*$p<0.05$) and higher tumor cell invasiveness (***$p<0.001$) relative to the unirradiated control. Tumor cell invasion was significantly reduced upon neutralization of IL-6 (**$p<0.01$).

These results establish that direct interaction between irradiated tissue and M2 macrophages drives TNBC cell invasiveness through IL-6 signaling. Our biomimetic spheroid model will be used to further elucidate mechanisms of macrophage-stromal interactions after irradiation to advance the discovery of new biomarkers and therapies to prevent recurrence. This work will have significant implications for immunocompromised TNBC patients receiving radiation therapy.

Emily C. Montgomery, Hydroxyapatite in combination with 2-heptylcyclopropane-1-carboxylic acid counteracts S. aureus planktonic growth and biofilm formation

Hydroxyapatite is a natural mineral component of dental tissue often used in synthetic biomaterials for its bone-like mechanical and chemical properties. Non-antibiotic methods for infection prevention, such as the use of short chain fatty acid signaling molecules like 2-heptylcyclopropane-1-carboxylic acid (2CP), are advantageous due to their structural stability, cell compatibility, and antimicrobial activity. In this study, 2CP was loaded onto hydroxyapatite coupons to assess the effect on microbial planktonic growth and biofilm formation. 2CP was dissolved in ethanol and deposited on hydroxyapatite coupons, allowing the ethanol to evaporate overnight. After 3 days of immersion in phosphate buffered saline (PBS) with daily media changes, Staphylococcus aureus was added to coupons and incubated at 37°C for 24h. The coupons were removed and washed three times in sterilized PBS before sonication in PBS to detach biofilm. Microbial viability for each group was quantified using Bactiter-Glo viability assay, which revealed a significant reduction in planktonic growth, biofilm on the coupons, and biofilm on the well plates with the use of 2CP ($p<<0.001$). This combination of hydroxyapatite and 2CP could potentially be useful for dental applications or in conjunction with ceramic implants such as bone graft substitutes. Future studies will evaluate the effects of 2CP-coated hydroxyapatite on other bacterial strains.

Sushant Prajapati, Delivering Senotherapeutics Using Hybrid Biomembranes for Osteoarthritis

Osteoarthritis is a common joint disorder that affects the diarthrodial joints in the knee, hip, and hand and is predominantly present in people over 50. Current therapies are mainly targeting the symptoms of OA, such as pain, rather than addressing the disease itself. Recent studies indicate that the accumulation of senescent cells in articular cartilage and synovium contributes significantly to OA development and progression. Senotherapeutics are drugs that can reduce age-related pathologies through selective

elimination of senescent cells. However, clinical trials and animal studies show only short-term benefits of these drugs in OA due to their limited intraarticular half-life. Our goal is to enhance the intraarticular half-life of these drugs through homotypic targeting and provide a therapeutic dosage through biomimetic delivery vehicles. Here, we have synthesized hybrid biomembranes (HyB) that can be loaded with senotherapeutics and systemically administered to target OA cartilage. We compared their efficacy for chondrocyte internalization and cytocompatibility with synthetic immunoliposomes (iLips) and pure biomembranes.

The extrusion protocol yielded a homogeneous mixture of iLips (PDI = 0.094, size =145±56 nm) and HyB (PDI = 0. 161, size =346±77 nm). The iLips were more stable in maintaining their unilamellar structure compared to HyB. When cultured with chondrocytes, iLips and HyB were readily internalized, presumably through receptor-mediated endocytosis. However, the HyB were internalized at an 8-fold higher rate than iLips by chondrocytes, indicating the homotypic targeting ability of HyB. It should also be noted that the pure biomembranes without the DSPE-PEG-mal molecules did not show enhanced uptake by chondrocytes or macrophages, indicating the importance of hybrid formulation. In addition, macrophages internalized both the HyB and iLips at a similar rate, indicating non-specific phagocytosis as the primary mode of uptake by macrophages. These results show the importance of vesicle formulations in determining their ability to target different cell types and evade the reticuloendothelial system.

Overall, the synthesized iLips and HyB showed high size homogeneity and cytocompatibility and were readily internalized by chondrocytes and macrophages. Also, our results indicate that HyB are more efficiently uptaken by chondrogenic cells and hence ideal for delivering senotherapeutics to OA joints. These hybrid biomembranes are a potent delivery system that can efficiently eradicate the senescent cells in OA joints and reduce disease progression and cartilage damage.

Rollie G. Mills, Utilizing Household Materials for the Synthesis of a Bio-functionalized Mask for Coronavirus Deactivation

The transmission of coronaviruses primarily occurs through airborne aerosol particles, thus improvements in respiratory face mask and closed-environment filter technology is vital to inhibit the spread of such viruses. Antiviral materials have been a promising option to address this problem and further increase the protection of individuals, but the materials created, such as masks coated in silver nanoparticles, can be toxic to humans. In this research, non-toxic materials (commonly found around a household) were utilized to create novel responsive biomaterials to capture and degrade viral pollutants. For the filtration of viral-sized aerosol particles, commercial water filters were functionalized with a protease enzyme, Subtilisin Carlsberg, that can cleave the spike glycoprotein (SGP) of the SARS-CoV-2 virus, rendering it unable to infect its host. This enzyme is not harmful to humans upon exposure and is commonly found in household laundry detergent. The material synthesis process also incorporated poly (methacrylic acid), commonly used in diapers and known for its water-retaining properties, thus enhancing the longevity of enzyme activity on the membrane surface without the presence of significant hydration. The produced material showed degradation of the SARS-CoV-2 SGP within 30 seconds of exposure time, indicating powerful and quick deactivation capabilities of coronavirus particles. The synthesis of these membranes shows exciting advances in creating the next-generation of non-toxic bio-based respiratory face masks and filtration systems for air remediation. This research is supported by the NSF-RAPID program and by NIEHS.

Emily Fabiano, Vinculin mediates migration and bioenergetics in breast cancer

Cancer cell migration, an early step of metastasis, is fueled by energy released during dephosphorylation of ATP to ADP. However, cellular energy needs in migration are poorly understood. Migration is directed by signals and mechanical anchorage from integrin mediated adhesion to the extracellular matrix. Integrins cluster to form focal adhesions (FAs), which transmit adhesive and traction forces between the cytoskeleton and the extracellular matrix. Thus, FAs are the primary mechanism through which cells

interact with the extracellular matrix, vital for cell migration. Our lab has shown that silencing an FA protein, vinculin, in MDA-MB-231 metastatic breast cancer cells impairs unidirectional migration. Instead, the vinculin deficient cells oscillated when placed in our collagen microtracks that mimic the tracks cell use when metastasizing in the body. This highlights the critical role vinculin is thought to play in affecting cancer cell dissemination with potential ramifications in metastasis, as vinculin is crucial for regulating traction forces and adhesion strength to the extracellular matrix. To more precisely determine how vinculin regulates cell bioenergetics and migration, we are manipulating vinculin expression and measuring cellular metabolic and migratory responses. We hypothesize that with reduced vinculin, the ability of cells to transfer force from the cytoskeleton to the matrix becomes impaired, and cells upregulate their energy to compensate. We demonstrated that transiently silencing vinculin increases energy utilization, suggesting that vinculin may mediate metabolism. We also found that ATP:ADP was significantly upregulated on softer substrates in MDA-MB-231 cells. On stiffer substrates, cells form mature FAs, allowing the cells to adhere. Thus, weakened FAs on softer surfaces correlating with increased energy suggests that cells may be compensating for reduced adhesion ability by increasing ATP:ADP. We generated a vinculin knockout MDA-MB-231 cell line using CRISPR/Cas9 to carry out a more in-depth analysis of the role of vinculin in bioenergetics and migration. Based on migration measurements in 3D collagen, our data suggests that the vinculin deficient population may migrate more slowly through the extracellular matrix. By uncovering the mechanisms that mediate metabolic changes that play a role in cancer cell migration, we can pave the way for a therapeutic avenue by targeting metabolic mechanisms.

Lucinda Pastora, Nanoparticle Delivery of Small Molecule Inhibitors to Treat STING-associated Inflammatory Diseases

The cyclic GMP-AMP synthase (cGAS)/Stimulator of Interferon Genes (STING) pathway is implicated in the development and progression of a myriad of inflammatory diseases including nonalcoholic steatohepatitis, colitis, sepsis, age-related macular degeneration, and cellular senescence. Thus, STING pathway inhibitors (in isolation or in combination with other therapies) could have wide therapeutic application in inflammatory conditions. The cGAS inhibitor RU.521 and the STING inhibitor H-151 have improved outcomes in mouse models of colitis, AKI, and ALS; however, these studies required frequent high-dose i.p. injections of the inhibitors, which limits translatability. Furthermore, long-term use of systemically administered cGAS or STING inhibitors may leave patients vulnerable to viral infections and tumor development. Thus, targeted inhibition of the cGAS/STING pathway may be an attractive, broadly applicable treatment for a variety of cGAS/STING-driven ailments. We hypothesize that targeted, nanoparticle-mediated delivery of RU.521 and H-151 to tissue-resident macrophages can enhance and sustain inhibition of cGAS/STING signaling in the inflamed tissue to slow or reverse progression of cGAS/STING-driven inflammatory conditions, while increasing the time between dosing and improving safety. In this work, we formulate and characterize PLGA and PPS nanoparticles containing RU.521 and H-151. These particles more effectively inhibit cGAS/STING signaling compared to dose-matched free drug in both human monocyte and murine macrophage cell lines in vitro. Treating M0 BMDMs with our inhibitor-loaded nanoparticles decreases M1-like gene expression and cell surface markers when co-administered with a cGAS agonist. In vivo, our nanoparticles decreased cGAS/STING signaling in an artificial model of liver interferonopathy. The therapeutic efficacy of our nanoparticle formulations is currently being evaluated in a murine model of colitis, which is a STING-associated inflammatory disease.

Jessalyn Baljon, A Nanocarrier Vaccine Platform for Co-Delivery of Peptide Neoantigens and Synergistic Adjuvants

Immune checkpoint blockade (ICB) has revolutionized cancer treatment and led to complete and durable clinical responses, but only for a minority of patients. Resistance to ICB can be largely attributed to an insufficient number and/or function of tumor antigen-specific T cells. Neoantigen-targeted cancer vaccines can activate and expand this T cell repertoire, but historically, clinical responses have been poor as

immunity against cancer peptide neoantigens is typically weak. Therefore, we have designed a pH-responsive polymeric nanovaccine that can overcome these barriers in several ways. First, the nanoparticle coordinates delivery of antigen and adjuvant(s) into the same antigen presenting cell and delivers the antigen to the cytosol of the cell, which enhances presentation on MHC class I molecules and the downstream CD8+ T cell response. Additionally, to make this platform more translatable we have developed a new method for neoantigen peptide loading wherein the peptide is anchored to a lipid and inserted post-assembly. Lastly, we explored potential synergy between two adjuvants, cGAMP and MPLA, to further increase peptide immunogenicity. We have successfully formulated these nanoparticles with a cGAMP encapsulation efficiency of 40-50%, an MPLA encapsulation efficiency of 100%, and a peptide insertion efficiency of 50-70%. Our results indicate that the nanoparticle co-loaded with cGAMP and MPLA has a more potent interferon and NF-kB response than nanoparticles loaded with either alone. Additionally, the co-loaded nanoparticle increases expression of dendritic cell activation makers (CD86, MHC-II) and secretion of pro-inflammatory cytokines (IFN-B, IL-6). In a B16.OVA murine model, the nanoparticle vaccine generated antigen-specific T cells, decreased tumor growth, and improved survival. Additionally, the formulation that included MPLA outperformed the formulation with only cGAMP and peptide.

Sumedha Kappagantula, Characterization of Alendronate Poly β-Amino Amide Polycation for Preventing Premature Graft Failure

Decompressive craniotomy, or removing part of the skull to relieve pressure from cerebral edema, is a common procedure in pediatric trauma patients. Removed bone is often frozen for preservation and re-implanted after edema resolution; however, these procedures suffer 50% failure rate in children likely due to overactive osteoclasts resorbing bone. This work describes the creation of a polymeric drug coating featuring a clinical osteoclast inhibitor, Alendronate (Ale), that can be formed on the surface of bone samples to prevent premature graft failure. Here, we synthesized the novel polycation Alendronate Poly β-Amino Amide (Ale-PBAA) through Michael addition polymerization. This polymer features cationic tertiary amines, making it amenable to formation of electrostatic film assemblies through a layer-by-layer (LbL) deposition method.

The monomers Ethylenebisacrylamide, Dimethylene dipipieridine, and Ale were used to synthesize Ale-PBAA (10% incorporation of Ale). The structure and covalent Ale incorporation of the Ale-PBAA polymer was confirmed by 1H Nuclear Magnetic Resonance (NMR) using Ale-specific peaks at approximately 1.7ppm and 2.7ppm, indicating conjugation of Ale into Ale-PBAA.

To assess this polymer's potential as an LbL film constituent, 2mg/mL solutions of cationic Ale-PBAA and anionic Polyacrylic Acid (PAA) in pH-5 acetate buffer were mixed to preliminarily confirm electrostatic complexation. This concentration of Ale-PBAA shows positive results in preliminary testing done with LbL films, a potential applicator source. Through alternation between PAA and 2mg/mL PBAA dip solution for three cycles, the polymer demonstrated that LbL films could form at this concentration. This finding is promising for the intended use of Ale-PBAA as a drug coating for at-risk autologous pediatric bone grafts.

Alexander Sorets, Lipid-siRNA conjugate broadly distributes in the CNS and silences Huntingtin expression following ICV delivery.

The restrictive properties of cerebrospinal fluid (CSF)-brain barriers preclude intrathecal delivery of unmodified biological medicines to deep brain regions, notably siRNAs, which have the potential to restore physiological function in many neurodegenerative diseases. To combat these delivery challenges, we engineered a fully-modified divalent lipid-siRNA conjugate (termed EG18), which distributes broadly in the CNS following intracerebroventricular (ICV) administration. We demonstrate that, unlike free siRNA, EG18 accumulates and is retained in the hippocampus, cortex, and striatum. After transport into the parenchyma, EG18 if effectively uptaken by cells, with preferential internalization in microglia and astrocytes, and to a lesser extent, neurons. Most excitingly, EG18 potentiates silencing of Huntingtin

(mRNA and protein) just one week after ICV injection, whereas an industry-standard cholesterol-siRNA fails to mediate knockdown at equivalent doses. In sum, we developed a novel lipid-siRNA construct that enters the brain parenchyma, is internalized by crucial CNS cells, and silences gene expression broadly. The modular EG18-siRNA delivery platform is a powerful tool for knocking down undruggable disease targets.

Mehjabeen Hossain, Multilayer Polymer Films for Interval Delivery of 5HT2A Agonists

Mental illnesses such as anxiety disorder, post-traumatic stress disorder (PTSD), and major depressive disorder (MDD) impact millions daily. Existing treatments including psycho- and pharmaco-therapy do no work in many (~50%) MDD patients. While new pharmacotherapies such as 5HT2A receptor agonists show significant improvement in treatment-resistant depression (TRD), a rigorous clinical setup is required to administer and many follow-up visits are required, resulting in poor patient compliance. Thus, there is an urgent unmet medical need for improved strategies to ensure patients suffering from mental illness have access to safe and efficacious therapeutics. Here, we seek to address this gap in treatment by developing a polymeric drug delivery device for precise, safe, and long-term interval delivery of 5HT2A receptor agonists to alleviate the symptoms of chronic mental illnesses outside of the clinical setting. We constructed polymer films of different thickness and of different weight ratio mixture (e.g., 70:30, 80:20 and 90:10) of cellulose acetate phthalate (CAP) and Pluronic F-127® (P) polymers, henceforth referred to as CAPP films. These films were then encapsulated with a “model drug” rhodamine or 5HT2A agonist 2,5-Dimethoxy-4- iodoamphetamine hydrochloride (DOI). We eroded these single layer films in PBS at 37° C, and assessed drug release profile of rhodamine (in microplate reader) or DOI (HPLC). Based on this, a multilayered polymeric film device-which had total three drug-loaded (0.1 mm thick, 70:30) film spaced by double blank films (0.4 mm thick, 90:10) was designed and made. In in vitro this multilayered device had three drug release peaks spaced ~50 hours. Whereas, in vivo this was much faster resulting in 2 distinct peaks at 2, at 20 hours within 28 hours of assay. The prototype multilayered film developed here shows promise for achieving consistent interval dosing from a safe and biodegradable material platform that could radically improve compliance to pharmacotherapies for depression and other mental illness.

Samantha Hall, In vivo evaluation of macrophage polarization in response to raspberry ketone-loaded chitosan membranes

Title: In vivo evaluation of macrophage polarization in response to raspberry ketone-loaded chitosan membranes

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Abstract: Nanofibrous electrospun membranes made from chitosan have shown promise for enhanced guided bone regeneration in alveolar defects when insufficient bone volume is present [1]. The nanofibrous structure provides an increased surface area to volume ratio that allows for local drug delivery to stimulate healing. A strategy that can be implemented to facilitate healing is the promotion of macrophage polarization from a pro-inflammatory phenotype (M1) to an anti-inflammatory phenotype (M2). Raspberry ketone (RK) is a natural phenolic compound that possesses antioxidant and anti-inflammatory properties [2]. Previous studies have shown that RK has potential to facilitate macrophage polarization [2]. This study used electrospun chitosan membranes (ESCMs) to locally deliver RK to an in-vivo bone defect site using a rat calvarial model. ESCMs were loaded with 0 (control), 100, or 500 μg RK. Membranes from each treatment group were implanted into rat calvarial defects (n=8). Each

animal received one control and one ESCM loaded with RK. Membranes and surrounding tissues were extracted in serial sections and immunohistochemically stained at 1, 2, and 4 weeks using individual markers for M1(iNOS), M2 (CD206), and total macrophages (CD68). Images of the stained tissues were obtained, and the percent-stained area was quantified using NIH ImageJ. Analysis was performed by a blinded observer. Results indicated that ESCMs loaded with 100 μg RK facilitated the M1 to M2 polarization in comparison to the 500 μg RK or control groups. Therefore, RK shows promise for the promotion of bone healing.

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Sk Arif Mohammad, Synthesis and Characterization of Stimuli Responsive Reactive Azlactone Containing Microgels for Drug Delivery Applications in both In-vitro and In-vivo

For a range of polymeric system applications, the relationship between chemical functionality, molecular design and stimulus-triggered reaction is crucial. Polymers composed reactive functional moieties have long been regarded as significant materials used in wide range of applications including therapeutics, biotechnology and engineering. An easy technique to add stimuli responsiveness in applications that benefit from the reversible integration of small molecules, stimuli responsive deflections, or bioinspired self-healing capabilities is to try to resolve the functional moieties along the chain. Nanoscopic or microscopic length microgels composed of reactive polymers are of special interest because they can be used as delivery vehicles. Therefore, a class of "small but smart" flexible materials known as reactive polymeric microgels is considered to be significant to both fundamental research and practical application. The dynamic amphiphilic block copolymers (BCPs) of poly(2-vinyl-4,4-dimethylazlactone)-block-N,N-dimethylacrylamide (PVDMA-b-PDMA) that we developed here were assembled into micelles included tert-amines. The PVDMA-b-PDMA BCPs spherical aggregates produced by self-assembly in a solvent combination of 2-propanol and THF (v:v = 19:1). To create core cross-linked azlactone-containing microgels, cystamine (Cys) was used to crosslink PVDMA. Non-crosslinked micelles of PVDMA-b-PDMA formed unimers when exposed to THF. However, the Cys-crosslinked BCPs microgels aggregates, this suggests that aggregate microgels or vesicles have formed. Because of their ability to be reactively modified as well as the ability to tailor their stability or disassembly aspects, azlactone-containing BCP microgels provide an exciting platform for applications in a variety of fields, including drug loading for specific delivery to catalysis, imaging etc.

Claire Rowlands, Varying formulation methods to achieve desired diameter of poly(caprolactone) microparticles and nanoparticles to treat disease

Polymeric particles show great promise for their sustained release profiles, tunable size, and loading. These drug delivery systems (DDS) can be used to treat a variety of diseases, requiring different routes of administration, ranging from allergic asthma to cancer. For example, allergic asthma is typically treated with inhaled aerosols requiring particles to be one to five micrometers in diameter while cancer is typically treated intravenously requiring particles ranging in diameter from 100-300 nanometers. Poly(caprolactone) (PCL) is a biodegradable, biocompatible, bioresorbable polymer that has been FDA approved for surgical implants, sutures, and long-term DDS. PCL has been found to be highly tunable, with diffusion dominating short-term drug release, independently of the time it takes to fully degrade the polymer. Two different solvents (chloroform and dichloromethane) along with varying sonication length, surfactant concentration, ratio of PCL to solvent, and ratio of oil to water phase were varied in the double emulsion solvent evaporation method to create different monodisperse nanospheres and microspheres of PCL DDS. Scanning Electron Microscopy (SEM) along with ImageJ quantified particle size and morphology. This method has generated particles as large as 3 micrometer and as small as 300

nanometers indicating we can generate particles suitable for both asthma and cancer treatments. Preliminary in vitro studies have indicated that blank particles do not induce harmful side effects. These results indicate that varying emulsion properties is sufficient at generating DDS with a wide variety of potential applications making this method suitable for advancing treatment in an array of diseases.

Sydney Neal, CELL ADHESIVE AND GROWTH FACTOR PEPTIDE MIMETICS COOPERATIVELY INFLUENCE OSTEOGENIC ACTIVITY OF MESENCHYMAL STEM CELLS IN 3D CULTURE

Regenerative medicine applications of growth factors typically require supraphysiological doses to achieve clinically meaningful benefits. This leads to dangerous off target effects, such as ectopic bone growth and nerve damage caused by recombinant human BMP-2 (rhBMP2). Matrix immobilized growth factor mimicking peptides have been studied as potential substitutes that would prevent off-target effects of growth factors. However, these short peptides often lack the potency of full-length growth factors. During normal development, growth factors work together with ECM to cause tissue formation at much lower growth factor levels. We hypothesize that combining immobilized cell adhesive peptides with growth factor mimetic peptides will enhance the potency of short peptide mimetics of rhBMP2. We grafted cell adhesive cyclo-RGD (cRGD) and growth factor mimetic BMP-2 knuckle epitope (KE) via two orthogonal click chemistries: strain promoted azide-alkyne cycloaddition (SPAAC; for cRGD) and maleimide-thiol for the knuckle epitope (KE) of BMP2 onto alginate polymers. FRET assays on fluorophore surrogates suggest that when KE and cRGD were coupled to the same polymer, their separation was 6.3± 0.7 nm. Clonally derived mouse mesenchymal stem cells (MSCs) were exposed either to rhBMP2 or KE. ALP assays and preliminary RUNX2 staining demonstrated that immobilized KE peptide was comparable to rhBMP2 in osteogenic potency. We also observed a trend towards higher osteogenic potency when cRGD and KE were presented in close proximity to one another by being conjugated to the same polymer molecules.

Alex Bryan, Wound healing analysis of human and porcine placental membranes in rat skin defect model

Abstract: Skin wounds like bed sores, diabetic ulcers, and burns affect more than 8.2 million patients in the United States, with annual Medicare cost estimated to be $90 billion [1]. Human-derived placental extracellular matrices (ECM) have been identified as alternative skin wound treatments due to their biodegradability, anti-immunogenicity, and delivery of pro-healing growth factors but has limited clinical usage due to high cost, low availability, and batch-to-batch- variation [2], [3], [4]. Porcine-derived placental ECM are gaining interest to replace human-derived placental wound treatments due to their low cost, abundance in availability, and low batch-to-batch variation [5].

This study aims to determine the potential for clinical use of porcine-derived ECM wound treatments compared to their human-derived counterparts in a pre-clinical rat skin defect model. Wound healing was evaluated by macroscopic wound perimeter image analysis on days 3, 7, and 14 and histological analyses. 24 Sprague-Dawley rats (12 male + 12 female) had two, 1 cm diameter excisional defects made on the upper back. One wound received either the human-derived placental ECM (FlowerAminoPatch™, Triad Life Sciences, USA) or the porcine-derived placental ECM (Innovamatrix™ AC, Triad Life Sciences, USA), while the other received the opposite placental ECM treatment. Results showed wound perimeter decreased significantly from days 3 to 14 ($p = 1 \times 10\text{-}15$) and no difference was found between groups ($p = 0.82$). H&E-stained tissue samples showed no difference in healing patterns between groups and presence of maturing dermal collagen at day 14. These results suggest that the potential for clinical use of porcine-derived placental ECM treatments is similar to the human-derived counterpart

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Ethan Wales, Evaluation of glutaraldehyde crosslinked chitosan-elastin electrospun membranes for skin wounds

Electrospun, chitosan membranes (ESCM) have seen success for guided bone regeneration applications [1]. Chitosan is a biodegradable, naturally occurring polysaccharide derived from crustacean exoskeleton that has many pro-healing properties applicable to other tissue. This biomaterial can also be mixed with other polymers, like elastin, to improve mechanical properties and bioactivity, increasing its healing capabilities [2]. Specifically, the elastin-polysaccharide nanofiber structure may serve as a template in skin tissue engineering applications. However, when untreated, the chitosan-elastin membrane's nanofiber structure is lost in aqueous environments leading to poor cytocompatibility due to a lack of extracellular matrix mimicking fibers. This has led many researchers to develop post treatments to retain fibrous morphology including amine group neutralization, hydrophobic treatments, and crosslinking [2], [3], [4].

This study explores the usage of glutaraldehyde as a crosslinker to prevent the loss of fibrous morphology of chitosan-elastin electrospun membranes in aqueous environments. After fabrication of electrospun membranes, samples were treated using glutaraldehyde vapor for 24 hrs. All samples were treated with a neutralization wash to quench the residual glutaraldehyde. Characterization included scanning electron microscopy imaging for fiber diameter and morphology, swelling ratio, and cytocompatibility with normal adult dermal human fibroblasts.

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Tibirni Yusuf, Cytocompatibility of Electrospun Chitosan Membranes Treated with Decanoic Anhydride and loaded with biofilm inhibitors and bupivacaine

Cellular responses to loaded electrospun membranes have previously been shown to promote healing in wound applications such as burns by reducing pain, preventing infection, and modifying inflammatory responses through the release of local anesthetics. In this study, we will use RAW264.7 mouse cells, a transformed macrophage-like cell line, to observe expression of inflammatory and anti-inflammatory cytokines and cell response to decanoic anhydride (DA) treated chitosan membranes loaded with therapeutics, both with and without stimulation with Lipopolysaccharide (LPS). RAW cells are generally loosely adherent and simple to detach from a plate, detaching after scraping the remaining adherent cells gently with a cell scraper. LPS is a bacterial polysaccharide that activates this macrophage cell type and drives them into inflammatory phenotypes present in wounded tissue. Macrophages are extremely sensitive to LPS endotoxin from Gram-negative bacteria. We will observe the effect of LPS on the cellular inflammatory response of RAW cells to DA treated chitosan membranes. Electrospun chitosan membranes treated with fatty acid decanoic anhydride were placed in a 24 well plate and loaded with either 0.15mg of anti-biofilm cis-2-decenoic aid (C2DA), 0.5 mg of local anesthetic Bupivacaine, or a combination of the two in ethanol. Each membrane was loaded with a total of 30 µL of solution, except for the unloaded membranes, which served as a control. Membranes will be exposed to RAW cells, both in the presence of LPS and not, to simulate acute response and inflammatory phases of wound healing. We hypothesize that these RAW cells will express inflammatory or anti-inflammatory proteins in the presence

of different concentrations of therapeutics. RAW264.7 cells showed increased IL-10 production after 72 hours in all groups that had high concentrations of C2DA, with minimal production at 24 hours. The RAW264.7 cells also showed increases in TNF-alpha after 72 hours in simultaneous delivery groups delivering low to medium concentrations of the therapeutic molecules.

Dylan Marques, Creation of dual-stage, ROS-responsive microparticles for critically-sized bone defects

Regenerating large-scale bone injuries or defects in the clinic remains particularly challenging. Segmental bone defects of greater than 2 cm are unlikely to heal spontaneously following skeletal stabilization and are thus deemed “critically” sized.1 Critically-sized defects require planned reconstruction. Additionally, there can be many technical and biological challenges in creating a fully functional vascular network within bone grafts. This work outlines an injectable, environmentally-responsive, dual-stage drug release system to controllably deliver multiple therapeutics to promote remodeling and healing of critically sized bone defects. We hypothesize that by doping in Poly(lactic-co-glycolic) acid (PLGA) into poly(propylene sulfide) (PPS) microparticles we can form highly stable particles that maintain reactive oxygen species (ROS) responsiveness, and are able to be coated via a layer-by-layer (LbL) process.

Polymer formulations with high ratios of PPS were consistently less stable in aqueous media when compared to those with doped in PLGA. However, due to the ROS-responsive nature of PPS, formulations with PPS showed greater sensitivity to ROS. These trends align with our hypothesis and will allow us to determine the optimal condition that combines particle stability with ROS-responsiveness. Based on SEM imaging, the particles were determined to be within 5-10 μm, small enough to be injectable while large enough to avoid rapid clearance.3 Using fluorescent microscopy we can confirm the capability of the polymer system to independently load multiple drug payloads. Preliminary data has shown that the formation of stable, ROS-sensitive, dual-stage microparticles is feasible. Testing needs to continue to determine the feasibility of independent dual-stage release, and to establish the optimal formulation that balances ROS-sensitivity and particle stability.

Jacob Thorn, Investigating physicochemical properties of liquid-state asthma drugs

One of the well documented advantages of the ionic liquid (IL) strategy, when applied to active pharmaceutical ingredients (APIs), is the potential for enhanced physicochemical properties of the target liquid-state pharmaceutical (API-IL). Several API-IL systems have been shown to have increased aqueous solubility and transdermal permeability when converted from their standard solid-state into a liquid-state. The research presented here aims to demonstrate the utility of task specific API-IL techniques when applied to asthma drugs. Montelukast (anion precursor) and albuterol (cation precursor) were selected as the pharmaceutically active drugs while choline cation and docusate anion were selected as the counterions due to their low toxicity, FDA approval, and targeted bioactive properties. Choline and docusate ions were paired with albuterol and montelukast counterions in varying molar ratios to produce several novel API-IL systems. The identity and thermal properties of each API-IL were determined using spectroscopic techniques and thermogravimetric analysis, respectively. Once each API-IL was thoroughly characterized, the most promising candidates were chosen to assess their potential transdermal permeability. Transmembrane diffusion studies were conducted using a static Franz diffusion cell apparatus under simulated physiological conditions with phosphate-buffered saline (pH 7.4) and a 0.01” thick silicone membrane barrier. The concentration of each API-IL in the Franz receiver cell at specific time points was determined using HPLC-DAD Analysis. Each API-IL was then evaluated in comparison to its starting material precursor to assess their relative permeability.

Haley Pruitt, Electrospun chitosan membranes loaded with raspberry ketone and simvastatin to stimulate osteogenic differentiation for guided bone regeneration

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Abstract: Bone graft procedures are required when there is significant craniofacial bone loss; however, soft tissue grows faster than bone disrupting the regeneration process. Therefore, guided bone regeneration (GBR) membranes have been used as a barrier between the bone grafting site and soft tissue. Electrospun chitosan membranes (ESCMs) have shown potential in being used as a GBR membrane due to their nanofibrous structure which allows local drug delivery to promote healing.
In this study raspberry ketone (RK) and simvastatin (SMV) were loaded onto ESCM discs that had been modified with one of three different fatty acid (FA) anhydrides: hexanoic anhydride, butanoic anhydride, and acetic anhydride. Combinations of RK (0, 100μg), and SMV (0, 50, 100, 250,500 μg), were loaded onto the membranes (0.5 cm diameter) and the release profiles were examined over 28 days. After the release period was finished, high performance liquid chromatography (HPLC) was used to determine the release rates of RK and SMV. It was found that RK and SMV release could be controlled by which FA treatment the membrane had received and by changing the amount of drug that was initially loaded on the membranes. The longer chain FAs released both drugs over a longer period than the shorter chain FAs, which released the majority of RK in the first few days. Additional research will be done to determine that the amount of RK and SMV loaded onto the ESCM discs did not have cytotoxic effects and to see if they induced osteogenic differentiation.

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Rachel Moen, Developing a scalable iPSC-based platform to improve extracellular vesicle (EV) production per volume and produce tissue specific EVs in Mesenchymal Stem Cells.
Extracellular vesicles (EVs) have widespread therapeutic potential. They can be used to treat a range of diseases such as graft versus host disease, rheumatoid arthritis, and Crohn's disease. Mesenchymal Stem Cells (MSCs) naturally produce therapeutically relevant EVs. However, collecting enough EVs for a therapeutic treatment is resource, cost, and labor intensive. Therefore, there is a need to adapt adherent MSCs to a "pseudo-suspension" culture to grow at the density needed for therapeutic levels of EV production. iMSCs differentiated from induced pluripotent stem cells (iPSCs) are a reproducible non-invasive source of MSCS. Therefore, EVs from iMSCs will not suffer the same obstacles as EVs from primary MSCs such as donor to donor variability or the scarcity of availability human tissue. To do this, first hTERT MSCs are adapted to a pseudo-suspension culture using GelMA-Cad microspheres. GelMA-Cad is a hydrogel that supports MSC growth in both 2D and 3D culture. The GelMA-Cad microspheres are formed using a custom microfluidic device to create consistently sized microspheres to best support cell growth and EV production. MSC growth and EV production has been shown in GelMA-Cad layers and compared to traditional adherent cell growth. EV release from the hydrogels is quantified for yield and particle size. Overall, the production of EVs from iMSCs embedded in GelMA-Cad should lead to higher production per volume and greater reproducibility.

Matthew Atwill, The Combinatory Effect of Raspberry Ketone and Simvastatin on Osteodifferentiation and Delivery via Electrospun Chitosan Membranes for Guided Bone Regeneration Applications
Guided bone regeneration (GBR) is a dental procedure in which a barrier membrane is placed over a maxillofacial defect filled with bone graft material to promote bone growth and prevent soft tissue infiltration. Current GBR procedures may be loaded with bone morphogenetic protein 2 (BMP-2) to supplement bone growth. However, supra-physiological loading of BMP-2 can lead to adverse effects, such as chondrogenesis or inadequate bone growth1. Thus, alternatives are needed to help heal dental

bone defects. Recently, raspberry ketone (RK), a natural anti-inflammatory compound, was shown to stimulate the transition of macrophage phenotype from pro-inflammatory (M1) to pro-healing (M2), leading to increased bone formation when locally delivered from electrospun chitosan GBR membranes (ESCM) in a rat calvarial model2. The anti-cholesterol drug simvastatin (SMV) was also shown to have significant positive effects on bone growth and regeneration when delivered from ESCM in in vitro and in vivo studies3.
The goal of this project is to evaluate the potential for combining RK and SMV for local delivery from an ESCM to promote bone healing. W-20-17 mouse mesenchymal cells were exposed up to 72hrs to combinations of RK and SMV ranging from 0-100μg/mL RK and 0-600ng/mL SMV. Cytotoxicity of the RK-SMV combinations was evaluated using a CellTiter-Glo viability assay. Results showed that SMV concentrations above 300ng/mL were significantly cytotoxic when combined with RK concentrations above 100μg/mL. Ongoing studies are aimed at evaluating non-cytotoxic RK+SMV combinations on the osteodifferentiation of W-20-17 cells based on ALP, osteocalcin and deposition of calcium-phosphate mineral when loaded in culture media and when delivered from ESCM.

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Ghiska Ramahdita, Symmetry breaking of cellular organization and contractile activity in engineered 3D Fibroblast Tissues

Mechanical forces are critical in activating fibroblasts into myofibroblasts during wound healing. Based on our previous studies (Alisafaei et al. Biorxiv 2022), we hypothesized that not only the magnitude, but also anisotropy of forces would be crucial to myofibroblast activation. To test this hypothesis, we used hydrogel-assisted double molding (Simmons et al. BioRxiv 2022) to create poly(dimethyl siloxane) (PDMS) pillars that control the geometry of engineered tissues. We engineered 3D tissues through collagen encapsulation of 3T3-fibroblasts in micro-wells containing either an anisotropic or isotropic arrangement of PDMS pillars. Based on our previous study, we expected anisotropic arrangements (2 or 4-posts arranged in rectangular distribution) to elicit higher myofibroblast activation compared to isotropic arrangement (8 posts in an octagonal arrangement).
Computational models predicted high stress anisotropy within the center of 2-posts and 4-post based tissues, but little to no anisotropy in the center of 8-post tissues. Surprisingly, however, we observed robust upregulation of myofibroblast markers F-actin and α-smooth muscle actin (αSMA) within the center of the 8-post tissues. Microscopic analysis of nuclear and cytoskeletal organization revealed symmetry breaking within the central region of the 8-post tissues. These findings are consistent with a symmetry breaking process that occurs within these tissues, suggesting that regions with high levels of isotropic tension are inherently unstable.

Katie Young, Mechanotypic Genome-scale CRISPR Knockout Screen of Metastatic Ovarian Cancer Cells

For many cancer types, as metastatic potential increases, cell stiffness will decrease. A limited number of genes have been shown to affect cell mechanics, but a genome-wide study of genes that modulate cell biophysical properties has not been attempted. To understand the gene networks that control cell mechanics and their role in the metastatic cascade, we used a high-throughput microfluidic mechanical screen in conjunction with a genome-scale CRISPR knockout (GeCKO) strategy in a metastatic ovarian

cancer cell line to investigate the role of protein-coding genes across the genome in cell mechanics. We transduced Cas9-expressing metastatic ovarian cancer cells with the human CRISPR lentiviral pooled library that targets 19,114 human genes, with 3-4 sgRNAs targeting each gene. There was a significant stiffness difference between cells transduced with the GeCKO lentiviral sgRNA library and non-transduced cells. Using a parallelized, high throughput version of a microfluidic stiffness-based sorting device, we were able to sort the library of single gene knockout cells (150 million cells in total) into five mechanical subsets. We identified 834 genes differentially distributed in the different mechanical subsets as compared to the inlet distributions, with 26 genes of interest that were significantly enriched >100-fold in the stiff outlets, were highly expressed in the cell line, and whose expression resulted in significant change in overall ovarian cancer patient survival. When we used siRNA against genes of interest related to the cell migration and the cytoskeleton, CCDC88A and RACGAP1, we saw an increase in cell stiffness that aligned with the GeCKO screen results.

Blass Watson, A Photopolymerizable Modified Chitosan PEG Bio-ink for use in Additive Manufacturing

The 3D printing of chitosan hydrogels has attracted wide interest because of their excellent biocompatibility, biodegradability, and low cost. [1] Chitosan has been shown to be able to be chemically modified with photo-polymerizable methacrylate group. However, printed chitosan scaffolds lack mechanical strength, limiting their use in tissue engineering. Polyethylene glycol (PEG)-based hydrogels have proven extremely versatile for tissue engineering applications with superb mechanical strength. [2,3] The aim of this work is to create a photopolymerizable methacrylate modified chitosan (N-MAC) and polyethylene glycol dimethacrylate (PEGDMA) bio-ink with improved strength characteristics for use in additive manufacturing.

To make the N-MAC-PEGDMA, chitosan in acetic acid solution was reacted with methacrylic anhydride in a nitrogen environment then neutralized with sodium bicarbonate to make N-MAC. To create a self-supporting bio-ink, N-MAC solution and PEGDMA ratios were varied from 1 wt% to 3 wt% N-MAC and 5 vol% to 25 vol% PEGDMA. To measure photopolymerization time, 365 nm wavelength light was used to irradiate bio-ink and hydrogel formation time was visually observed. One cm diameter cylinder test prints were made using an Allevi 2 Bioprinter. Mechanical characteristics of compression strain, stress and modulus were evaluated using an Instron testing frame.

Results showed a mixture of a 2.5 wt% solution of N-MAC made with DI water containing a 0.2 wt% lithium phenyl-2,4,6-trimethylbenzoylphosphinate photoinitiator and 8 wt% PEGDMA produced a self-supporting bio-ink. Average compressive stress was 62 ± 12 kPa with 25 ± 1.3% compressive strain. Future work will aim to incorporate aligned collagen fibers to further aid in alignment of cells for tissue engineering applications.

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Taylor Sheehy, Polymeric Drug Conjugates for STING Pathway Activation to Improve Cancer Immunotherapy

Cancer remains the second leading cause of death in the United States. Immune checkpoint blockade is revolutionizing treatment of diverse cancer types; however, these treatments only benefit a minority of cancer patients. Low response rates are often correlated to immunogenically “cold” tumors, which lack sufficient tumor antigen-specific CD8+ T-cell infiltration. Therefore, there is a need for therapeutic systems that shift tumors towards an immunogenically “hot” phenotype. One promising strategy is to activate the Stimulator of Interferon Genes (STING) pathway which triggers a type I interferon (IFN-I)-driven

inflammatory response that stimulates dendritic cell cross-presentation of tumor antigens, leading to mobilization of tumor-specific CD8+ T cells to reduce tumor progression and potentially lead to immunological memory. Although STING agonists are currently being explored in clinical trials, most are limited to intratumoral routes of administration, which are not ideal for all patients and tumor types. Systemic, small molecule STING agonists have also been developed; however, these activate STING indiscriminately and can lead to systemic inflammation and toxicity that limits safety and efficacy. Therefore, we aim to develop a tumor-targeted polymeric delivery platform for a chemically-modified STING agonist, suitable for I.V. administration. This will allow for enhanced circulation, tumor-targeting, and environmentally-responsive drug release. A small molecule STING agonist was modified with a chemical linker that allows for chemical conjugation to a delivery platform and intracellular drug release. Specifically, the drug was functionalized to include a cathepsin-cleavable linker for enzyme-mediated intracellular release and a terminal DBCO reactive group for conjugation to free azides on a carrier platform. The prodrug's capacity to activate STING signaling in vitro was demonstrated in IFN-I reporter cell lines, along with ELISAs utilizing primary splenocytes and bone-marrow derived dendritic cells (BMDMs). Its in vivo activity was evaluated in E0771, CT26 and B16 murine tumor models. Polymeric carrier platforms of various molecular weights were synthesized through reversible addition-fragmentation chain-transfer (RAFT) polymerization and characterized through NMR. The polymers' ability to accumulate in the tumor was evaluated through IVIS imaging after conjugating a DBCO-fluorophore via click chemistry and cell uptake was measured via flow cytometry. The same chemistry was utilized to conjugate the prodrug to the platform and reaction efficiency was measured via UV-Vis spectrophotometry and NMR. The conjugate's in vitro activity was demonstrated through IFN-I reporter lines and ELISA and its in vivo activity was measured in an E0771 tumor model.

Julia Strecker, Analysis of calcium sulfate bone graft materials with the incorporation of antibiotics and antifungals

Joint arthroplasty is a surgical procedure that is performed to restore the function of a joint by either replacing, remodeling, realigning it. One of the greatest complications that can take place in joint arthroplasty is periprosthetic joint infection (PJI). Antimicrobials can be delivered locally to these affected areas using dissolvable calcium sulfate beads. These beads provide the non-exothermic environment for the utilization of many antimicrobials.

The aim of this study was to incorporate combinations of antibiotics and antifungals into the calcium sulfate graft materials and measure the release of antimicrobials over 28 days. Antimicrobial release was measured on days 1, 3, 5, 7, 10, 14, 17, 21, 24, and 28 and evaluated with changes in mass, pH, and antimicrobial and antifungal properties of released agents. The study consisted of 16 testing groups that consisted of various combinations of the synthecure calcium sulfate beads, vancomycin, tobramycin, amphotericin B, isoniazid (INH), Cerament, Gentamicin, and Genex. Each group consisted of 35 g of beads and 70 ml of the 25% bovine serum solution and incubated at 37 °C. Every group received a complete fluid refreshment under aseptic conditions on each of the days measurements were collected. The collected solutions were measured for pH and calcium ion release using a pH meter and a commercial calcium assay kit. Antibiotics and antimycotics released were measured using HPLC protocols. Data was analyzed using a two factor ANOVA with post-hoc testing with a level of significance set at alpha = 0.05.

Tian Zhu, The irradiated extracellular matrix promotes breast cancer recurrence

Patients with triple negative breast cancer (TNBC) continue to suffer local recurrence after radiation therapy (RT), and previous work suggests a link between radiation damage and TNBC recurrence under immunocompromised conditions. How radiation impacts the microenvironment, especially the extracellular matrix (ECM) and its role in locoregional recurrence, is unknown. We hypothesize that the radiation-damaged ECM facilitates pre-metastatic niche formation and leads to tumor cell recruitment and

retention. In this study, we developed ECM hydrogels to characterize the influence of RT on breast cancer cell behavior. This work represents a crucial step toward illustrating how modulation of the ECM after RT contributes to breast cancer recurrence.
To explore the influence of the irradiated ECM on breast cancer cell behavior, we fabricated an irradiated ECM hydrogel model to recapitulate in vivo conditions. Murine mammary fat pads (MFPs) were irradiated to a dose of 20 Gy ex vivo. Following 48h incubation, the resulting unirradiated control and irradiated MFPs were decellularized and digested to form ECM hydrogels. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were utilized to characterize ECM hydrogel structure and stiffness, respectively. Luciferase-labeled murine 4T1 TNBC cells were encapsulated within these hydrogels, and cell proliferation was evaluated by bioluminescence measurements. Cytoskeletal organization and invasion of the encapsulated tumor cells were analyzed by phalloidin staining of F-actin and cortactin immunofluorescence staining. We also collected the condition media from 4T1 cells cultured in the ECM hydrogels and analyzed the cytokine secretion by a Luminex multiplex immunoassay. Finally, we co-cultured GFP-labeled 4T1 cells and bone marrow derived macrophages (BMDM) stained with CellTrace Far Red to evaluate the interactions between tumor and immune cells in irradiated ECM hydrogels.

SEM analysis revealed thinner and denser ECM fibers following RT, which may allow for tumor cell adhesion and retention. Irradiated ECM hydrogels also exhibited elevated stiffness compared to unirradiated controls consistent with increased ECM deposition. Bioluminescence imaging confirmed enhanced tumor cell proliferation in irradiated ECM hydrogels ($p<0.01$). We further demonstrated that irradiated ECM hydrogels promote a higher invasive capacity in tumor cells through quantifying cell elongation using F-actin staining. Additionally, tumor cell invadopodia, as determined by the colocalization of F-actin and cortactin, increased in irradiated hydrogels, suggesting that tumor cell invasion is enhanced in the radiation-damaged microenvironment. We observed an increase in granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion following 4T1 tumor cell encapsulation in irradiated hydrogels, suggesting that macrophages influence recurrence after radiation damage. 4T1 cells co-cultured with BMDMs exhibited enhanced proliferation by 3-fold compared to the proliferation of 4T1 cells cultured in ECM hydrogels alone, highlighting the role of immune cells in promoting cancer cell proliferation in the irradiated microenvironment.

Our study examines ECM modulation after RT and establishes that the irradiated microenvironment may promote recurrence post-therapy. We demonstrate the importance of using ECM hydrogels derived from mammary tissue to understand how the breast tissue environment responds to radiation damage. Our results suggest that the irradiated ECM promotes tumor cell proliferation and invasion directly as well as through altering tumor-immune cell interactions. Future studies will develop ECM hydrogels from in vivo irradiated mouse models and further evaluate the role of the ECM in TNBC recurrence following RT.

Alyssa Questell, A Bone Marrow Mimetic 3D Culture Platform for Simulating the Effects of Stiffness on Breast Cancer Metastasis
Breast cancer (BC) is the second most diagnosed form of cancer. Metastasis of breast cancer tumor cells to distant organs remains a significant hurdle in reducing morbidity and mortality. In the majority of metastatic BC cases, metastases form within the bone marrow. Colonization of the bone marrow is highly correlated with mortality, yet regulators of BC cells within the bone marrow microenvironment are not well understood. A growing number of studies highlight the importance of extracellular matrix stiffness in regulating cell behavior. Disseminate BC cells encounter a wide range of stiffnesses when moving throughout the marrow, and the effects of these unique mechanical environments on metastatic progression are unknown. To study BC tumor interactions with the wide-ranging, heterogenous stiffness environment of the bone marrow, a biomimetic, tunable hydrogel for 3D cell culture with defined stiffness

regions spanning the bone marrow's stiffness range has been designed and characterized. Hydrogels of low, medium, and high stiffness are fabricated using Matrigel (4.5 mg/mL) and alginate (10 mg/mL) crosslinked with 5, 10, and 50 mM Ca2SO4, respectively. Alginate-Matrigel hydrogels of discrete stiffnesses are injected in adjacent sub-wells created by a thin-walled insert 3D printed in biocompatible resin. The insert is then promptly removed before gelation to form a gradient. Quantification of the distribution of fluorescent microbeads reveals a high integrity of stiffness interfaces over 7 days of incubation with media. Preliminary studies with GFP-labeled 4T1 murine triple negative BC cells embedded in the substrate suggests biocompatibility, with moderately sized colonies forming by day 7. Interestingly, string-like, elongated colonies were observed forming along the medium to high stiffness transition zone with parallel orientation to the interface. Future work will focus on quantifying migration, invasion, and proliferation of embedded cells to elucidate the influence of stiffness on metastatic progression in a bone marrow-mimetic microenvironment.

Abdullah Al Masud, Optimizing Liposomal Azithromycin for the Treatment of Cardiovascular Diseases

Liposomal azithromycin (L-AZM) is promising immunotherapy for improving cardiovascular outcomes in the context of myocardial infarction (MI). We observed a 50% increase in the survival rate of mice in post-MI upon treatment with L-AZM. Thus, an optimized L-AZM formulation in terms of quality attributes along with a reproducible and high throughput manufacturing process for the liposomes are vital for its transition to the clinic. While our parent formulation is composed of an equimolar mixture of DSPC, DSPG, and cholesterol, it exhibits a moderate level of AZM encapsulation efficiency (30 - 50%) primarily because AZM being comparatively heavy (MW 785) leaks out of the liposomal bilayer. To improve upon the retention of AZM in the bilayer membrane, we adopted an ion pairing approach between the cationic AZM and anionic lipids. The liposomes were made by nanoprecipitation in microfluidics which is well known for reproducible and high throughput manufacturing of liposomes. By optimizing the charge and concentration ratio of AZM and various anionic lipids, the lead formulation is found to be composed of DSPC:DSPG:Chol: AZM (1:1:1:0.5) in terms of mole ratio. Adjusting the microfluidic parameters, i.e., flow mixing rate, and mixing ratio, the lead formulation yields the EE% exceeding 90%, along with an acceptable size (100 – 200 nm) and PDI (< 0.1). A thorough comparison of the lead formulation made by microfluidics yields significantly higher EE% and slower release of AZM compared to the identical formulation manufactured by conventional thin film hydration. An optimized L-AZM formulation backed with positive pre-clinical data would make it a potential candidate for the clinic

Marsalas Whitaker, Optimizing Affinity Purification of Small Vesicles using Bio-functionalized Magnetic Particles

The level of epidermal growth factor receptor (EGFR) activation is reflected in subpopulations of extracellular vesicles (EVs) originating from cancerous tissue that contain EGFR. Such EVs can be assessed using methods like immuno-blots and Flow Cytometry. One pitfall of current EV purification techniques is relying on size and density-based purification of small-EVs that are labor intensive. Additionally, other affinity-based techniques lack the ability to release captured EVs in a non-destructive manner. Here, we developed antibody-conjugated superparamagnetic microparticles with cleavable DNA linkers to enable the capture and non-destructive release of EGFR+ EVs. We utilized DNA linkers using conventional copper-free "click" chemistry synthesis methods to generate cetuximab-DNA (CTX-DNA) conjugates. Linkers contain two unique restriction sites to provide selective labeling and liberation of EVs mediated by BAMHI and ClaI restriction enzyme cleavage. Superparamagnetic microparticles were decorated with different concentrations of CTX-DNA to test conditions leading to efficient purification and release of EGFR+ EVs from complex biological samples. To do this we loaded different amounts of CTX-DNA (6, 3, and 1 ug) on magnetoparticles. Quality control parameters were evaluated (volume yields, degree of labelling DBCO/Antibody and Nanodrop IgG concentrations) and immuno-blot analysis

of released analytes were performed to show method validation of optimized enzymatic cleavage at the BamHI restriction sites that increased the signal of EGFR+ EVs. In conclusion, we will determine optimal conditions for improved efficient EV capture from biofluids with intact EV release thus allowing our group to efficiently capture and label EV subsets for flow cytometric and proteomic analysis. This will provide an efficient alternative to ultracentrifugation that allows specific interrogation of EV subsets containing unique analytes for which we have affinity reagents, such as EGFR antibodies.

Oluwaseyi Shofolawe-Bakare, ROS-responsive, Glycopolymeric nanoparticles for enhanced drug delivery to macrophages

Macrophages play a prominent role in the pathogenesis of a variety inflammation-related diseases (asthma, Crohn's disease, cancer) and are therefore an important therapeutic target for these diseases. Therapies that modulate macrophage activity have shown promising results, however, they are plagued by poor pharmacokinetics and off-target toxicities that hamper their efficacy. Drug delivery platforms that can improve the transport these therapeutics to macrophages are therefore essential to improve the efficacy of these drugs. Here, we synthesized poly (propylene sulfide)-b-poly(methacrylamidoglucopyranose) (PPS-PMAG) diblock copolymer-based nanoparticles that can target macrophages through polymeric glucose that forms the corona and selectively release drugs in response to oxidative species. This smart drug delivery platform shows the potential for enhanced delivery to macrophages.

Madison Bates, Identifying Hyperglycemic Microenvironment Determinants of Cancer-Associated Fibroblast Immunosuppressive Phenotypes

Cancer patients with a history of diabetes experience worse prognosis than nondiabetic cancer patients. Hyperglycemia, characteristic of the diabetic microenvironment, increases the presence of reducing sugars, such as glucose, which stiffen tissue by non-enzymatically crosslinking collagen fibers. Tissue stiffening is known to promote tumor progression by inducing aberrant cell behavior through altered mechano-signaling. Cancer-associated fibroblasts (CAFs) are a mechanosensitive and heterogeneous cell population within the tumor microenvironment thought to play major roles in regulating tumorigenesis and metastasis by matrix remodeling and immunosuppression. Three CAF subtypes have been identified within human samples of luminal A/B and triple negative breast tumors based on relative expression of CAF biomarkers: fibroblast specific protein 1 (FSP1), alpha smooth muscle actin (a-SMA), and fibroblast activation protein (FAP), where the immunosuppressive CAF subtype expresses all three biomarkers. Our preliminary data indicate that mammary tumors from diabetic mice have decreased infiltration of CD8+ cytotoxic T-cells compared to nondiabetics. As such, we hypothesize that a hyperglycemic microenvironment shifts CAFs to an immunosuppressive phenotype. In order to parse the relative effects of hyperglycemia from hyperglycemia-induced tissue stiffening on CAF phenotype, we utilize engineered matrices with tunable mechanical properties to examine the effects of soft (1 kPa) and stiff (20 kPa) substrates in the presence of normoglycemic (5 mM) or hyperglycemic (25mM) glucose levels on CAF biomarker expression in 3T3 fibroblasts. Our results indicate that hyperglycemic glucose levels work synergistically with substrate stiffness to increase the expression of CAF biomarkers associated with the immunosuppressive CAF subtype. This work has the potential to provide a mechanistic understanding of how diabetes shapes the biochemical and mechanical cues that govern immunosuppressive CAF phenotype shift and provide potential therapeutic targets to recover immune function in diabetic cancer patients.

Kristopher M Castillo, **Nanomedicine Drug Combinations for the Treatment of Tumor-Induced Bone Disease**

Tumor-induced bone disease (TIBD) is a collection of morbidities including osteoporosis, fractures, and pain arising from bone metastasis. The Gli2 transcription factor plays a pivotal role in regulating TIBD

through transcription of parathyroid hormone-related protein which promotes osteoclast activity and bone resorption. Release of growth factors from the bone matrix further potentiate tumor growth, creating a vicious cycle of bone degradation/cancer invasion. Gli2 additionally promotes chemoresistance and is highly upregulated in cancer stem cells populations. GANT58 has been identified as an inhibitor of Gli2 capable of inducing cell cycle arrest in vitro and blocking TIBD in vivo. Paclitaxel (PTX) is the first line treatment for bone metastasis, but is dose limited by bone marrow side-effects (neutropenia). Herein, we evaluate the combination of PTX and GANT58. We hypothesize that co-administration of synergistic GANT58/PTX ratios will improve overall therapeutic effect by preventing TIBD, PTX-resistance and PTX side-effects. PTX-alone potentially kills parental MDA-MD-231 cells (IC50 = 0.003 uM) but bone-metastasized (bone-clone) cells, which upregulate Gli2 are highly PTX-resistant (IC50 = 10 μM). GANT58-alone was able to kill both parental and bone-clones with an IC50 ranging from 12.76-15.88. GANT58/PTX combinations demonstrated strong synergy when GANT58 was above a concentration of 1 M, with an optimal drug-ratio of 10:1 GANT58:PTX. We have further confirmed that GANT58 and PTX can be coloaded into polysulfide-based micelles with loading capacities (LCs) above 35 wt.% and at ratios that reflect their feed ratio. In summary, GANT58/PTX combinations are highly synergistic and can be co-loaded at ultrahigh LCs into micellar nanocarriers.

Kate Reardon, Engineered Muscle Constructs to Elucidate the Effects of Inflammation on Muscle Atrophy

Skeletal muscle atrophy is a common and debilitating condition associated with various inflammatory disorders, including rheumatoid arthritis, chronic obstructive pulmonary disease, and sarcopenia. Although acute inflammation is essential to promote muscle regeneration after injury, a dysregulated inflammatory response can lead to muscle loss and atrophy. The myopathic effects of inflammation, primarily arising from elevated levels of interferon-g (IFN-g), have been identified as a significant contributor to muscle dysfunction and atrophy. To study the underlying mechanisms of IFN-g-mediated muscle dysfunction, we have engineered 3D muscle constructs that mimic skeletal muscle biology. We fabricated the 3D constructs using natural matrices that enable myoblast differentiation and myobundle formation. Our goal is to elucidate underlying mechanisms of IFN-g-mediated muscle dysfunction and identify therapeutic targets to address inflammation-induced muscle atrophy. Here, we investigated the effects of IFN-g treatment on muscle phenotype through gene expression studies and immunofluorescence analyses.

To fabricate muscle constructs, a custom 12x12 mm PDMS well was made and lined with a thin felted nylon fabric (1-2 mm in width) at the edges. Then, hydrogels (collagen, fibrin, or collagen+fibrin) containing myoblasts were cast on the well. The nylon fabric enabled the matrix to anchor and facilitate the directional alignment of differentiating myoblasts. All hydrogel formulations supported multinucleated myotube formation within 5 days of serum starvation. However, collagen matrices formed a more robust muscle construct with denser myotubes that remained intact for more than a week without detachment. Fibrin matrices supported relatively lower myotube formation and often contracted within 5 days, presumably due to fibrinolysis. Hence, collagen constructs were used to investigate inflammation-induced atrophy. When collagen constructs were treated with the inflammatory cytokine IFN-g, most of the myotubes stayed intact, and no significant morphological differences were noticed compared to the untreated control. But the gene expression analysis showed that there was downregulation of Myog, a marker for myogenesis, and anabolic myokines like glucose transporter type 4 (Glut4), a significant contributor to glucose homeostasis, and follistatin (Fst), a signaling molecule that promotes muscle growth. The IFN-g treatment also resulted in the upregulation of Il15, a pleiotropic myokine that can cause muscle atrophy. Overall, the IFN-g treatment resulted in a muscle phenotype consistent with muscle atrophy seen in chronic inflammatory diseases.

Overall, our in-vitro collagen constructs supported multinucleated myotube formation and enabled us to study the effects of inflammation on skeletal muscles. Our studies show that IFN-g-treatment disrupts skeletal muscle phenotype mainly by downregulating the expression of anabolic myokines, which can lead to muscle atrophy. Our muscle constructs are a valuable tool for investigating inflammation-induced muscle atrophy and can help identify therapeutic targets to ameliorate inflammatory myopathies in the long term.

Kyra Smart, Glycation-Mediated Matrix Stiffening Due to Diabetic Hyperglycemia Promotes Tumor Progression Independent of RAGE Signaling

Cancer cells preferentially use glucose as an energy source, but knowledge of the impact of the hyperglycemic microenvironment on cancer progression is not complete. Glucose can crosslink tissues through non-enzymatic glycation resulting in a stiffer extracellular matrix (ECM). Our lab has shown that ECM stiffness alters cellular energetics to increase invasion and proliferation. Here we show that hyperglycemic ECM stiffening increases tumor burden. A mouse model of diabetic breast cancer was created from the MMTV-PyMT transgenic line. Non-enzymatic glycation due to sugars was assessed by quantifying the concentration of advanced glycation end products (AGEs) in diabetic and non-diabetic tumors; diabetic tumors showed increased glycation and cancer cell proliferation. This effect was preserved even in experiments where implanted cells lack the receptor for AGEs (RAGE). Diabetic mice with orthotopic RAGE-knockdown tumor cells also exhibit enhanced tumor growth, increased tumor stiffening, and an increase in migratory cell phenotype when compared to nondiabetic mice. Here we parse apart the contributing factors of the diabetic tissue microenvironment to establish a mechanistic role in promoting cancer progression.

Anastasiia Aronova, Glutathione triggered swelling and degradation of disulfide-containing poly (β-amino esters) hydrogels for Drug Delivery Applications

Oxidative stress is a ubiquitous pathophysiological process that occurs in concert with the inflammatory pathway. During this process, highly reactive free radicals partake in a series of chemical reactions with cellular components and biomolecules, substantially damaging them, and further affecting the function of tissues as organs. Glutathione (GSH) is a tripeptide that plays a critical role in scavenging and neutralizing free radicals and serves as a cofactor for multiple enzymatic systems. Commonly found in the cytosol, GSH also serves as a thiol buffer maintaining sulfhydryl groups of many biomolecules in their reduced form. We have previously developed disulfide-containing PBAEs as a means of regulating and responding to oxidative stress. However, while these materials were shown to serve as a protective agent, we have not evaluated the direct interaction of these materials with GSH. In this work, we demonstrate that these hydrogels are sensitive to physiological levels of glutathione (GSH), a key component in the regulation of cellular oxidative stress, and can be used to achieve tunable release in the presence of varying GSH concentrations. Consequently, these hydrogels might be advantageous for oxidative stress treatment and prophylaxis.

Cameron McHargue, The effect of exercise mimetics on inflammation-induced muscle atrophy in Type-2 diabetes

Type II diabetes (T2D) is a common metabolic disorder associated with chronic low-level inflammation throughout the body. Exercise training has been shown to have beneficial effects in improving symptoms of T2D, reducing the associated chronic inflammation, and increasing cellular uptake of glucose. Although, the underlying mechanisms are not well understood. Our long-term goal is to elucidate these mechanisms and identify novel therapeutic targets that can mimic the effects of exercise training in reducing chronic inflammation and muscle wasting. Here, we have developed 3D muscle constructs to investigate the effects of different exercise regimens (Endurance (END) and High-Intensity Interval

Training (HIIT)) on myokine secretion and evaluated their protective effects on muscle phenotype under chronic inflammatory conditions and hyperglycemia.
The myoblasts seeded in collagen matrices formed muscle constructs with visible multinucleated myotubes within 3-5 days of serum starvation. When the contructs were subjected to 1-hour bouts of a cyclic linear strain of 10% at 1/30 Hz (HIIT) for 6 hours, we saw a significant upregulation of the anabolic myokine irisin (Fndc5, $p=0.013$). But, no significant changes were seen when stimulated for shorter periods. When the constructs were subjected to a continuous cyclic linear strain of 2% at 1/30 Hz (END) for 6 hours, there was a significant increase in the expression of Il15 ($p=0.014$), which is a pleiotropic myokine that can cause muscle atrophy. These results show that HIIT is more conducive to promoting muscle hypertrophy than the END exercise regimen. Then we investigated the change in muscle phenotype under inflammatory conditions.
When the muscle contructs were treated with IFN-γ, a significant downregulation of Fndc5 and upregulation of Il15 was noticed, which is consistent with muscle atrophy. When the constructs were subjected to IFN-γ treatment followed by a HIIT regimen, a significant downregulation of most myokines, including Fst, Fndc5, Igf1, and Fgf2, and upregulation of Il15 was noticed when compared to control samples subjected to HIIT alone. This indicated that HIIT training does not provide protective effects in the presence of IFN-γ, in the tested settings, against muscle atrophy but may exacerbate the pathology.
Overall, our in vitro model enabled us to study the effects of different exercise regimens on muscle atrophy seen in T2D. Further, under hyperglycemic conditions, the HIIT exercise regimen is more conducive to promoting muscle hypertrophy than END. But, when combined with chronic inflammation, the beneficial effects of HIIT may be reversed. Together, a combinatorial exercise and anti-inflammatory treatment approach may offer a therapeutic avenue for diabetic muscle atrophy.

Jamie Ahmed, Erodible Thermogelling Hydrogels for Localized Mitochondria Delivery to Spinal Cord Injuries

Spinal Cord Injury (SCI) is considered a real health concern to human body. Not only due to the seriousness of physical damage that it causes to neural and motor functions but also the series of cascading events following the primary physical trauma poses additional complications. The leakage of cytosol and blood from ruptured neurons and blood vessels cause ions imbalance and excitotoxicity leading ultimately to the loss of neuronal functions and cellular death. The delivery of mitochondria to the site of SCI proved to be a viable solution to this issue, however. Mitochondria transplantation improves cellular bioenergetics and reduces concentration of reactive oxygen species achieving homeostasis and neuroprotection. Nonetheless, keeping mitochondria viable outside cell environment for anytime longer than a few minutes, the time required for transplantation, proves to be challenging. In addition, localized delivery to the injury site is also limited by other factors including flow of cerebrospinal fluid that washes away mobilized organelle and drift them from the SCI site. In this work, we overcome these challenges using an erodible system of thermogelling delivery. The combination of methyl cellulose, which possesses reverse thermogelling properties, and hyaluronic acid hydrogels proves to be viable solution to the aforementioned challenges. Hyaluronic acid-methyl cellulose hydrogels (HAMC) allow for controlled release around the SCI site utilizing the reverse thermogelling property of MC which allow mitochondria to be released in a pace that increase their absorption into affected neurons. We have used ultraviolet visible spectroscopy to measure optical density of hydrogels are they solidify at elevated temperature. Differential scanning calorimetry was also used to study hydrogels phase change. Fluorescence microplate reader is utilized to study dye-labeled hydrogel release over time. Finally, seahorse assay was used to study released mitochondria respiration and viability.

Emily Berestesky, Sorting of 4T1 Metastatic Breast Cancer Cells Based on Metabolism

Cancer cells exhibit a distinct change in their metabolism compared to healthy cells, which is thought to aid in tumor progression and metastasis. Even in the presence of oxygen, cancer cells have an increased

dependence on glycolysis for ATP generation, rather than the more efficient energy generation method of mitochondrial oxidative phosphorylation. While differences in the metabolism of cancer and healthy cells point to a potential therapeutic avenue, the plasticity of cancer cells to adapt their metabolism to their environment proves to be an obstacle in identifying key molecular targets. Our goal is to sort metastatic 4T1 murine breast cancer cells based on their NADH:NAD+ ratios to observe how metabolic heterogeneity affects their behavior and ability to metastasize. The Peredox probe is a genetically-encoded fluorescent probe that allows for real time measurement of the cytoplasmic NADH:NAD+ ratio in live cells as a metric for glycolysis. Using human MDA-MB-231 metastatic breast cancer cells expressing the Peredox probe and fluorescence activated cell sorting (FACS), we sorted cells into populations of high and low NADH:NAD+ ratios. However, because the MDA-MB-231 culture media contains a high glucose concentration and the probe signal is highly dependent on glucose, the probe signal was saturated and no detectable differences were observed between the sorted populations. To overcome this challenge, we looked to the 4T1 cancer cell line as glucose levels in culture media are lower and allow for more variability in the probe. Further, since FACS requires cells to be suspended in a sorting media for the sorting process, the media formation has to be optimized to result in the smallest fluctuations in the probe's readings for an heterogenous population of cells. Once cells have been stably sorted into high and low NADH:NAD+ expressing subpopulations, cells will be characterized based on their ability to migrate in 2D culture and on collagen scaffolds, and to metastasize in a mouse model. Sorting based on metabolic profile has the potential to parse apart the roles of metabolic pathways in breast cancer metastasis.