Oral Presentation – Cell-Biomaterial Interactions

Cherie Stabler- Professor, Plenary Speaker
Email: CStabler@bme.ufl.edu
Affiliation: University of Florida
Title of Talk: Modulating Immunological Responses to Cellular Transplants for Type 1 Diabetes

Roxanne Glazier – PhD Candidate
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Title of Abstract: Investigating Integrin Mechanics in Podosomes Using Fluorescence Spectroscopy
Presenting Author: Roxanne Glazier
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Complete Author List:
Roxanne J. Glazier (1); Joshua M. Brockman (1); Khalid Salaita (1,2) All Author Affiliations:
Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University (1); Department of Chemistry, Emory University (2)
Abstract:
Receptor spatial organization and molecular mechanics work together to drive cell decision making in a variety of biological processes, including immune cell activation, cancer invasion, and bone resorption. An important feature regulating these phenomena is substrate stiffness. Despite the influence of material mechanics on cell signaling, few tools exist to image receptor tension and clustering on fluid substrates. Existing technologies suffer from low signal to noise ratio and probe multivalency. To address these problems, we developed Molecular Tension- Fluorescence Lifetime Imaging Microscopy (MT-FLIM) for mapping piconewton receptor forces at the cell surface. Here, MT-FLIM was used to study integrin tension in podosomes, protrusive

adhesive structures. FRET-based DNA tension probes were attached to a supported lipid bilayer. Each probe consisted of a folded DNA hairpin strand hybridized to two dye-labeled DNA strands: a donor-labeled ligand strand and an acceptor-labeled anchor strand. Resting probes were closed, with quenched fluorescence and short fluorescence lifetime. Upon integrin binding, probes were dragged and clustered, which increased local density and intensity. Integrin tugging opened the hairpin, yielding a massive increase in both fluorescent intensity and lifetime. Importantly, FLIM allowed us to unambiguously distinguish between hairpin opening and probe clustering. MT-FLIM provides new capabilities to untangle the interplay between receptor clustering and mechanics at fluid interfaces.
Using MT-FLIM we mapped integrin tension and clustering in podosomes. Podosomes play a key role in bone resorption and antigen sampling; they also share many structural elements with invadopedia in metastatic cancer. Although podosomes typically form in monocytic, endothelial, and smooth muscle cells, their formation is also triggered by fibroblast spreading on fluid scaffolds. In podosomes, a densely crosslinked actin core surrounded by a ring of accessory proteins drills into the substrate. Our work provides the first direct evidence that podsomes not only push on their substrate, but they also pull. In addition to elucidating the mechanism of podosome force balance, our study provides broad insight into the mechanics of integrin adhesion to mobile ligands. MT-FLIM will be useful in probing a wide array of cell-cell and cell-material interactions in immunology, developmental biology, and cancer biology.

Rapid Fire Talks

Kristen Parratt– PhD Candidate
Email: kparratt4@gatech.edu
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Title of Abstract: Screening Microfabricated Biomaterials Using Flow Cytometry

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Abstract:
To study how materials control cell function, samples should be standardized across all conditions. This reduces experimental variability which exists across technical and biological replicates. At the same time, the number of replicates should be large such that the statistical power of studies is high. This will result in more reproducible data and define small differences between samples. Currently, many methods to study this rely on arrays spotted on glass slides and microscopy-based assays. However, these methods are difficult to make high replicate and high throughput, while also enabling multiplexed analyses.
Flow cytometry, which is used primarily for the study of cells in suspension, can be modified to instead analyze cell-containing microparticles fabricated from biomaterials of interest. Flow cytometers automate the collection of thousands of individual events in a short period, which can be combined to characterize the entire population and collect data with high statistical power. Inherent heterogeneity between individual cells can result in a range of responses from the population and therefore high replicate number will better characterize the population. Microparticle shape, size, and fluorescence were used as variables and a rapid assay system was developed for the study of material-encapsulated cells. These variables were used to barcode encapsulating materials such that samples could be pooled for culture and analysis.
The high-throughput screening platform was demonstrated using a popular model system; poly(ethylene glycol) diacrylate hydrogels and human mesenchymal stem cells (hMSCs). PDMS mold arrays of >10^4

replicates were fabricated and hydrogel microparticles were collected in suspension. The ImageStreamX MarkII flow cytometer was used to investigate test microparticle barcodes. Test populations consisted of three sizes (20, 40, and 60 μm length) and four shapes (square, right triangle, circle, and equilateral triangle). A data handling template was designed in IDEAS software to separate the different barcodes. True members of each population were selected from collected images and image features that gave the greatest separation between groups were identified. The accuracy of the gating scheme was evaluated to be greater than 73% for all experimental groups consisting of 40 and 60μm lengths. Cells were encapsulated in microparticles and cultured. Microscopy was used to determine the cell loading distribution and viability.

Christian Macks– PhD Candidate
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Title of Abstract: Combinatorial Therapy of Rolipram and pNGF for Traumatic Brain Injury
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Abstract:
Traumatic brain injury (TBI) continues to represent one of the leading causes of disability and death following injury. The primary injury, a direct result of the traumatic event, is accompanied by an inflammatory response. The expression of several key potentially neurotoxic cytokines can be reduced by increasing levels of cyclic adenosine

monophosphate (cAMP). Rolipram (Rm), a phosphodiesterase inhibitor, prevents the degradation of cAMP and is able to inhibit production of potentially neurotoxic cytokines and also lead to increased axonal regeneration. Exogenous nerve growth factor (NGF) has neuroprotective functions and can reduce edema following primary injury. We developed a novel polymeric carrier system combining the delivery of Rolipram and plasmid DNA encoding NGF (pNGF) to neural cells present at the injury site. This carrier will be formed from amphiphilic copolymers composed of poly(lactide-co-glycolide)-g-polyethylenimine (PgP). PgP was synthesized from branched PEI (25kDa) and PLGA (4kDA) and characterized by 1H- NMR. The transfection efficiency of PgP was evaluated using pBLAST44-hNGF (pNGF, (InvivoGen), 2ug/mL) in C8-B4 (ATCCŒ¬ CRL-2540) in the presence of 10% serum at N/P ratios 25/1 and 30/1. The NGF concentration was determined by ELISA assay and cytotoxicity using MTT assay. Rm was loaded in PgP micelles (Rm-PgP) by solvent evaporation method and loading efficiency was measured by high performance liquid chromatography (HPLC). Neuron survival, neurite length, and cAMP level of primary rat cerebellar neurons (CBNs) was assessed under a hypoxia condition (95% Nitrogen and 5% CO2) as an in vitro TBI model. The cells were treated in vitro with Rm-PgP (10ug Rm/10 ŒµL) after 24 hours in hypoxia and then returned to hypoxia incubation for an additional 24 hours. CBN cells in normoxia were used as a positive control. Neurite projection length was measured using ImageJ software after staining cells using antibody against neuron-specific beta-III tubulin, and cAMP level was evaluated via ELISA assay (R&D Systems). An in vivo controlled cortical impact (CCI) TBI model was generated and Rm-PgP was injected (16ug Rm / rat) locally following injury. The tissue was harvested 1 day after and cAMP level was analyzed via ELISA. Our results demonstrate that the PgP is a promising carrier for pNGF to C8B4 microglia cells in 10% serum condition with persistent elevation of NGF expression over a course of 120 hours. We successfully demonstrated that after Rm-PgP treatment

both neurite length and the cAMP level were increased above untreated primary rat CBN cells cultured under hypoxia. Following Rm-PgP treatment, the cAMP level in the in vivo TBI model was successfully restored to sham animal levels. Future work will be to evaluate the synergistic effect of Rm-PgP/pNGF polyplexes on the in vivo rat CCI TBI model.

Michelle Gaines– Postdoc
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Title of Abstract: Controlling the Adhesion of Microgels Particles Adsorbed to a Surface for Improved Biomaterial Function
Presenting Author: Michelle Gaines
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Abstract:
Platelet-Like-Particles (PLPs) are polymer n-isopropylacrylamide (pNIPAM) microgel particles, coated with a fibrin-specific antibody fragment, which allows them to function as artificial platelets. The functionality of PLPs depends on the microgel particles ability to bind and contract the surrounding fibrin matrix, thereby forming clot. These biomaterials have demonstrated wound-trigger homeostasis and have reduced bleeding time due to strong fibrin-binding and contraction in vitro. This work further explores how the materials properties of the pNIPAM microgel particles influence surface adhesion. Our hypothesis is that soft, deformable microgel particles will spread more along an adhesive surface than stiff microgel particles, which will thereby increase the binding strength between the particles and the surface,

compared to stiffer particles. The first step in this work is to analyze the adhesion between pNIPAM microgel particles and a functionalized hard surface by employing the well-characterized biotin/streptavidin system. pNIPAM microgel particles are biotinylated and presented to adhere to a streptavidin-functionalized polystyrene bead. Particle stiffness is modulated by titrating in N-Methylenebis(acrylamide) (BIS) crosslinking agent, during microgel particle synthesis. Atomic Force Microscopy (AFM) is used to characterize the intermolecular interaction forces between the two surfaces by measuring the force between surface of a polystyrene bead, glued to the tip of an AFM cantilever, and a microgel particle. The adhesion between pNIPAM microgels functionalized with albumin bovine serum (BSA) proteins and BSA-functionalized polystyrene beads were first used as a baseline experiment, where no adhesion is expected. In-liquid force curves were collected on the samples, where the modified-cantilever (BSA-polystyrene bead) approached the BSA-microgel surface, indented the surface 12 nm, and then retracted. The force curves associated with the approach and retraction were measured and reflected that no adhesion had taken place between the BSA-pNIPAM microgels and the BSA-polystyrene beads. Now that a baseline has been established, the work will move on to analyzing the adhesion and binding strength between biotinylated pNIPAM microgel particles and streptavidin-polystyrene beads. The impact adhesion has on particle stiffness will also be included in the study by creating microgel particle arrays with (0.1-2) molar %BIS crosslinking agent. In the future, the work will move toward characterizing the adhesion between fibrin surfaces and PLPs. These studies will provide design guidance for synthesizing PLPs, as well as other soft biomaterials whose functionality depends on adhesion strength.

Oral Presentation – Rationally Designed Biomaterials

James Dahlman – Professor, Faculty Presentation
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Title of Talk: Barcoded Particles

Taylor Kavanaugh – PhD Candidate
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Title of Abstract: ROS Scavenging for the Prevention of Post-traumatic Osteoarthritis
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Abstract:
Osteoarthritis is characterized by the degeneration of cartilage, bone, and other tissues leading to chronic joint pain and debilitation. Post-traumatic osteoarthritis (PTOA) occurs after a traumatic injury to the bone or soft tissue, including ligament and meniscal tears, and there is currently no cure. Reactive oxygen species (ROS) are elevated at sites of joint injury and can cause cell-damaging and pro-inflammatory oxidative stresses that propagates the tissue degenerative process of PTOA. We hypothesize that local, sustained activity of Tempol, a powerful superoxide dismutase (SOD) mimetic, from microparticles comprising poly(propylene sulfide) (PPS), a powerful H2O2 sponge and catalase mimetic, can prevent the onset of PTOA after a joint injury. We developed a polymeric form of Tempol (polyTempol) through the use of reversible addition-fragmentation chain-transfer (RAFT) polymerization. Co-polymers of Tempol and dimethylacrylamide (DMA) were synthesized to decrease the hydrophobicity of polyTempol with the goal of increasing Tempol bioavailability while maximizing

antioxidant potency. DMA:Tempol co-polymers at ratios of 50:50, 75:25, and 90:10 have increasing water solubility. DMA:Tempol 75:25 and 90:10 reduced ROS by 30% using a cell free dihydroethidium (DHE) assay. PolyTempol was encapsulated in PPS microparticles via oil-in-water emulsion; The combination of polyTempol and PPS is intended to mimic the functions of the catalase-SOD system and provide comprehensive scavenging of multiple ROS.Œæ Measurement of intracellular ROS in stimulated RAW 264.7 macrophages using DCFDA dye showed that DMA:Tempol 75:25 scavenges 84% while DMA:Tempol 75:25-PPS microparticles scavenge 97% of intracellular ROS. These data confirm a synergistic effect of our PPS carrier and polyTempol.
Using an established mechanical loading model of PTOA in mice, we delivered polyTempol-PPS microparticles via intraarticular (IA) injections. MMPSense 750 and a collagen II specific antibody (MabCII 680) are used to determine the progression of OA. Microparticles reduced MMP activity and MabCII 680 binding by 40% and 31% respectively following IA injections in mechanically induced PTOA. PolyTempol-PPS microspheres are able to effectively reduce ROS in vitro and reduce cartilage damage in an in vivo model of PTOA. We have shown that Tempol and PPS work synergistically, mimicking physiological processes performed by superoxide dismutase and catalase to control oxidative stress.

Rapid Fire Talks

Bryan Dollinger – PhD Candidate
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Title of Abstract: PPS-based ABC Triblock Polymer Thermo-Hydrogels Protect Pancreatic Islets from Cytotoxic ROS
Presenting Author: Bryan Dollinger
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Abstract:
Transplantation of primary islets or engineered glucose-responsive/insulin-producing cells represents a potential route for curing type 1 diabetes. However, transplanted cells suffer from poor survival post-transplant due to suboptimal cell implant sites, host immune response, and innate production of high levels of cytotoxic reactive oxygen species (ROS). We hypothesize that islet encapsulation within a cytoprotective, ROS-absorbing hydrogel will promote islet survival and function. To test this hypothesis, we recently developed the ABC triblock polymer poly[(propylene sulfide)-block-(N,N-dimethyl acrylamide)-block-(N-isopropylacrylamide)] (PDN). The hydrophobic PPS block drives polymeric self-assembly into micelles at room temperature in aqueous solutions. It also reacts irreversibly with ROS such as hydrogen peroxide (H2O2), providing an ROS-sensitive hydrogel degradation mechanism, and imparting inherent anti-oxidant properties to the system. The hydrophilic PDMA block ensures swelling/hydration of the resulting hydrogel, while PNIPAAM triggers a thermally-induced transition of micelles into a supra-assembled 3D hydrogel at ~34Œ_C. We've previously demonstrated that this hydrogel protects fibroblasts from cytotoxic levels of H2O2Œæin vitro, and herein we have further explored this gel's capacity to protect murine '_-cells and primary human islets.
A control PCL-PDMA-PNIPAAM polymer was also synthesized to form thermoresponsive hydrogels without inherent anti-oxidative activity. MIN6, mouse-derived '_-cells, were formed into pseudo-islet aggregates (PIs). PIs were: (1) embedded in 5 wt% PDN Œ± 0.3 wt% type 1 collagen (T1C), (2) embedded in 5 wt% PCL-triblock (ROS-inert)

hydrogels ± 0.3 wt% T1C, or (3) cultured directly on tissue culture polystyrene (TCPS). The PIs were cultured ± 100 Œµm H2O2 to mimic in vivo ROS levels post-transplant, and relative cell number was measured over 5 days. Also, primary human islets were seeded onto T1C and either: overlaid with (1) + 0.3 wt% T1C, (2) + 0.3 wt% T1C, or with no overlay and cultured Œ± 100 µm H2O2 for 48 hours
PIs cultured in normal cell media showed no significant difference in relative cell number between material treatments, indicating the non-cytotoxic nature of these hydrogels. When treated with 100 µm H2O2, PIs embedded within the PPS hydrogels ± T1C showed approximately two-fold greater relative viability in comparison to the control PCL hydrogels, demonstrating that PPS provides protection against cytotoxic H2O2. Similarly, in primary human islets, PDN + T1C maintained approximately two-fold greater relative viability in 100 µm H2O2 in comparison to controls. Therefore, PDN is a promising vehicle for retention and protection of delivered cell therapies due to its ability to protect cells from cytotoxic ROS.

Jessica Petree – PhD Candidate
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Title of Abstract: Building a Nanozyme for RNA Gene Therapy
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Abstract:
Transcribed RNA must be processed through the splicing of introns and joining of exons to produce the mature messenger RNA (mRNA)

sequence for protein synthesis. Errors in this process result in 60% of disease-causing genetic mutations. Current technologies to correct mutant RNA include ribozymes and hijacking of the spliceosome to perform novel splice steps. These techniques suffer from off-target effects, instability and difficulty in delivery to cells. Therefore, there is a need to develop novel RNA gene therapy techniques to correct splicing errors. To address this problem, we developed a nanozyme platform that is capable of excising segments from RNA. This multifunctional nanozyme consists of catalytic DNA (DNAzymes) and a ligating enzyme (RtcB) attached to a gold nanoparticle (AuNP) scaffold. To perform splicing, two DNAzymes are designed to bind a target RNA, cleaving specific phosphodiester bonds and allowing RtcB to reattach the two exons, generating a corrected RNA. In principle, this technique obviates the need for genetic engineering and solves the delivery problem, as DNA-functionalized AuNPs are readily taken up by mammalian cells. Nanozymes are also protected against degradation by sequestration on the AuNP, allowing for a longer half-life in vivo.

To demonstrate proof-of-concept for this nanozyme platform, active RtcB and DNAzymes were shown to cooperatively splice a 20 bp intron from an RNA/DNA stem-loop in vitro with a 40-60% yield. Splice product of the correct size was observed using polyacrylamide gel electrophoresis (PAGE). DNAzymes were then assembled on AuNPs via 3-thiolated poly-thymidine linkers. DNAzyme-AuNP conjugates were also active for splicing in the presence of excess soluble RtcB. Work is ongoing to couple all three enzymes to a single AuNP and show splice activity. The next phase will be to introduce the complete conjugates into mammalian cells to determine splice efficiency. This work provides a new tool for RNA manipulation and correction in vitro and inside cells. Potential applications include synthetic editing of RNA, correction of mis-splicing diseases and analysis of protein structure/function through alternative splicing events.

Thomas Werfel – PhD Candidate

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Title of Abstract: Optimizing Architecture and Hydrophobic Content of RNAi Polyplexes Enables Selective mTORC2 Therapy In Vivo
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Abstract:
The PI3K/Akt/mTOR signaling cascade is dysregulated in over 60% of breast cancers across all three major clinical subtypes, where there is mounting evidence that mTORC2 plays a unique role in driving tumor cell survival and motility. Although mTORC1/2 dual kinase inhibitors exist, no current therapeutics selectively inhibit mTORC2 while sparing mTORC1. Here, specific mTORC2 therapy was enabled by genetic inhibition of the mTORC2-specific co-factor Rictor using potent siRNA nanopolyplexes with ternary architecture and hydrophobic content optimized through a combinatorial approach. Screening of 30 ternary nanopolyplex formulations yielded a lead candidate that was small and stable in size (~100 nm), resistant to cargo unpackaging in the circulation, and able to overcome endosomal delivery barriers through pH-dependent membrane disruption. The lead ternary nanopolyplexes

exhibited greater blood circulation time, reduced renal clearance, increased tumor biodistribution, and greater silencing of the model gene luciferase compared to our previously-optimized, binary parent formulation following intravenous delivery. The optimized nanopolyplexes containing Rictor siRNA significantly decreased mTORC2 activity and tumor growth in HER2+ breast cancers through induction of cell death. Combination therapy of Rictor siRNA with the HER2 tyrosine kinase inhibitor lapatinib resulted in greater inhibition of Akt, increased tumor cell killing, and reduced tumor growth as compared to either agent alone. These collective data demonstrate that optimized ternary nanopolyplexes can effectively modulate targets previously considered undruggable and underscore the therapeutic potential of blocking mTORC2 in PI3K/Akt/mTOR dysregulated cancers.

Oral Presentation – Biomaterials for Tissue Regeneration

Jeoung Soo Lee – Professor, Faculty Presentation
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Title of Talk: Multi-functional Polymeric Micelle Nanocarriers for Central Nervous System Regeneration

Liane Tellier – PhD Candidate
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Affiliation: Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University
Title of Abstract: Localized SDF-1 Delivery Promotes Endogenous Cell Recruitment to the Supraspinatus Muscle After Severe Rotator Cuff Injury
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Affiliation: BME
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W.H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University (1); Department of Orthopaedics, Emory University School of Medicine (2); Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology (3).
Abstract:
Degeneration of the supraspinatus muscle after severe rotator cuff tear correlates with increased re-tear rates. Thus, methods to promote muscle healing may significantly improve clinical outcomes.Œæ The hypothesis of this study was that stromal cell-derived factor-1a (SDF-1a) delivered via heparin-based microparticles to the supraspinatus muscle after severe rotator cuff injury would significantly enhance the recruitment of pro-regenerative cell populations to the injured muscle.

Rotator cuff injury was induced in rats through transection of the rotator cuff tendons and the suprascapular nerve. Microparticles were fabricated via free radical polymerization with 10 wt% N-desulfated heparin methacrylamide and 90 wt% poly (ethylene glycol) diacrylate and subsequently loaded with 60 ng SDF-1a.Œæ Immediately following injury, the supraspinatus muscle was injected with no microparticles (injury-only), unloaded microparticles, or SDF-1a-loaded microparticles. 7 days later, flow cytometry was used to quantify cell types recruited to the muscle (N=6).

More fatty infiltration was identified in the injured supraspinatus than the uninjured contralateral control 42 days post-injury, indicating that muscle degeneration was induced in our model. Furthermore, compared to unloaded microparticles and injury-only controls, significantly more total leukocytes (CD11b+, 5.75Œ±1.14 fold change over uninjured contralateral), total macrophages (CD11b+CD68+,

6.8Œ±0.23), anti-inflammatory macrophages (M2, CD11b+CD68+CD163+, 5.79Œ±0.67), and MSCs (CD29+CD44+CD90+, 4.78Œ±0.84) were recruited to the supraspinatus muscles containing SDF-1a-loaded MPs 7 days post-injury.

This study indicates that SDF-1a-loaded microparticles are able to recruit significantly more pro-regenerative cells, specifically M2 macrophages and MSCs, to the supraspinatus muscle after severe rotator cuff injury, which may lead to enhanced muscle regeneration and improved clinical outcomes.

Rapid Fire Talks

Joshua Walters – PhD Candidate
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Title of Abstract: Partially Restored Kinematics of Spinal Units Following the Repair with Novel Annulus Fibrosus and Nucleus Pulposus Biomaterials
Presenting Author: Joshua Walters
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Department of Bioengineering, Clemson University (1); Department of Orthopaedic Surgery, Spartanburg Regional Healthcare System (2);
Abstract:
Annually, over 5.7 million Americans are diagnosed with intervertebral disc (IVD) pathologies;1 IVD herniation and/or degeneration (IVDD). These conditions detrimentally alter the nucleus pulposus (NP) and annulus fibrosus (AF), and compromise the functional spinal unit (FSU) kinematics. Thus, biomaterials developed to repair these tissue structures must above all function mechanically to re-establish FSU

kinematics while resisting herniation. Our lab has previously developed novel, acellular biomaterials for the repair of both the AF and NP regions of the IVD.2,3 These materials have been characterized previously; however, they have yet to be assessed in tandem for their ability to restore in situ kinematics of FSU's. Thus, the objectives of the research were to: 1) evaluate the maximum burst strength of the AF repair patch biomaterial when attached to an FSU, and 2) assess the ability of both the NP and AF biomaterials to re-establish FSU kinematics following discectomy.

Herein, testing was completed on FSU's (vertebrae-IVD-vertebrae) harvested from 2 caudal levels (cc2-3 and cc3-4) of skeletally mature bovine tails obtained from a local abattoir. Following the excision of superficial tissues, the FSU's were potted in urethane resin for mechanical evaluation. AF patch burst strength was evaluated in accordance with ASTM D3786/D3786M with minor modification. A 6.25mm annular defect was created via drill bit followed by the attachment of an AF patch over the defect using 4-0 FiberWire suture at the four corners (sample size n=6). The FSU was then secured in a custom designed apparatus and tested on a servo-hydraulic testing frame using a 300mm/min displacement rate. Biomechanical testing of FSU's were evaluated based on previous literature.4äóñ6 Testing was performed on an electromechanical test system. Potted FSU segments (n=5) were submerged in a saline/protease inhibitor solution and tested consecutively within four treatments: Intact, Annulotomy (6mm biopsy punch perforating AF), Discectomy (removal of all NP), and Repair (NP replacement and AF patch). The axial testing regime of each specimen began with a 1-hr. creep period at maximum peak compression (0.5 MPa) followed by 35 tension-compression cycles (peak stresses of 0.25 and 0.5 MPa, respectively). Samples were then loaded to their mean amplitude load before a constant rate slow ramp to maximum peak compressive stress. Tensile and compressive stiffness were calculated using the slope of a linear fit of the force-displacement curve from 60-100% of the peak load during the unloading phase of the

35th cycle. Axial range of motion (RoM) was calculated as the total peak-to-peak displacement. Neutral zone length was determined using a third-order polynomial equation to fit the cyclic load-displacement data and the derivative of the equation was taken as described previously.5 The constant rate slow ramp compression stiffness was calculated using the slope of a linear fit of the slow ramp load-displacement response. Creep data was analyzed using a non-linear constitutive model fit to the data as described previously.5 Short-term and long-term creep time constants were derived from constitutive model coefficients using. All statistical comparisons were performed in relation to intact conditions using a one-way analysis of variance (ANOVA) with significance denoted as (p

Elda Trevino – PhD Candidate
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Title of Abstract: Heparin-based microparticles for release of protease inhibitors after rotator cuff tear
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Abstract:
Rotator cuff tears (RCT) can lead to the development of osteoarthritis in the shoulder. Cathepsin proteases are thought to be partially

responsible for cartilage degeneration characteristic of osteoarthritis. Cystatin-C is the endogenous protein inhibitor of cathepsins that can interact with heparin (Hep) via electrostatic interactions. The goals of this project are 1) characterize arthritic changes in cartilage following RCT in an animal rat model and 2) develop an injectable heparin-based microparticle system to inhibit cathepsins.
Adult male rats (n=8) underwent suprascapular nerve denervation and infraspinatus/supraspinatus tendon transection. Twelve weeks post-operation, humeral heads were treated with 10% Hexabrix (Covidien) and scanned (45kVp, 200ŒµA, 600ms) via contrast-enhanced microCT (ScancoMedical). For cystatin-C release, fully desulfated heparin (Hep-) and PEG-diacrylate particles were used as controls that should interact less with cystatin-C. Microparticles were fabricated via water-in-oil emulsion and release (n=3) was tracked over 7 days.
In 4 out of 8 injured shoulders, focal defects (average: 0.12Œ±0.09 mm^3) were observed, whereas no focal defects were found in control samples. Hep- microparticles loaded significantly more cystatin-C than the PEG control (PEG: 35.4±8 ng, Hep: 50.4±12.7 ng, Hep-: 64.3±8.3 ng). However, after 7 days, heparin microparticles released significantly more cystatin-C (PEG: 6.28±1.48 ng, Hep: 9.86±1.18 ng, Hep-: 3.69±0.10 ng).
Cartilage loss indicates the rat model can replicate osteoarthritic changes seen following RCT in humans. Hep microparticles released the most cystatin-C, possibly due to their ability to prevent protein denaturation during release. Hep microparticles achieved sustained released of cystatin-C over 7 days, making this a potential injectable intra-articular therapy to preserve cartilage following RCT.

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Title of Abstract: Improving Neural Stem Cell Transplantation in Stroke with a Sulfated Chondroitin Sulfate Glycosaminoglycan Hydrogel Carrier

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Abstract:
Ischemic stroke is a debilitating disorder that affects millions of people each year, yet there are limited successful treatments. Stem cell transplantation has emerged as a potential regenerative treatment for brain injury. However, suboptimal cell survival after transplant and poor differentiation hinder current cellular therapies. Sulfated chondroitin sulfate glycosaminoglycans (CS-GAGs) are an integral component of the brain extracellular matrix (ECM) and are known to regulate endogenous neural progenitor cells (NPCs). We hypothesize that CS-GAG hydrogels can be used to enhance stem cell transplantation following ischemic stroke, leading to improved cellular and behavioral outcomes compared to traditional cell transplantation. We employed a mouse stroke model to study the effectiveness of CS-GAG hydrogels as NPC carriers in stroke. Adult C57BL/6 mice were subjected to focal cerebral ischemia targeting the sensorimotor cortex. To study the stability of the gel in vivo, we injected 2Œµl rhodamine-infused hydrogel into the infarct core 1 week after stroke and monitored it over 6 months. To evaluate the efficacy of the hydrogel in transplantation, 200k induced pluripotent stem cell-derived NPCs in 2Œµl medium or hydrogel were injected into the stroke core 7 days

after stroke. Behavior was tested before and 1 week after infarct, and weekly after transplantation. Brains were processed for immunohistochemistry. Positively-stained cells or blood vessels were counted and glial scar width was measured using ImageJ. Statistical analysis was performed using GraphPad Prism 6.0.
Gross and microscopic images of brains injected with rhodamine-infused hydrogel revealed that it is stable in the stroke core up to at least 8 weeks. Blood vessels appear to infiltrate the gel and it is highly cellularized. Immunostaining of mice brains injected with cell + hydrogel showed that the average glial scar width and vascularity surrounding the stroke region were not significantly affected compared to cell-only treatments. Latency to fall in a rotarod motor function test was significantly greater in cell + hydrogel treated animals after transplantation compared to mice with cell-only injections. Furthermore, mice treated with the gel tended to have fewer sensory deficits as observed in the corner test.
CS-GAG hydrogel cell carriers are stable in the stroke core and improve sensorimotor outcomes after ischemic insult. Further experimentation will clarify the mechanisms of improved functional recovery seen in hydrogel + cell treated stroke mice. Ongoing studies will explore the utility of CS-GAG hydrogels in promoting endogenous neuro-regeneration, retaining growth factors in the stroke region, and increasing transplanted stem cell survival.

Oral Presentation – Biomaterials for Immune Modulation

Julie Champion – Professor, Faculty Presentation
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Title of Talk: Protein Assembly to Create Therapeutic Materials

Rikhav Gala – PhD Candidate
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Title of Abstract: Needle less Measles Vaccine Delivery: Preclinical Evaluation of Oral Disintegrating Films in a Juvenile Pig Model
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Abstract:
Measles is a highly contagious infection that is caused by the measles virus. It mainly affects children and can be fatal as well. The disease causes about 100,000 deaths every year worldwide although it is completely preventable by vaccines. It affects people primarily in the developing areas of Africa and Asia causing the most vaccine-preventable deaths of any disease. Since the primary targets of this disease are children, we aimed to formulate an oral vaccine that would prevent the use of needles making the vaccine more patient-compliant. The oral

cavity of the mouth is covered by a lining that is rich in immune cells. These immune cells help the body to distinguish between harmful and harmless foreign material entering the body through the mouth. Oral Disintegrating Films (ODF) are films that dissolve when placed in the mouth. These films can be an inexpensive and an effective means to deliver drugs/vaccines orally without the use of needles. On dissolution, the microencapsulated vaccine antigen will be recognized by the immune cells in the mouth and further processed to produce protective antibodies against measles virus. Later, whenever the body is exposed to the virus, the protective antibodies present will be capable of combating the measles infection. The goal of this study was to evaluate the potential of Oral Disintegrating Films (ODF) loaded with measles vaccine nanoparticles as a viable immunization strategy. In this preliminary study, 2 pigs were used in the in-vivo model in order to evaluate the immunogenicity of the measles vaccine formulation when administered via the buccal route using ODFs.

Methods:

A 1% w/v solution of sterile BSA in sterile water was prepared and cross-linked overnight with glutaraldehyde. Excess glutaraldehyde was neutralized with sodium bisulphite the following day. Live attenuated measles virus (antigen) and Aluminium Hydroxide (adjuvant) were added to the solution and spray dried using a Buchi Spray Dryer B-290. These nanoparticles were then incorporated in the ODF. The film solution was prepared at room temperature by incorporating the film forming polymer (Lycoat RS720) in an aqueous

plasticizer solution under continuous mixing for 10-15 minutes until the suspension was monodisperse. The measles vaccine nanoparticle powder was then added to this suspension. The film formulation was cast using a BYK- Gardner, mechanical drive Resource I equipment and dried air dried. The measles ODF vaccine was tested in-vivo in 2 pigs by delivery via the buccal route. Blood serum samples were collected every 2 weeks and a specific ELISA was performed to quantify the amount of specific antibody present.

Results:

The size of the vaccine nanoparticles was in the range of 400 to 1200 nm. Using the pre-gelatinized hydroxypropyl pea starch, Lycoat RS720, we obtained the desired film strength and disintegration properties. Upon immunization of pigs, we observed a 5-fold increase in the antibody titers after 2 weeks. This increased to a 7-fold increment after 6 weeks which proved the efficacy of the vaccine. There was a significant increase in the specific antibody against measles virus as seen after 2 weeks of dosing and remained elevated until the end of the 6 week study period.

Conclusion:

The buccal delivery of ODF loaded with vaccine nanoparticles is a promising immunization delivery system. These encouraging preliminary results, will lead the way for further research in this area.

Rapid Fire Talks

Christopher Johnson – MD/PhD Candidate
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Affiliation: Georgia Institute of Technology
Title of Abstract: Lysostaphin-presenting Biomaterial Reduces Staphylococcus aureus Infection and Improve Bone Healing in a Mouse Model for Bone Repair
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Abstract:
Over one million medical device associated infections occur each year, with infection often leading to device failure and removal. In the US, nearly 112,000 orthopedic device infections occur annually, with Staphylococcus aureus being the most common pathogen, motiving the development of bifunctional materials that promote regeneration and prevent failure due to infection. We have recently engineered proteolytically degradable poly(ethylene) glycol (PEG) based hydrogels that deliver BMP-2 and the integrin specific adhesive ligand GFOGER, to promote bone repair in a critical-sized mouse radial segmental defect. However, few robust animal models exist allowing for controlled and reproducible infections in the context of bone repair. We have extended the mouse radial segmental defect model to include an infection component with the bioluminescent strain of Staphyloccocus aureus, Xen29. Lysostaphin is an anti-staphylococcual enzyme produced by the bacteria Staphylococcus simulans that is

recombinantly expressed in E. coli. Lysostaphin functions as a glycine-glycine endopeptidase cleaving the petna-glycine linkages in the peptidoglycan cell wall of Staphylococcal species leading to lysis. This is advantageous in that the enzymes efficacy is not dependent upon the metabolic activity of the target organism in the way traditional antibiotics are. We set out to engineer a lysostaphin delivery system to reduce infection in our mouse radial segmental defect model. PEG hydrogels functionalized with the adhesive ligand GFOGER and cross-linked with the cross-linking peptide VPM were synthesized. Hydrogels with and without lysostaphin were contaminated with of S. aureus Xen29. A 2.5mm segment of the mouse radius was removed and hydrogels were implanted. After seven days, the mice were taken down and assayed for viable bacteria. The results indicated a four log reduction in recovered viable Xen29 in the lysostaphin group compared to the no treatment control. To evaluate the potential for these materials to improve bone healing, PEG hydrogels functionalized with GFOGER, carrying of BMP-2, with or without lysostaphin were implanted. After 8 weeks, uCT reconstructions demonstrated improved healing in the lysostaphin treated group. Furthermore, all lysostaphin treated hydrogels were clear of pathogenic bacteria. This demonstrates that lysostaphin therapy can eliminate bacteria and improve bone repair in our mouse infection model.

Matthew Lawler – Undergraduate
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Title of Abstract: Investigation of immunomodulation on myofibroblast activation: implications for biomaterial

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Abstract:
The implantation of biomaterials such as hydrogels or extracellular-matrix derived scaffolds elicit a foreign body response (FBR) often characterized by immune cell recruitment and fibrous encapsulation. Macrophages are highly involved in FBR and wound healing, and express either an inflammatory (M1) profile, promoting cell debris clearance and early tissue regeneration, or express an anti-inflammatory (M2) profile, being involved in the later stages of tissue regeneration. In the M2 spectrum of macrophages, there are a range of diverse phenotypes, which can be classified most succinctly by cytokine secretory profiles, and encompass the M2a and M2c subtypes. Key contributors to fibrous encapsulation are myofibroblasts, which secrete Type I collagen to facilitate wound heading but also can cause fibrosis if there is persistent myofibroblast activity. Fibroblasts can differentiate into myofibroblasts in response to transforming growth factor-beta (TGF-beta), among other signaling pathways. While it is clear that both macrophages and myofibroblasts are involved in wound healing, the interplay between the two cell populations and its contribution to fibrosis has not been thoroughly investigated. In vitro experiments of M1, M2a, and M2c macrophage

phenotypes with 10T1/2 fibroblasts were conducted, with 10T1/2s incubated in macrophage-conditioned media for 72 hours. Expression of alpha-smooth muscle actin (alpha-SMA) was probed via immunofluorescence techniques since high alpha-SMA expression is indicative of myofibroblast activation. Through confocal microscopy and image intensity analysis, it was determined that fibroblast interaction with M2a and M2c soluble factors lead to significantly higher levels of alpha-SMA expression within fibroblasts. 10T1/2 fibroblasts that were exposed to M1 macrophage media exhibited similar levels of alpha-SMA expression to those that interacted with no macrophages. Therefore, controlling the macrophage populations present after biomaterial implantation may be used to reduce fibrous encapsulation and scarring. Ongoing research is underway investigating contact dependent interactions between macrophage subsets and fibroblasts.

Alexandra Tsoras – PhD Candidate
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Title of Abstract: Oncofetal Antigen Cancer Vaccines in Peptide Nanocluster Form Have Enhanced Immunogenicity

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Abstract:
Oncofetal antigen (OFA) is a protein found on on many cancer cell surfaces including myeloma, lymphoma, myeloid leukemia, and some solid tumor cells, yet it is not expressed on most normal cells. OFA has been shown to have multiple immunogenic epitopes. Similarly to how bacterial or viral vaccines are made, proteins unique to cancer cell surface markers can be used as antigens in cancer vaccines to enhance the immune system's ability to kill cancer cells. A unique 9-mer peptide epitope on OFA, known as OFA2, is currently being investigated as a vaccine along with soluble adjuvants. The goal in this work is to improve the delivery and immunogenicity of OFA2 by packaging it as a nanoparticle. Nanoparticles provide benefits to vaccines by increasing stability and retention in tissue as well as allowing higher uptake rates owing to the nanoparticles' natural adjuvancy and tunable sizing to preferred DC uptake ranges and higher amounts of presentable antigen available for every particle internalized. To enable particle fabrication from OFA2, it was modified with the addition of one cysteine onto the C-terminus, making OFA 2C. Desolvation was used to make nanoclusters ~200 nm in diameter, consisting of OFA 2C crosslinked with a thiol crosslinker. Peptide concentration, solvent:desolvent pairings and volume ratio, crosslinker type and concentration were among the parameters optimized in nanocluster synthesis. 200 nm was the target size to maximize dendritic cell antigen

recognition and uptake as well as to minimize passive diffusion away from the injection site before uptake by antigen presenting cells can occur. A reversible thiol crosslinker was used to maintain the immunogenic epitope sequence after intracellular processing and breakdown of the nanocluster. OFA 2C nanoclusters are shown to have increased DC uptake in vitro in comparison to OFA 2C peptide administered in soluble form as well as successful DC activation and surface presentation of internalized peptide nanoclusters. In vivo, the nanoclusters are retained 2.7-fold more in an intradermal injection site (an area high in localized immune cell uptake and trafficking) than the injected soluble peptide. Fully processed peptide from nanoclusters may result in a better presentation to T-cells by APCs than typically seen in loose presentation of non-processed peptides, and this will be investigated in future studies. The peptide nanocluster formation of immunogenic peptide OFA 2C holds potential to increase the efficacy of OFA cancer vaccines.

Jordan Green – Professor, Plenary Speaker
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Title of Talk: Engineered Biomaterials for Biomimicry: Gene Delivery Nanoparticles and Artificial Antigen Presenting Cells

Posters

Kimberly Gomes – PhD Candidate
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Title of Abstract: Immunological Assessment of a Transdermal Human Papillomavirus Micro Particulate Vaccine

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Abstract:
Clinical trials of currently available prophylactic Human Papillomavirus (HPV) vaccines demonstrated high efficacy in preventing cervical disease associated with vaccine type HPV. These vaccines have great potential to reduce cervical cancer worldwide, particularly in low-income countries that have the highest burden of disease and no screening. However the high costs associated with the vaccine and with administration (need for cold-chain and 3-dose injections by trained personnel) are significant barriers to adoption in the areas of the world that are in most need of the vaccine. By contrast, transdermal vaccine delivery combined with micro particulate HPV antigens are simple to administer and could avoid the need for cold-chain.
Purpose: The purpose of this study was to develop a micro particulate HPV 16 virus-like particle (VLP) vaccine and assess its immunogenicity in transdermal delivery using a pre-clinical mouse model.
Method: HPV-16 L1/L2 VLPs were produced in human embryonic kidney cells 293TT. The VLPs were incorporated into a biodegradable polymer matrix with the addition of an infrared dye, indocyanine green and two FDA approved adjuvants, Alum and Monophosphoryl Lipid A. The formulation was sprayed using a Buchi B-290 spray dryer. The

micro particulate vaccine was characterized for size and charge using the Malvern Zetasizer. The size distribution and surface morphology of the micro particulate vaccine was established using Phenom Pure Desktop ¬ scanning electron microscopy (SEM). To determine encapsulation of VLP, transmission electron microscopy (TEM) and Western Blot analysis were used. For immunogenicity evaluation, female Balb/c mice (6-8 week old) were given 4 doses of the vaccine using AdminPatch¬ transdermal delivery: 1 prime dose given at week 0 and 3 booster doses given at weeks 2, 4 and 8. . One group received solution of HPV-16 VLP and the second group received micro particulate HPV-16 vaccine. Blood samples were collected prior to dosing at week 0 and at weeks 5, 7, 10, 12, 14, 20, 24, and 28. Antibodies were measured using a direct HPV-16 VLP based IgG ELISA. At week 40, spleen and lymph node will be collected and analyzed for memory B and T cells.
Results: The micro particulate vaccine yield was found to be 95.7 % w/w with size range of 2.0-4.0‘_m and zeta potential around -22.1 mV. The VLP encapsulation efficiency was 87% based on Western blot detection of HPV-16 L1 protein. TEM showed VLP conformation integrity was sustained after spray drying. The antibody levels were found to be approximately 9-fold higher in the solution group in the early weeks after dosing. In later weeks, specifically from week 12-28, the micro particulate vaccine group showed higher antibody titers, compared to the solution vaccine group.
Conclusion: The transdermal delivery of a micro particulate HPV-16 VLP vaccine was capable of producing a robust antibody response, higher than that observed in the solution group. The current study utilized a high dose of VLPs in the microparticulate vaccine. Future studies will evaluate dosing amounts and further characterization of the immunological response.

Kameron Kilchrist – PhD Candidate
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Affiliation: Vanderbilt University
Title of Abstract: Elucidation of the Delivery Mechanism of MK2 Inhibitory Peptide Nano-polyplexes for Improving Long-term Vascular Graft
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Abstract:
Electrostatic complexation of a cationic MAPKAP kinase 2 inhibitory peptide (MK2i) with the anionic, pH-responsive polymer poly(propylacrylic acid) (PPAA) yields MK2i nano-polyplexes (MK2i-NPs) that significantly increase peptide uptake and intracellular retention. This study focused on elucidating the mechanism of MK2i-NP cellular uptake and intracellular trafficking in vascular smooth muscle cells. Poly(propylacrylic acid) and MK2i were synthesized and formulated into MK2i-NPs as previously reported. Small molecule inhibitors of endocytosis were purchased from Sigma-Aldrich. A7r5-Gal8-YFP cells were generated with plasmids from Dr. Randow (Gal8-YFP) and Dr. Weinburg (retroviral packaging). Small molecule inhibition of various endocytic pathways showed that MK2i-NP cellular uptake involves both macropinocytosis and clathrin mediated endocytosis, whereas

the free peptide utilizes clathrin mediated endocytosis alone for cell entry. Scanning electron microscopy studies revealed that MK2i-NPs, but not free MK2i peptide, induce cellular membrane ruffling consistent with macropinocytosis. TEM confirmed that MK2i-NPs induce macropinosome formation and achieve MK2i endo-lysosomal escape and cytosolic delivery. Finally, a novel technique based on recruitment of Galectin-8-YFP was developed and utilized to quantify MK2i-NP induced endosomal disruption within 30 minutes of uptake. MK2i-NP is proposed to be internalized via macropinocytosis and clathrin mediated endocytosis, whereas MK2i peptide is internalized via clathrin mediated endocytosis. The enhancement in cellular uptake and endosomal escape is proposed to be driven by NP hydrophobicity and pH-responsive character of PPAA, respectively. These new insights on the relationship between NP physicochemical properties and cellular uptake and trafficking can potentially be applied to further optimize the MK2i-NP system and more broadly toward the rational engineering of nano-scale constructs for the intracellular delivery of biologic drugs.

Frances Knight – PhD Candidate
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Title of Abstract: Stimulating Pulmonary Immunity via Intranasal Delivery of Nanoparticle Vaccines
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Abstract:
Stimulating immunity by vaccinating at mucosal surfaces such as the lungs mimics natural infection and is more likely to generate a protective response at the site of pathogen entry. Pulmonary immunization with subunit vaccines is an attractive approach because they are safer than vaccines based on attenuated or inactivated microbes. However, they are also typically less immunogenic and are poor at generating CD8+ T cell (CD8T)-mediated immunity, which is necessary for defense against many intracellular pathogens and cancer. We have developed a pH-responsive nanoparticle (NP) delivery system that can be loaded with subunit protein antigen and nucleic acid adjuvant. The system leverages endosomal acidification after uptake to release antigen into the cytosol, where it can be processed by the MHC-I pathway, resulting in a CD8T-biased immune response.
Micellar NP were self-assembled from diblock copolymers synthesized by RAFT polymerization. A thiolated model protein antigen, ovalbumin (OVA), was covalently conjugated to NP via disulfide exchange, and a nucleic acid adjuvant, CpG DNA, was electrostatically complexed with NP. To assess the vaccine's ability to elicit a robust pulmonary CD8T response, C57BL/6

mice were immunized intranasally with formulations containing 7.5 ‘_g OVA with or without 1.4 ‘_g CpG and/or 25 ‘_g NP. On day 14, lungs, bronchoalveolar lavage fluid (BALF), and spleens were collected. Numbers of OVA-specific CD8T in these tissues were determined via MHC-I tetramer staining and flow cytometry. To evaluate whether this vaccine also induces a humoral response, sera were collected on day 12 and antibody titers for anti-OVA IgG were determined with ELISA. One-way ANOVA comparison and Tukey’s post-hoc test were used to determine statistical significance between treatment groups. Reaction of NP and thiolated OVA at a 5:1 molar ratio (NP:OVA) resulted in 90-95% protein conjugation, and reaction with CpG at a 6:1 charge ratio (+/-) resulted in complete complexation. Dual-loaded NP were ~40 nm in diameter. In vivo immunization with OVA-NP/CpG formulation elicited significantly higher OVA-specific CD8T counts relative to mice receiving a mixture of OVA+CpG, OVA-NP conjugate, or a mixture of NP/CpG complex+OVA. Mice showed negligible weight loss and morbidity in response to the formulation, and pathology analysis revealed only minor local inflammation. Immunization with OVA-NP/CpG also increased levels of anti-OVA IgG after a single dose, relative to controls.

Significant increases in CD8T response and IgG titer from the OVA-NP/CpG formulation after a single dose indicate that co-delivery enhances pulmonary immune response over control formulations that do not deliver antigen and adjuvant on the same particle. Combined with lack of toxicity in mice, these results show the delivery system holds promise for use as a vaccine platform. Future work will evaluate the effects of

different nucleic acid adjuvants on the pulmonary immune response, as well as the formulation's ability to elicit CD4+ T cells.

Jessica Weaver – Postdoc
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Title of Abstract: Vasculogenic cell delivery hydrogel vehicle enhances islet engraftment, survival, and function in diverse extrahepatic transplant sites
Presenting Author: Jessica Weaver
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Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology (1); Woodruff School of Mechanical Engineering, Georgia Institute of Technology (2); Department of Biomedical Engineering, Emory University and Georgia Institute of Technology (3)
Abstract:
Clinical islet transplantation is a promising cell replacement therapy for the treatment of Type 1 Diabetes, with the potential to eliminate secondary complications common to this patient population. Widespread application of this therapy has been limited by suboptimal graft survival, due in part to the inhospitable intrahepatic islet transplant site. The development of a highly vascularized extrahepatic transplant site that supports greater islet graft survival and function would enhance the clinical success of islet cell replacement therapy, and vastly widen its applicability. To that end, we have

developed a vasculogenic, proteolytically degradable hydrogel vehicle capable of delivering islets to diverse extrahepatic sites. We examined the vasculogenic potential of this VEGF-labeled hydrogel within the subcutaneous (SUBQ), small bowel mesentery (SBM), and epididymal fat pad (EFP) sites, as well as site-specific innate immune responses to the delivery vehicle. We found that VEGF-labeled gel delivery to the EFP exhibited the greatest vasculogenesis of all three sites, as well as minimal innate immune responses. Additionally, delivery of a minimal islet mass to the EFP + VEGF resulted in the fastest rate of diabetes reversal of all three sites. Whole mount imaging of functional vasculature via lectin labeling and insulin staining enabled visualization of islet integration within each site, and the EFP + VEGF site exhibited the greatest density of islets integrated with local vasculature. To evaluate whether this site provided superior post-transplant islet survival to intrahepatic delivery, we utilized in vivo bioluminescent imaging to track luciferase-expressing islets delivered to the intrahepatic, EFP, and SUBQ sites. Islets were tracked for a period of 5 weeks, where signal intensity provided quantitative analysis of graft survival over time. Previous studies have indicated significant intrahepatic islet loss upon delivery, and bioluminescent tracking supports this assessment. By contrast, islets delivered to the EFP exhibited relatively stable signal for 5 weeks, with the EFP + VEGF exhibiting the greatest signal overall. This study highlights the significant impact of transplant site selection on islet graft survival and function. In particular, our data demonstrates that islet vascularization plays a key role in graft survival and function, and that delivery via VEGF-labeled

hydrogel vehicle has the capacity to enhance islet transplant outcomes.

Hynhee Ahn – Research Associate
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Title of Abstract: Development and Biological Evaluation of Porous poly(para-phenylene) for orthopaedic implants
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Abstract:
Current approaches to lumbar interbody fusions (LIFs) use devices made from traditional solid materials to restore disc height and provide stability for fusion; however, these designs do not evenly distribute loads across the vertebral endplates and can lead to micro-fractures and subsidence. Polyparaphenylene (PPP) is a new class of aromatic polymers with higher strength, stiffness and superior manufacturability. We have previously developed PPP scaffold with bulky porosity to promote bone ingrowth for better fusion and match

trabecular bone stiffness. Here, this study was to demonstrate in vitro cellular compatibility and in vivo osseointegration of the material in the tibia bone defect site using a rat model. The tailored porous scaffolds were fabricated with 420-500 ‘_m of pore size and scaffolds were divided into 4 different groups for comparison: 1) Solid PPP, 2) Solid PEEK, 3) Porous PPP and 4) Porous PEEK. To assess cellular compatibility, mouse pre-osteoblasts (MC3T3-E1) were seeded on each samples and the metabolic activity was measured at d1, d3 and d5 for cell proliferation. To determine pre-osteoblast differentiation, cells were treated with differentiation media and measured alkaline phosphatase (ALP) activity at d7 and total calcium amount at d21. The bilateral drill holes were made in tibia metaphysis, and cylindrical implants (6 mm length and 2.3 mm diameter) were implanted in the defect to determine osseointegration. MC3T3 cells were able to adhere and grow on all scaffolds and the proliferation rate was not different between PPP and PEEK. Cells grown on porous scaffolds showed a significant increase in ALP activity and calcium deposition (p

Angela Alexander-Bryant – Postdoc
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Title of Abstract: Nanotherapeutics for combination drug and gene therapy in treating glioblastoma multiforme
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Abstract:
Gliomas represent approximately 80% of all malignant brain tumors, and glioblastoma multiforme (GBM), the most aggressive type, accounts for nearly half of all gliomas. Despite treatment strategies including surgery, radiation, and chemotherapy, the 5-year survival rate for brain cancer is only 35%.ŒæŒæ New therapeutic strategies are necessary to improve the outcomes of this disease. Chemotherapy with temozolomide (TMZ), a DNA alkylating agent, is used as a first-line of treatment for GBM. However, GBM tumors develop resistance to TMZ over time due to increased expression of O6-methylguanine-DNA methyltransferase (MGMT), a gene responsible for DNA repair. We previously developed cationic, amphiphilic copolymer poly(lactide-co-glycolide)-g-polyethylenimine (PgP) and demonstrated its utility for nucleic acid delivery. Here, we examine the ability of PgP polyplexes to overcome TMZ resistance and improve therapeutic efficacy through combination drug and gene therapy for GBM treatment. PgP micelles were designed and synthesized for delivery of hydrophobic drugs in the PLGA core and negatively charged nucleic acids through electrostatic interactions with positively charged PEI. RNA binding and polyplex stability assays were performed using agarose gel electrophoresis.Œæ Cytotoxicity of TMZ and/or PgP/siRNA polyplexes was determined by MTT assay. Silencing of MGMT on the protein and mRNA level was determined using western blotting and

qPCR, respectively. Our results demonstrated that PgP effectively forms stable complexes with siRNA and protects siRNAs from serum- and ribonuclease-mediated degradation, confirming the potential of the polyplex for in vivo delivery. Results from MTT assays showed that PgP/siRNA polyplexes exhibited minimal cytotoxicity compared to untreated cells when incubated with T98G human GBM cells.Œæ We also demonstrated that PgP/siMGMT polyplexes mediate knockdown of MGMT protein as well as a significant ~56% and ~68% knockdown of MGMT mRNA in T98G GBM cells compared to cells treated with PgP complexed with non-targeting siRNA (siNT) at a 60:1 and 80:1 nitrogen:phosphate (N:P) ratio, respectively. In future studies, we plan to apply combination therapy to GBM cells through co-treatment with PgP/siMGMT polyplexes and TMZ to enhance therapeutic efficacy compared to treatment with the polyplex or TMZ alone. ŒæSuccessful combinatorial drug and gene therapy using PgP may overcome drug resistance and improve therapeutic outcomes for patients with glioblastoma.

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Title of Abstract: A Sticky Situation: Protein Purification on Barnacle Adhesion
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Abstract:
As the demand for biofouling increases, so does the need to understand what makes barnacles adhere underwater the first place. To do this, speculated adhesive proteins 19 and 20 from Megabalaus rosa was used. If proteins 19 and 20 are purified and tested, antimicrobial and adhesive properties will be found due surface coupling. Proteins were purified using sonicator, french press, elution and dialysis. SDS PAGE was used to view protein bands. Results showed that proteins 19 and 20 can be purified on the small scale. With the right amount of purified protein, a Quartz Crystal Microbalance (QCM) can be used to measure adhesion properties. To test the proteinäó»s antimicrobial properties, various protein dilutions can be placed on a 96 well plate and incubated overnight. The wells with low ODs and no growth on the plate would show antimicrobial properties. This research is important because understanding barnacle adhesion can lead to biomedical applications, such as a waterproof superglue for bones.
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Title of Abstract: Functionalized Silk Hydrogels for Controlling Cell Adhesion
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Abstract:
Controlling cell adhesion is a critical milestone for tissue engineering success. Studies show that in order for cells to adhere to surfaces, they must be able to form an adequate number of focal adhesions, which is dependent on many factors including surface stiffness and ability to spread. Here, we functionalize silk biomaterials to improve its adhesive properties with cells. Silk is a good candidate as a biomaterial because of its high material strength, biocompatibility, and biodegradability in addition to being non-immunogenic and largely biologically inert. We have chosen silk hydrogels as our platform for use in our studies of cell adhesion. The hydrogels are fabricated using a chemical cross-linking method which allows for multiple functionalization methods. The silk gels can be non-specifically and specifically functionalized with integrin binding domains. Specific functionalization is accomplished through patterning of the surface of the silk hydrogels with integrin binding proteins via microcontact printing. Patterned lanes of fibronectin were used here to show preferential cell alignment in the direction of the lanes in comparison to unmodified hydrogels. The mechanics of these hydrogels were also characterized and found to span nearly two orders of magnitude depending on hydration and protein concentration conditions. These studies have shown that the bioactivity of silk can be controlled, both in terms of cell adhesion as well as

mechanical properties. The platform developed is highly versatile, as a variety of integrin binding proteins can be used to functionalize gels of varying stiffness to accommodate application specific demands. By demonstrating these capabilities on a biocompatible surface, these methods can have the potential to be applicable in in vivo studies in addition to their potential in vitro uses.

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Title of Abstract: Investigating the impact of poloxamer-fabricated nanoparticles on efflux of drugs by breast cancer cells
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Abstract:
Investigating the Impact of Poloxamer - Fabricated Nanoparticles on Efflux of drugs by Breast Cancer Cells
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Development of resistance to chemotherapeutic agents is a major hurdle in the treatment of cancers and it is known to be

mediated by P-glycoprotein (P-gp). The over-expression of P-gp in tumor cells leads to a reduction in the intracellular concentrations of the drugs, thereby reducing the cytotoxic effect of many antitumor agents. Anticancer agent must be available at the tumor site in sufficient concentrations in order to exert the required activity. The use of nanoparticles for targeting tumor cells has become an attractive area of research due to its ability to deliver a high pay load of drug, influence drug solubility, sustain the drug load in the in-vivo environment as well as control the concentration of the drug in certain tissues. The aim of this study was to compare the uptake of dye loaded nanoparticles and dye solution, investigate the effect of poloxamer-fabricated nanoparticles on efflux and to establish the involvement of P- glycoprotein in the efflux of drugs from cancer cells.

Method: Polycaprolactone (PCL) nanoparticles containing coumarin (fluorescent dye) was prepared by the nanoprecipitation method using three different poloxamers and characterized. Cellular uptake of coumarin by two breast cancer cell lines (MDA-MB-231 and HCC-70) was determined by flow cytometry. Influence of poloxamers on efflux of internalized nanoparticles was determined by measuring the fluorescence of culture media after cellular uptake of fluorescent loaded nanoparticles and additionally by the measurement of the mean fluorescent intensities of cells after internalization of coumarin. P-(gp) involvement in the efflux process was established by its inhibition before the analysis of accumulation and efflux using verapamil.

Results: The nanoparticles formulated had an average size of 190 Œ±20 nm, with a narrow size distribution and a zeta potential of +18 ± 3mV. Uptake of nanoparticles was significantly different from solution (0.05). Efflux of coumarin loaded nanoparticles from the cell lines was less compared to the dye solution. The degree of efflux of dye from the cell lines was different for all three poloxamer surfactants used. Conclusion: Nanoparticles delivered a higher payload of drug than solution of equivalent concentrations. Poloxamers have some influence on efflux. Further studies need to be done to optimize the concentration of poloxamer needed to totally inhibit P- glycoprotein.

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Title of Abstract: Antifibrotic effects of vibratory mechanical stimulation in scleroderma fibroblasts
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Abstract:
Fibrotic disorders can lead to permanent functional loss in every major organ system and account for 45% of deaths in the United States; examples include pulmonary fibrosis, renal fibrosis, liver cirrhosis, arteriosclerosis, and scleroderma. However, recent studies indicate that fibrosis can be reversed

depending upon the extent of its progression and suggest that mechanical forces may be an important therapeutic target that is able to modulate the homeostasis of tissues in combating specific pathophysiologies and diseases. Previous studies have shown that vibratory mechanical stimulation significantly down-regulated the TGF-‘_ signaling pathway and inhibited collagen synthesis. Moreover, vibratory stimulation blocked induction of collagen production and mechanical stiffening of polyurethane substrates in response to cyclic tension or exogenous TGF-‘_1 supplementation for a week. Thus, understanding the fundamental mechanisms by which mechanical forces are sensed, transduced, then transformed to gene and protein changes in a clinical model is essential to develop a novel mechanotherapy for improving wound healing and treating fibrotic diseases. In this study, normal and scleroderma fibroblasts (1.2 x 10e6 cells per substrate) were seeded in porous polyurethane sponges and cultured under static conditions for 4 days. After 4 days of pre-incubation, the substrates were subjected to either further static, cyclic strain (0.25 Hz, 5% strain amplitude) or cyclic strain in combination with vibration under tension (100 Hz frequency, 3.8 Vrms, 1.28 ± 0.07 mm amplitude). After exposure to mechanical stimuli for 7 days, mRNA levels were analyzed to see the molecular changes in fibrotic phenotype markers including profibrotic cytokines and collagens relative to normal control group. The results show that vibratory stimulation down-regulated pathological markers (TGFBR1, TGFBR2, LOX1, FBN1, IL1A, IL4, TIMP1, COL1A1, and COL3A1 that are over-expressed by fibroblasts isolated from scleroderma lesions), and up-

regulated antifibrotic regulators (FLI1 and SMAD7) compared with the cyclic strain alone group. These studies suggest that vibration may be applicable as a novel mechanotherapy approach for treatment of fibrotic diseases in multiple tissues and organs.

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Title of Abstract: Targeted Nanoparticles For Delivery Of siRNA To Sites Of Early Onset Post-Traumatic Osteoarthritis
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Abstract:
Post-traumatic osteoarthritis (PTOA) occurs after a traumatic injury to the bone or soft tissue including ligament and meniscal tears, and there is currently no cure, only medications to relieve the pain. Type II collagen (CII) is normally shielded by superficial structural polysaccharides but becomes exposed in the early stages of OA when the overlying polysaccharides are lost at sites of injury. Subsequent degradation of exposed CII, primarily by matrix metalloproteinase 13/collagenase 3 (MMP-13), represents the irreversible step in OA pathogenesis that causes permanent cartilage damage. We are developing a

siRNA nanocarrier that targets the earliest PTOA biomarker (exposed CII) to deliver therapy to prevent PTOA progression by blocking MMP13Œædegradation of CII.
Targeted nanoparticles (MabCII-NPs) were synthesized by efficient conjugation of a CII specific antibody (MabCII) known to specifically bind at early PTOA damage sites to a siRNA nanoparticle recently developed in our lab to overcome in vivo delivery barriers for siRNA. This nanoparticle comprises a hydrophobically-stabilized, siRNA-packing, and endosomolytic core and a 5kD polyethylene glycol (PEG) surface corona. The present studies focused on optimization of the antibody conjugation chemistry and analysis of resultant MabCII-NP size, siRNA loading efficiency, pH-dependent membrane disruptive function, bioactivity in silencing MMP-13, binding to trypsin damaged pig cartilage samples over non-damaged tissue ex vivo, and in vivo targeting to joints with mechanically-induced PTOA in mice.
MabCII conjugations were very efficient with no residual free antibody detectable at a molar ratio of 10:1 (polymer:peptide). The resulting MabCII-NPs efficiently loaded siRNA at a charge ratio ofŒæ10:1 and reliably formed with a hydrodynamic radius of ~110 nm. FRET assays verified sufficient stability of the micellar structure in the presence of heparin and fetal bovine serum, and hemolysis assays verified the pH-dependent membrane disruption (as a marker for endosomal escape function). In vitro studies demonstrated MabCII-NP effective knockdown of MMP-13 expression (stimulated by TNF‘± in ATDC5 chondrogenic cells). Cartilage explant binding assays demonstrated significantly increased affinity of MabCII-NPs to

damaged pig cartilage over control, non-targeted NPs coupled to an isotype control antibody. Finally, pilot in vivo experiments showed the accumulation of MabCII-NPs in PTOA joints resulted in twice the area under the curve (AUC) in the first six hours relative to the unloaded, contralateral control limb.
We have developed MabCII-decorated nanoparticles with potent MMP-13 gene silencing function and that are proven to preferentially bind to damaged cartilage. Ongoing studies are expected to establish this promising targeted nanomedicine for inhibition of PTOA progression in vivo.

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Title of Abstract: Using Near Infrared Imaging to Assess Knee Clearance in a Rats
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Abstract:

Osteoarthritis (OA) is a degenerative disease of the joint that leads to joint instability, degradation of the articular cartilage surface, and eventually joint failure. The synovial membrane, which contains vascular and lymphatic capillaries, regulates the synovial fluid by controlling the influx and efflux of molecules. Protein clearance from the synovial fluid characterized as a lumped parameterÂ that involves starling forces, synovial permeability, and lymphatic drainage. In humans, inflammatory conditions precede the distinctive radiographic changes that confirm the loss of cartilage. The retention or clearance of inflammatory molecules like Interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-Î±) is critical to joint health as these molecules up-regulate the synthesis of matrix metalloproteinases, which accelerate the degradation of cartilage. The objective of this study was to utilize a lymphatic specific biomaterial tracer and near-infrared (NIR) imaging to determine the clearance of proteins within the knee space. NIR dye conjugated to 13kDa polyethylene glycol (PEG) can be used to simulate proteins like IL-10 and TNF-Î±; therefore, we have proposed a biomaterials based strategy for the determination of protein clearance. NIR imaging offers a higher temporal resolution, is low cost, minimally invasive and innocuous when compared to previously established radiolabeling techniques of intra-articular clearance. OA can be surgically induced in rats via the medial meniscus transection (MMT) surgery, which involves the transection the meniscus and the MCL. This leads to joint instability, altered loading, and presents the phenotypical cartilage degradation seen in OA at 3 weeks. The sham MMT involves only the transection of the MCL. We

hypothesize that the invasive nature of the surgery and suturing technique in the sham surgery may have an effect on the lymphatic vessels that clear the knee and thus the clearance of tracer/proteins from the knee space. Sham surgeries were performed and NIR tracer was injected into the left knees of rats to assess over the course of 90 minutes. Data are currently being collected and analyzed, however initial results after two weeks show there are no significant changes in clearance of tracer between control (n=5) and sham MMT (n=3). These methods will be used in future experiments where we perform MMT surgery to investigate how lymphatic function and OA are linked, both in regards to the initial severity of the disease and its progression.

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Title of Abstract: Crosslinked Angle-Ply Annulus Fibrosus Repair Patch Resists Enzymatic Degradation While Maintaining Mechanical Properties and Cell Viability
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Abstract:
Annually, over 5.7 million Americans are diagnosed with intervertebral disc (IVD) pathologies:1 IVD herniation (IVDH)

and/or degeneration (IVDD)- both can mechanically disrupt the annulus fibrosus (AF). In IVD pathologies, enzymatic degradation of IVD tissue occurs via collagenases, specifically matrix metalloproteinases (MMP-1, MMP-13), contributing to the destruction of the AF. Thus, the development of a biomaterial which can be used to repair and regenerate AF tissue in patients with IVD pathologies should be biomechanically equivalent to native mechanical properties and support cell viability while resisting enzymatic degradation. Our lab has previously developed a cytocompatible, collagen-based multi-laminate angle-ply AF patch biomaterial which mimics the native architecture and mechanical properties of the human AF.2 The objectives of this research were to identify a chemical crosslinking treatment that would improve AF patch resistance to collagenase without detrimentally altering tensile properties and cytocompatibility.

Herein, AF patches were crosslinked at ambient temperature for 24 hours using four crosslinking solutions: Solution 1) 6mM (1-Ethyl-3-(3-dimethylaminopropyl)) Carbodiimide HCL (EDC) / 1.2 mM N-Hydroxysuccinimide (NHS), Solution 2) 30mM EDC / 6 mM NHS, Solution 3) 0.2% glutaraldehyde (GLUT) and Solution 4) 0.6% GLUT. EDC/NHS and GLUT crosslinking were carried out in 50mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer at (pH 5.5) and 50mM 4-(2-hydroxyetheyl)-1-piperazineethanesulfonic acid (HEPES) buffered saline (pH 7.4), respectively. Differential scanning calorimetry (DSC) was used to verify crosslinking by determining the thermal denaturation temperatures (Td) (n=3 samples/non-crosslinked and native) (n=4 samples/crosslinked groups). Each sample was heated

from 20 to 120C at a rate of 10C/minute. Tensile testing was performed to determine elastic modulus (EM) and ultimate tensile strength (UTS) of crosslinked and non-crosslinked patches using methods described by Green et al. with minor modification.3 Resistance to collagenase degradation was performed on crosslinked and non-crosslinked patches as well as native bovine AF (n=3/group). Samples were removed from TRIS buffer, blotted dry, frozen at -80C, lyophilized, and weighed. Samples were then incubated in 2mL of 33.6 U/mL collagenase type I at 37ŒÁC for up to 14 days. Sample mass loss was determined by comparing the dry mass of each sample before and following enzymatic digestion. Day 7 samples were further incubated for an additional 7 days in an increased concentration, 336 U/mL, due to minimal initial degradation. Cytocompatibility was evaluated following seeding of bovine AF cells onto AF patches at a density of 9 x 106 cells/cm3.4 Briefly, cells were injected into the interior layers using a 20G syringe and seeded drop-wise onto the outer surfaces of the patch. Patches were cultured under standard conditions for up to 12 days. Cytotoxicity was assessed via a lactate dehydrogenase (LDH; n=3 patches/time-point/group) assay. Positive control AF patches were seeded and cultured in parallel, and 100% cell death was created by snap freezing cultured patches 3 days prior to LDH analysis on the culture media. LDH values are expressed as a percentage of positive (100%) cell death control. Histological analysis of cell infiltration and distribution for cell-seeded patches (n=3/time point) was completed on paraffin embedded, 5µm sections stained with H&E. Quantitative results are expressed as mean standard error of the mean

(SEM) and were statistically compared via one-way analysis of variance (ANOVA) with significance denoted as (p

Brittany Cotton – Masters Candidate
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Title of Abstract: An Immunohistochemical Analysis of Large-pore and Small-pore Polypropylene Surgical Mesh
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Abstract:
An Immunohistochemical Analysis of Large-pore and Small-pore Polypropylene Surgical Mesh
Brittney Cotton, Xinyue Lu, Megan Hanschke, Todd Heniford, Melinda Harman
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Statement of purpose: Polypropylene surgical mesh is an implantable biomaterial intended to reinforce soft tissue for repair of abdominal hernias, and has been increasingly used to treat stress urinary incontinence and pelvic organ prolapse in women. Surgical meshes induce a variety of inflammatory responses in the body, which may lead to the patient rejecting the mesh. In 2008, the Food and Drug Administration (FDA) issued a Public Health Notification,

warning clinicians and patients of the detrimental effects of surgically implanted mesh such as hernia recurrence, scarring, chronic inflammation, pain, and infection. Research is needed to explain why some patients are having such adverse reactions to the implanted mesh.

Methods: Using immunohistochemical techniques, local tissue reactions to meshes removed from patients will be analyzed for signs of inflammatory response. Specifically, large-pore and small-pore mesh properties, suturing stress associated with surgical mesh implantation methods, and patient immunologic factors linked to mesh erosion will be considered in the study.

Results: Explanted defective mesh/tissue samples have been acquired and are ready to be analyzed. A Biosafety Level 2 lab has been acquired for the analysis.

Conclusion: This research might explain recurrence of surgical mesh failure in patients. The findings can be useful for improving mesh biocompatibility in the treatment of abdominal hernias, stress urinary incontinence, and pelvic organ prolapse.

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cytokine levels offer a prognostic factor for polypropylene mesh erosion after suburethral sling surgery for stress urinary incontinence? Int Urogynecol J Pelvic Floor Dysfunct. 2009;20:69äóñ74

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Title of Abstract: Evaluating the role of polymer structure on cell uptake and endosomal escape of nanopolyplexes for peptide drug delivery
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Abstract:

Polymers with pH-selective membrane disrupting capability are a valuable component in nanocarriers for intracellular biologic drug delivery, as endosomal escape is a critical barrier against cytosolic bioactivity. We are interested in the intracellular delivery of therapeutic peptides because they can disrupt intracellular protein-protein interactions with greater specificity than small molecule drugs. However, peptides are extensively trafficked and degraded in the endolysosomal pathway, which limits their cytoplasmic bioavailability. We have previously designed electrostatically bound nanopolyplexes of peptides and poly(propylacrylic acid) (PPAA), which effectively facilitated peptide uptake and translocation to the cytoplasm. However, the structural role of the PPAA polymer in facilitating membrane disruption and drug unpackaging is not thoroughly understood, and potentially not optimized. This is primarily because propylacrylic acid is challenging and expensive to synthesize, which prohibits systematic variations of its chemical structure, specifically the length of the hydrophobic side chain and the acid:hydrophobe ratio. To further examine the effect of polymer structure on peptide uptake, we developed a strategy for synthesizing a series of graft copolymers with controllable, well-defined anionic and hydrophobic content to investigate the relationship between polymer structure and pH-dependent membrane disruption. Copolymers containing fixed ratios of anionic acid groups and alkyl side chains were synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. A total of 15 polymers were screened to provide complementary understanding on the role of hydrophobe length and density in modulating the acid pKa,

which in turn controls the pH-responsive membrane disrupting character of the polymer. A red blood cell hemolysis assay was used to model membrane disruption at pH corresponding physiological conditions as well as increasingly acidic pH found in early endosomes (6.8), late endosomes (6.2), and lysosomes (5.6). Flow cytometry was used to quantify uptake of polymer alone and polymer complexed with peptide for a series of formulations.Our results identified a subset of polymers with an anion-hydrophobe ratio that optimizes pH-dependent endosomal escape and matches the behavior of PPAA. Flow cytometry revealed that PPAA and the copolymer library exhibit both dose and formulation-dependent uptake in vitro, and that polymer hydrophobicity is a strong indicator of uptake behavior.

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Title of Abstract: Evaluating the role of polymer structure on cell uptake and endosomal escape of nanopolyplexes for peptide drug delivery
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Abstract:
Degradable hydrogels to deliver bioactive proteins represent an emerging platform for promoting tissue repair and vascularization in various applications. However, implanting these biomaterials requires invasive surgery, which is associated with complications such as inflammation, scarring, and infection. To address these shortcomings, we applied microfluidics-based polymerization to engineer injectable poly(ethylene glycol) microgels of defined size and crosslinked with a protease degradable peptide to allow for triggered release of proteins. The release rate of proteins covalently tethered within the microgel network was tuned by modifying the ratio of degradable to non-degradable crosslinkers, and the released proteins retained full bioactivity. Microgels injected into the dorsum of mice were maintained in the subcutaneous space and degraded within 2 weeks in response to local proteases. Furthermore, controlled release of VEGF from degradable microgels promoted increased vascularization compared to empty microgels or bolus injection of VEGF. Collectively, this study motivates the use of microgels as a viable method for controlled protein delivery in regenerative medicine applications.

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Title of Abstract: Enhancing the Delivery of Therapeutic Antibodies to Tumor Draining Lymph Nodes via Lymphatic-draining Poly(propylene sulfide) Nanoparticles for Cancer Immunotherapy

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Abstract:
Immunotherapies continue to emerge as the most promising new treatment for an array of advanced cancers. In particular, monoclonal antibodies (mAbs) are increasingly utilized immunotherapeutic agents that recognize specific cell markers on immune cells or tumor cells to either modulate anti-tumor adaptive immunity or facilitate antibody-dependent cell-mediated cytotoxicity, respectively. Although responses are often durable, a large majority of patients, however do not respond to such treatments. Many of these mAbs therapies, including checkpoint blockade inhibitors and anti-HER2 antibodies, could be improved by targeting them to lymph nodes rather than tumor sites as it is home to a high population of immune cells and is the initial tissue of metastatic dissemination. As such, we seek to improve the immunotherapeutic potential of mAbs by increasing their bioavailability within lymph nodes by leveraging biomaterial nanoparticles that, by virtue of their formulation and size,

exhibit greatly enhanced profiles of lymphatic uptake into lymphatic tissues and accumulation within lymph nodes post administration. Post emulsion polymerization of these Pluronic-surface stabilized poly(propylene sulfide) nanoparticles, a solvent- and denaturation-free disulfide displacement chemistry is used to tether Traut's treated mAbs to the nanoparticle core, resulting in antibody-conjugated nanoparticles that preserve the binding properties of the Pluronic-tethered IgG. To date, we have found that as a result of nanoparticle conjugation, IgG accumulates to greater extents in lymph nodes post administration in the skin relative to free IgG in a lymphatic drainage-dependent manner. In ongoing work, we seek to determine the resulting effect of nanoparticle conjugation on the bioavailability of IgG to LN-resident cells post administration and the therapeutic mAb efficacy in treating breast cancer using advanced preclinical mouse models of mammary carcinomas.

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Title of Abstract: Cationic Polymeric Micelle as a RhoA siRNA Carrier for Axonal Regeneration in Rat SCI model
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Abstract:
The regenerative capacity of the injured adult central nervous system (CNS) is extremely limited due to both extrinsic microenvironmental factors and intrinsic, age-related changes in neuronal biochemistry. Many studies have shown that diverse extracellular inhibitors of neuroplasticity including both myelin associated inhibitors (MAIs) and chondroitin sulfate proteoglycans (CSPGs) may act through common intracellular signaling pathways. Neurite growth inhibition in response to MAIs and CSPGs has been shown to be associated with activation of RhoA and Rho kinase (ROCK) and can be overcome by Rho/ROCK inhibitors. The goal is to develop neuron-specific multi-functional polymeric nanotherapeutics for combinatorial delivery of multiple bioactive molecules targeting different barriers to plasticity and axonal regeneration. Previous study was reported the synthesis and characterization of novel cationic, amphiphilic copolymers (poly (lactide-co-glycolide)-g-polyethylenimine: PgP) that provides efficient nucleic acid delivery in vitro and in vivo. Here, we show that PgP can deliver RhoA siRNA and efficiently knockdown RhoA gene expression in B35 cells and in a rat compression spinal cord injury model in vivo. PgP/RhoA siRNA polyplexes were prepared at various N/P ratios. B35 cells were transfected with PgP/RhoA siRNA in B35 cells and total RNA was isolated at 72hrs post-transfection. The efficiency of RhoA gene knockdown was measured by real-time PCR. To generate rat compression SCI model, laminectomy was

performed on the back of Sprague Dawley rats and the T9 spinal cord region was exposed and vascular clip was placed for 10 min. PgP/RhoA siRNA polyplexes were prepared and injected at the T9 spinal cord injury (SCI) region. At 1, 2, and 4 weeks after polyplex injection, the rats were sacrificed and total RNA was isolated and RhoA gene knockdown is measured by real-time PCR. For histological evaluation, rat were perfused with 4% paraformaldehyde at 4 weeks after injection of PgP/RhoA siRNA. The sections were stained using antibodies against neurofilament and GFAP.RhoA gene knockdown of approximately 45% was achieved after transfection of PgP/RhoA siRNA at N/P ratio of 30/1in B35 cells. In untreated SCI animal group, RhoA gene expression was increased 2.6-fold compared to that in sham animal group at 1week. In PgP/RhoA siRNA polyplexes treated SCI animal groups, the RhoA expression was reduced to levels not significantly different from the sham group and significant knockdown was maintained up to 4 weeks. In untreated SCI group, extensive necrotic cavity was formed in injury site, while axonal growth was observed in PgP/RhoA siRNA treated group.These studies demonstrate that PgP is a promising therapeutic siRNA delivery carrier in vitro and in vivo SCI model. Currently, we are evaluating the effect of PgP/RhoA siRNA on functional recovery by Basso-Beattie-Bresnahan (BBB) locomotor rating scale in SCI model.

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Title of Abstract: Adhesion and Alignment of Stem Cells on a Spider Silk Scaffolds after UV Sterilization

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Abstract:
Spider silk is known for its high strength properties and it has recent studies have shown that it has the potential to encourage cell growth in some cultures. Mesenchymal stem cells can act as a source of pluripotent cells that can be manipulated to differentiate into neurogenic cells. An important trait of this differentiation is the alignment and morphology of the cells themselves. The current study aims to look at how these cells align, proliferate, and behave when placed in contact with spider silk scaffolds that have been exposed to different levels of UV radiation.
The drawline silk was obtained from a spider (Nephila clavipes äóñ golden silk orb-weaver spider) using a series of motors that pull the silk from the spider's spinneret. The spider was sedated prior to silking by placing it in a low temperature environment in order to put it into a more dormant state. Silk was reeled directly onto a sterilized glass slide to act as a scaffold for cell growth, then sterilized under UV light for varying lengths of time from 0-60 minutes. 15,000 cells (current studies are using human adipose derived stem cells, (hADSCs) are plated onto

the slide and allowed to grow for a period of 3 weeks. Microscopy images are taken throughout the trial in order to qualitatively observe cell behavior during trial. Proliferation assays are done to ensure that cells are continuing to multiply, and DAPI and phalloidin staining is done to monitor the alignment of actin in the cell along the spider silk. In addition to the cell testing, spider silk samples were characterized after UV exposure to measure the surface roughness and mechanical testing with tension tests.

The tests showed that there was no significant difference in the cell response on different samples. Light microscopy revealed that the cells in all UV conditions had a tendency to align to the silk when it was plated near it and there was no evidence of cell death being concentrated in location of silk. There was less incidence of bacterial or fungal growth on plates that had been exposed to longer UV radiation times when compared to those with shorter radiation times. Proliferation assays revealed that the cells were continuing to proliferate throughout the trials, and microscopy imaging showed that cells in most plates reached confluency by the end of their trials. Fluorescent imaging showed that there was adhesion and alignment of the cells to the silk in each of these trials. Figure 1 demonstrates this alignment on different scales of magnification.

The hADSCs in this study adhered and aligned to spider silk in cell culture while continuing to proliferate. This is consistent with data found on other cells types that have been studied. It should be noted that prolonged UV exposure damages the silk; the mechanical properties decrease and surface roughness decreased after more than 15min of UV exposure. We have

shown that fibroblasts are sensitive to these changes; however, it appears that the hADSC are less sensitive to changes in the fiber structure seen they grew, aligned and proliferated on all samples in this study. Our future studies are aiming to assess how silk fiber scaffold density and structure can be altered for optimal cell growth and differentiation towards varying lineages.

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Title of Abstract: Biofunctional Hydrogel for Skeletal Muscle Satellite Cell Delivery
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Abstract:
Function and regenerative potential of skeletal muscle decline with age, where loss of skeletal muscle quality is attributed to reduced muscle satellite cell number and function. Inadequate treatments of traumatic muscle injuries in an aging population further exacerbate the progression of sarcopenia, and therefore reduced independence and quality of life. While

direct transplantation of satellite cells contributes to muscle regeneration to some degree, direct cell transplantation is limited by sub-optimal engraftment, survival, and function. Therefore, the objective of this project is to engineer a hydrogel-based bioactive cell delivery vehicle to facilitate delivery, survival, engraftment, and differentiation of muscle satellite cells, and ultimately to promote muscle regeneration in the aging muscle.

Sca-1- CD45- Mac-1- Ter-119- CXCR4+ beta-1 integrin+ primary satellite cells were obtained from rodent muscles via fluorescent-activated cell sorting. The sorted cells expressed Pax-7, a marker specifically expressed in quiescent and proliferating satellite cells, and were capable of differentiating into myoblasts in 2D culture. The cells also proliferated in 3D and formed myogenic colonies when encapsulated within a 20 kDa poly(ethylene glycol) hydrogel containing cell adhesive ligands in culture. Satellite cells cultured in hydrogels containing RGD or YIGSR peptides formed the largest myogenic colonies compared to scrambled control (RDG) and other laminin peptide (AG73, C16, and A2G10). Furthermore, the cells cultured in hydrogels containing RGD peptides also exhibited differentiated myogenic phenotypes after 9 days of culture in vitro.

We further demonstrated that this hydrogel platform containing luciferase+/eGFP+ satellite cells can be injected into the injured intramuscular space, and induce the stem cells to proliferate, differentiate, and engraft in vivo. Collectively, the results illustrate a promising opportunity for the stem cell

therapy for skeletal muscle disorders and dysfunctions in an aging population

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Title of Abstract: Wear Resistance Characterization of CoBlast Surface Modifications for Metallic Implants
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Abstract:
To help reduce infection rates, which are as high as 91% in the treatment of battlefield fractures (Mody), and improve clinical outcomes, surface modification of implants has been a promising proposed solution.Œæ Materials that have been used to affect the formation of biofilm on various materials include polytetrafluoroethylene (PTFE), chitosan, and vancomycin. A novel surface treatment technology, CoBlasting, has been proposed by ENBIO, Ltd. as a delivery process to deposit materials such as PTFE onto implant materials.Œæ These surface treatments can be used to regulate bone adhesion and bacterial growth on the surface of the fracture fixation implants. The aims of this research are to assess the wear resistance of these proposed surface modification techniques to compare their properties to the base material,

stainless steel. Results from this work, combined with surface roughness analysis, biofilm quantification, and material composition analysis will then be used to validate these surfaces using a bacterially challenged rabbit model using fracture fixation implants called dynamic compression plates (DCPs).
Stainless steel (316L) coupons, either blank, coblasted with 50 µm grit alumina with PTFE or 90 µm grit alumina with PTFE, were scratched using 3 mm diameter tungsten carbide ball bearings. Wear tests of 1 cycle, 10 cycles, 50 cycles, and 100 cycles were performed at 10 N normal force, 67 µm/s, 23Œ_C, with PBS as a lubricant. The wear tracks were optically examined at 50x (HUVITZ, Clemson, SC) and examined in n=9 locations per wear track with an optical white light interferometer (WYKO NT-2000, Bruker). Track width and depth were measured in micrometers and statistically compared between blank stainless steel and coblasted samples (‘±=0.05). Evaluating the 1 cycle wear track, the stainless steel blank produced a width of 110.27(+/- 3.28) µm and depth below the intact surface of 0.91(+/- 0.07) µm. The 50 µm and 90 µm coblasted samples showed a surface damage in the wear track, however it did not penetrate below the roughened coBlasted surface, rather it showed evidence of material plowing. This was also the result for the 50 µm and 90 µm coblasted samples at 10 cycles, however the stainless steel blank produced a width of 130.77(+/- 4.31) µm and depth of 1.32(+/- 0.08) µm. Evaluating the 50 cycle wear track, the stainless steel blank produced a width of 148.83(+/- 0.76) µm and depth of 2.17(+/- 0.22) µm. At 50 cycles, the coblasted surfaces finally showed

measurable wear depths, with the 50 µm grit alumina with PTFE coblasted producing a width of 131.73(+/- 10.57) and depth of 1.18(+/- 0.28) µm and the 90 µm grit alumina with PTFE coblasted producing a width of 80.35(+/- 23.69) µm and depth of 0.93(+/- 0.33) µm. Evaluating the 100 cycle wear track, the stainless steel blank produced a width of 243.63(+/- 2.69) µm and depth of 4.05(+/- 0.35) µm, whereas the 50 µm grit alumina with PTFE coblasted produced a width of 116.4(+/- 14.28) and depth of 1.12(+/- 0.16) µm and 90 µm grit alumina with PTFE coblasted produced a width of 177.15(+/- 8.27) µm and depth of 2.66(+/- 0.20) µm. Statistical difference between wear measures are shown in figures 2-3.
The stainless steel blanks produced wear tracks that were statistically significantly (p

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Title of Abstract: Understanding the Phase Transition of Recombinant Fusion Proteins into Hollow Vesicles
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Abstract:
In this work we trace the detailed phase transition for self-assembly of recombinant fusion protein complexes into hollow

vesicles as a function of concentration and temperature. The fusion protein complexes are composed of a fluorescent globule protein, which can serve as a model therapeutic protein, conjugated to a glutamic acid-rich leucine zipper (mCherry-ZE) and a counter, arginine-rich leucine zipper fused with a thermo-responsive elastin-like polypeptide (ZR-ELP). The main driving force behind self-assembly of the fusion protein complexes into vesicles is the thermally-induced inverse phase transition of the ELP block. Since the ELP transition temperature is strongly dependent on protein concentration, different self-assembled structures are created at different protein concentrations at the same solution temperature. The detailed structures ranging from spherical coacervated particles to hollow vesicles with sizes from 500 nm to 2 Œµm are confirmed by dynamic light scattering (DLS), transmission electron microscope (TEM) and small angle neutron scattering (SANS) measurements. The results enable us to design the self-assembled materials with target size and structures from the globule-zipper-ELP fusion protein complexes by tuning temperature and concentration. Furthermore, the fundamental studies on the phase transition of the recombinant fusion proteins are expanded to develop functional drug delivery vehicles containing antibody fragments for cancer therapy.

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Title of Abstract: Anti-inflammatory effects of PEG-bis-AP/HA-DXMin rat traumatic brain injury
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Abstract:
Traumatic brain injury (TBI) occurs when an external force injures the brain and initiates an inflammatory response by resident brain cells and circulating inflammatory cells. Neuroinflammation after TBI can be detrimental to repair and neuroprotective treatments Based on anti-inflammatory drugs may minimize the neurotoxic effects and improve recovery. Dexamethasone (DXM) is a synthetic glucocorticoid, which is known to attenuate inflammation. In this study, we investigated the effects of DXM conjugated hyaluronic acid (HA-DXM) combined with hydrolytically degradable, photo-cross-linkable PEG-bis-(2-acryloyloxy propanoate) (PEG-bis-AP) hydrogel in a controlled cortical impact (CCI) TBI rat model. To generate TBI animal model, Sprague Dawley rats were placed in a stereotaxic frame and then a 5mm craniotomy was made between the bregma and lambda. The exposed cortex was injured using the CCI device armed with a 3mm tip. The CCI device was set at a velocity of 3.5m/sec and to a depth of 2mm. Animals were divided to 4 groups: 1)Sham group (n=7) : craniotomy only, 2) TBI group (n=7) : TBI untreated group, 3) PEG-bis-AP/HA gel treated group (n=7) after TBI, and 4) PEG-bis-AP/HA-DXM gel treated group (n=7) after TBI. Functional

recovery was evaluated by beam walk test performed one day before surgery (-1) and 1, 3, and 6 days post-injury. After behavioral test at 7 days, the rats were sacrificed and cortices harvested for analysis. PCR and IHC were conducted to evaluate expression of cytokines and activity of astrocytes and microglia. In the behavioral tests, time to traverse the beam was significantly increased at 1 day after injury and gradually decreased during 6 days after surgery in TBI untreated group. However, time to traverse the beam in PEG-bis-AP/HA-DXM gel treated group at 1 day after injury was not significantly different with that in sham animal group. In RT-PCR analysis, PEG-bis-AP/HA-DXM exhibited significantly reduced levels of inflammatory cytokines, IL-1b, IL-10, TGF-‘_1 and TNF-‘± relative to TBI untreated group. By histological analysis, substantially fewer ED1+ cells (activated microglia) were observed in the PEG-bis-A/HA-DXMP treated group compared to untreated TBI group. In conclusion, this study demonstrated that PEG-bis-AP/HA-DXM gel showed anti-inflammatory effects and enhanced functional recovery. In future studies, we will evaluate the long-term effect of HA-DXM/ PEG-bis-AP gel on inflammation and functional recovery.

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Title of Abstract: Carbon dots as biocompatible probes for in vivo imaging
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Abstract:
Currently there are myriads of fluorophore on the market. The most common ones are the organic dyes and fluorescene proteins which emit relatively low signals, photobleach and areŒæsensitive to biological milieu. The more advanced fluorophores, quantum dots, have superior brightness, photostability, biodegradation resistance but contain hazardous cadmium and are unlikely to advance to most human trials5. Our group has developed novel biocompatible carbon nanodots with comparable emission intensity and Photostability to quantum dots. Our nanodots compose of carbon core passivated with either EDA (2,2äó_-(ethylenedioxy)bis(ethylamine)) or EPA (3-Ethoxypropyl amine). Our in vitro tests using chick-embryo neuron cells has demonstrated that both EDA and EPA carbon dots may be used as fluorescence dye at concentration as low as 0.5 micromolar and provide good image contrast. MTT assay shows both dots post no significant cytotoxic effect within concentration range of 0.5 -Œæ50 micromolar. EDA and EPA dots also display clear distinct signal after being injeted inside baby sprague dawry rats at concentration of 0.18 mg particle/animal weight. Although our in vivo experiments are still on going, the

cumulative results display good evidence for novel carbon nanodots to be utilized as in vivo imaging probe, and developed to gain specific binding for further imaging and diagnostic uses.

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Title of Abstract: Carbon dots as biocompatible probes for in vivo imaging
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Abstract:
Peptide functionalized poly(ethylene glycol) (PEG)-based hydrogels with are being widely developed for tissue engineering, regenerative medicine, drug delivery, bioimaging, and biosensor applications. The bioactivity of peptide-conjugated hydrogels depends on the accessibility of the tethered peptides at the hydrogel surface in order for them to carry out their intended bioactive function (e.g., targeting for drug delivery and bioimaging or cell-receptor binding for regenerative medicine/tissue engineering). As a copolymer system, the presence of the peptide at the hydrogel surface is dependent on the thermodynamics controlling its partitioning within the hydrogel matrix. This partitioning is difficult to assess

experimentally, with experimental methods essentially being limited to trial-and-error-based approaches (e.g., combinatorial methods) for hydrogel design to optimize bioactivity. To provide insight into the thermodynamic behavior of peptide-functionalized hydrogels, we are developing multiscale molecular modeling methods to visualize, predict, and understand their molecular structure as a tool for hydrogel design optimization. The modeling approach involves: (1) on-lattice modeling at experimentally determined cross-link density, (2) off-lattice coarse-grained modeling to efficiently equilibrate the system, and (3) reverse-mapping the equilibrated CG models to all-atom models with final equilibration. Model validation includes comparison with X-ray structure-factor analyses. The resulting all-atom models are analyzed to characterize the distribution of the peptides within the hydrogel and their accessibility and structure at the hydrogel surface. CG and all-atom modeling is performed based on the PCFF force field along with TIGER2 (CG) and TIGER2A (all-atom) accelerated sampling for efficient equilibration. This work was supported by RESBIO The National Resource for Polymeric Biomaterials funded under NIH Grant No. P41 EB001046 and the Center for Advanced Fibers and Films (CAEFF) at Clemson University, Clemson, SC. Computational support was provided by the Palmetto High Performance Computing Resource at Clemson University, Clemson, SC.

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Title of Abstract: Glioma Cell Invasion is Significantly Enhanced in Composite Hydrogel Matrices Composed of Chondroitin 4- and 4,6-Sulfated Glycosaminoglycans

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Abstract:
Glioblastoma multiforme (GBM) is an aggressive astrocytoma accounting for a majority of diagnosed primary malignant brain tumors in the United States. Chondroitin sulfate proteoglycans and their glycosaminoglycan (CS-GAG) side chains are key constituents of the brain extracellular matrix (ECM) implicated in tumor invasion. However, the mechanisms by which sulfated CS-GAGs promote glioma invasion are unknown. We hypothesize that glioma invasion is triggered by the altered sulfation of CS-GAGs within the tumor ECM, and that this is potentially mediated by independent mechanisms involving CXCL12/CXCR4 and LAR signaling, respectively. Sulfated CS-GAG and unsulfated agarose, or hyaluronic acid hydrogel matrices with similar biophysical properties were introduced as choices to U87MG-EGFP glioma cells within microfluidics devices designed to facilitate quantification of cell preference, migration, and colocalization of focal adhesion proteins. Our

results demonstrated enhanced preferential cell invasion into sulfated CS-GAG hydrogels when compared to other unsulfated hydrogel matrix controls (p

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Title of Abstract: Protein Nanocarrier for Targeted Intracellular Delivery of Functional Antibodies
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Abstract:
The cell membrane remains a formidable barrier for antibody-based therapeutics, and efficient intracellular delivery of functional antibodies may be critical for modulating important cell signaling mechanisms and protein interactions that are involved in various disorders. This study utilizes protein engineering techniques to develop a nanocarrier that is capable of delivering functional antibodies to the intracellular environment. Each nanocarrier is capable of delivering up to six functional antibodies. This novel design has great potential for many diverse applications due to its simplicity and biocompatibility.Œæ
The nanocarrier is produced through self-assembly of six recombinant proteins into a hexameric coiled coil. Six SPAB domains, which are connected to the central HEX domain via flexible linkers, are able to noncovalently bind to the constant region of antibodies, thus allowing

the nanocarrier to bind different types of targeting and therapeutic antibodies with good affinity. Three iRGD domains enhance the specific targeting properties of the nanocarrier by interacting with integrins and may also contribute to cellular uptake. Experimental results indicate significant cellular uptake of nanocarrier bound to IgG by HeLa and SK-BR-3 cells. Current work is focused on further enhancing specific trageting properties of the nanocarrier as well as endosomal escape efficiency.

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Title of Abstract: Ratiometric tension probes for mapping receptor forces and clustering at artificial intermembrane junctions
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Abstract:
Short-range communication between cells is required for the survival of multicellular organisms. One mechanism of chemical signaling between adjacent cells employs surface displayed ligands and receptors that only bind when two cells make physical contact. Ligandäóñreceptor complexes that form at the celläóñcell junction and physically bridge two cells likely experience mechanical forces. Herein, I will describe the development of ratiometric tension probes for direct imaging of receptor tension, clustering, and lateral transport within a model

celläóñcell junction. These probes, which are displayed on supported lipid membrane, employ two fluorescent reporters that quantify both the ligand density and the ligand tension and thus generate a tension signal independent of clustering. As a proof-of-concept, we applied the ratiometric tension probes to map the forces experienced by the T-cell receptor (TCR) during activation and showed the first direct evidence that the TCR-ligand complex experiences sustained pN forces within a fluid membrane junction. We envision that the ratiometric tension probes will be broadly useful for investigating mechanotransduction in juxtacrine signaling pathways such as Ephäóñephrin, cadherins, and Notch-Delta.

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Title of Abstract: Characterization of Compressive- & Tensile-Stressed Orthopaedic Ti6Al4V Corrosion Under Static Load
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Abstract:
TKA & THA show high global demand with 1.4/1.3 mil. knee/hip replacements performed in 2015. Approx. 10% of primary replacements fail & require revision. Mechanical loosening is the most common failure mode & is enhanced by stress. Stress-assisted corrosion at modular necks has previously been characterized yielding surface corrosion data for FEM application.
Depassivation & passive layer cycling effects on crack development are not well characterized. It is thought micron-scale passive layer cracking allows underlying metal oxidation, yielding sheet polarization & passive

layer repulsion. Micromotion is a known contributor to depassivation via this method but it confounds subtle underlying processes.
We constructed a potentiometric cell with Ti6Al4V (Ti(VI)) strips under variable 2-point bending stress to evaluate stressed corrosion behavior using linear polarization resistance, electrochemical impedance spectroscopy, & cyclic polarization. Results describe general & passivation corrosion behavior, which have suggested interesting passivation resistance properties for Ti(VI) at approx. 50%-75% “€Y. This study’s goal is to characterize corrosion processes of metallic strips in physiologically-similar electrolyte solution under stress, with micromotion removed as a confounding variable.
A 3-electrode system was made to test deformed strips using an electrolyte cell (1X PBS, pH 7.4). Ti* strips under two-point bending acted as primary electrodes. Plastic holders (4.29 & 5.29 in) held bent, variable-length Ti(VI) strips to strains proportional to experimental yield stress (0 to 125% “€Y in 25% intervals, Figure 1). Strip surfaces were epoxy-coated leaving 1 in2 apical sections exposed.
Tensile force/corrosion effects have been studied and current efforts study static compressive load effects. Strips are placed in holders, coated & brought to electric equilibrium. Polarization resistance (RP) is determined by LPR (-10-10 mV, 0.125 mV/s) & EIS (10-2-105 Hz). EIS collects impedance for equivalent circuit fitting & finds corrosion rate (icorr). CP measures active/passive icorr and breakdown potential by anodic sweep (ASTM F2129).
Tensile results showed increased passive & active corrosion at increased tensile strain. Greatest active/passive icorr and fastest repassivation occurred at 52% & 78% “€Y. Lower RP was observed for stressed samples compared with control. Current efforts study compressive effects on corrosion via this method. Greater understanding of the passivation/stress relationship will allow prolonged corrosion onset via design considerations.
Ti(VI) is chosen for orthopedics because its stable passive layer causes high corrosion resistance. Passivation differences can cause drastic

corrosion rate changes leading to failure. Characterizing static stress influence on passivity will enable optimization by avoiding risk states.

Jiro Nagatomi – Associate Professor
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Title of Abstract: Mechanical Characterization of Tetronic Hydrogel-Based Surgical Adhesive
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Abstract:
Previously, in our lab, Sanders and co-workers developed a synthetic hydrogel-based adhesive by modifying the structure of Tetronic 1107 to introduce acrylate (ACR) groups for chemical crosslinking and N-hydroxisuccinimide (NHS) to improve tissue bonding, improving off of the original work done by Cho et al. Tetronic polymer is an amphiphilic block copolymer of polypropylene oxide (PPO) and polyethylene oxide (PEO) with a central ethylene diamine that connects the 4 arms. One of the main reasons Tetronic polymer was selected was due to its attractive ability of thermal gelation. The problem with T1107-ACR is that it tends to gel at room temperature and interferes with the contact between the tissue microstructure and the adhesive. To correct this, a lower molecular weight Tetronic, T304, was incorporated to control gelation temperatures and to reduce hydrogel swelling. However, increased amounts of T304- ACR can lead to lower adhesive strengths. The goal is to determine the right ratio to be able and isolate the wanted properties to produce a tissue adhesive that has the most

balanced blend of mechanical strength, a useful degradation profile, and a reasonably controlled gelation temperature.
In order to test which ratio of T1107 and T304 has the most useful combination of properties, a lap shear test was conducted with two different blends. The first blend tested being 50:25:25 (T1107- Acrylate: T1107-NHS-Acrylate: T304 Acrylate): and the second being 75:25:0 by weight percent. In order to convert this hydrogel into an adhesive the addition of Dithiothreitol (DTT) is applied directly before application. In preparation of the lap shear test, we attached a collagen layer to 4x1 cm aluminum metal strips using a gel superglue. We then allowed the collagen sheet to cure onto the aluminum sheet overnight. Two strips were then attached using 60 ul of adhesive over a 1 cm squared area using the different blends on the collagen side. The main emphasis on running this test compared to past lap shear tests was to compare how the different blends responded to being soaked in a PBS solution for an extended period of time after application. In order to test this variable the adhesive was let to cure for an hour in an incubator, then half of both blends were placed in a PBS bath overnight (16.5 hours) while the other half were left in the incubator as a control. The PBS was then removed and bond strength was tested using a performing a tensile lap shear test, we recorded the bond strength for the different blends for both soaked and non soaked samples. The samples soaked in PBS had lower bond strength with the 50:25:25 blend giving us a mean value of 77.84 kPa, while the 75:25:0 blend showed a mean bond strength of 23.69 kPa. The dry samples with the 75:25:0 blend had a mean bond strength of 23.69 kPa, while the 50:25:25 blend had a mean bond strength of 77.84 kPa. These results provide evidence that incorporation of T304 not only slows down thermal gelation, but also prevents early swelling and weakening of the hydrogel-based adhesive. Further in vivo investigation, however, is needed to determine the efficacy of our surgical adhesive.

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Title of Abstract: Understanding nuclei formation and growth of gold nanoparticles within large- and giant- unilamellar liposomes (LUVs and GUVs)
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Abstract:
Liposomes are excellent candidates for the compartmentalized synthesis of AuNPs especially if the end-applications are in the field of biological probes, biosensors, and drug delivery. Such compartmentalized synthesis offers the possibility to: control particle size, control the environment of synthesis to include catalytically active components, and carry out synthesis in a highly organized solvent structure. Recently, Gudlur et al. (2015), described a simple method for the controlled synthesis of spherical AuNPs with an average size of 2.8 ± 1.6 nm, inside 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) liposomes using a photochemical method of synthesis which was first reported by McGilvray et al. (2006). This large difference in AuNP sizes between those synthesized within liposomes and those in the bulk solvent is presumably due to an effect of confinement as well as the presence of a lipid bilayer. The project aims to elucidate the role of (1) particle diffusion rates (2) peptides as capping agents, and (3) membrane lipid composition and surface properties, such as curvature on AuNP formation within large- and giant- unilamellar vesicle (LUVs and GUVs).

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Title of Abstract: Qualitative Regional Wear Analysis of Novel 3D-Printed Variable-Hardness Foot Orthotics

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Abstract:
Introduction: Foot ulcers due to chronic focal plantar pressure and poor circulation affect 25% of all diabetics1. Recent clinical outcome research supports the findings that the stresses that are felt by the feet that cause Diabetic Foot Ulcers (DFUs) can be lessened through the use of orthotics and encourage circulation to the feet with activity. It has been found that the use of available 3D printed materials in combination with a specialized design in the orthotic structure will enable the rapid fabrication of an orthotic that provides similar comfort to a traditional foot orthotic2. This recently developed novel 3D printed foot orthotic must also have the durability comparable to the traditional foot orthotic. The purpose of this study was to evaluate the wear of the novel 3D printed foot orthotic following a two-week volunteer trial.

Materials and Methods: Twenty (n=20) healthy male subjects (age 18-26 years, 160-250 lbs, 10-13 shoe size) were fitted with novel 3D printed orthotics and diabetic running shoes (i-Runner, HyLan Shoe Company, Owensboro, KY) to be worn during the duration of a two-week study, after which the novel orthotics were evaluated for signs of wear by specific region. Four different modes of wear were analyzed,

though only one is discussed here. The first mode evaluated is that of Mode A: visible tears or cracks in the material. Each of the three layers of the novel 3D printed orthotic was evaluated for this mode of wear: (A) top cover of Plastazote, (B) 3D printed insert with variable geometries, and (C) 3D printed base with variable hardness.

Results and Discussion: Components A and B were sub-divided into 10 anatomical regions (metatarsals, metatarsal heads (MTH), arch and heel), and component C was sub-divided into 14 regions that included 4 regions of the supportive rim. The majority of wear (by severity) was seen in the 3D-printed base with the 3D-printed insert (Figure 1B) as a close second with 7 of 9 regions showing Mode A wear. In 80% of the 20 3D printed bases, regions 13 and 14 of the base (Figure 1C) saw visible tears/cracks in the material. This suggests a need for design improvements to be made in this region. Of the surrounding anterior regions, region 4 saw wear in 30% of the 20 orthotics, while the regions 7-12 saw the majority of the rest of the wear in 30% of the 20 orthotics. The top-cover plastazote layer (Figure 1A) saw the least amount of wear, with only 5.3% of orthotics experiencing Mode A wear in regions 4, 9, and 10.

Conclusions: The regions that experienced the heightened incidence of qualitative wear are being further evaluated for design changes to allow for improved wear, comparable to that of the traditional orthotic.

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2Przestrzelski, B.T. et al., Novel Additive-Manufactured Foot Orthoticäó_, Proceeding of the 2016 ORS.

Mathew Stanford – Master's Candidate
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Title of Abstract: Development of Tissue Phantoms and a Surgical Simulator for Testing Mesh Implants in the Abdominal Wall
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Abstract:
Hernias are caused by the weakening of muscles in the abdominal wall and can be repaired with surgical mesh, a biomaterial implanted through open or laparoscopic surgery to reinforce the weak tissues. In the United States, more than 350,000 hernia repairs are performed each year making them the most common general surgical procedure. As hernia repairs and other open and laparoscopic procedures have developed, complications still remain related to tension and position of the implanted mesh, often requiring revision surgery. These complications prove the need for a handheld instrument to quantify mesh tension in situ and remove the subjectivity of probing with fingers. The purpose of this study is to use a prototype hand-held instrument to measure mesh tension implanted in simulated tissue phantoms.
The prototype instrument uses proprietary instrumentation to determine mesh-tissue composite stiffness calculated through MATLAB scripts. Tissue phantoms will be created using silicone gels with a range of densities and stiffness that simulate abdominal wall properties. Three different types of surgical mesh will be implanted into the tissue phantoms and a custom apparatus will also be developed to apply relevant surgical and physiological conditions. The prototype

instrument will be used to measure mesh tension under various in vitro conditions. Subsequent stiffness measurements will be acquired in vivo on porcine models with surgically induced hernias reinforced with mesh.
Preliminary results show a predictable relationship between mesh tension and stiffness in the simulated tissue phantoms. The instrument was able to detect changes in stiffness in response to changing mesh conditions on tissue phantoms. This relationship is reflected in the statistical difference between four mesh conditions overlaying the phantoms. This predictable relationship will be used to calculate a normalized mesh stiffness for a range of tissue phantom properties. The opinions of medical professionals and preliminary validation of the current instrument prototype have proven that a handheld surgical tool can be used as an alternative to larger, more complex machines for the in situ mechanical characterization of hernia mesh. This study reports further preliminary validation using mesh-gel composites, customized lab equipment, and animal models for the development of a more precise and refined instrument.

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Title of Abstract: Development and characterization of a microparticulate vaccine formulation for metastatic breast cancer
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Abstract:

Breast cancer is the most fatal form of cancer in females worldwide. In the United States alone, 1 out of 8 females suffer from breast cancer. Even with early diagnosis, 20% of women still develop metastases. Current therapies against breast cancer are mostly invasive and pose numerous adverse effects. Immunotherapy is being explored to provide a better treatment option for patients suffering from breast cancer. With this in mind, our purpose has been to formulate and evaluate a micro particulate therapeutic vaccine to provide a new line of therapy for metastatic breast cancer.

Murine breast cancer cell line 4T1 was used as source of antigens. Vaccine microparticles were prepared by encapsulating 4T1 tumor associated antigens in cellulose polymer using spray dryer technology. Total protein concentration of the whole cell lysate was determined by Bio-Rad total protein assay. In vitro characterization of microparticles (Scanning electron microscopy, Particle Size and Zeta potential) was conducted. In vitro innate immune response was determined by performing nitric oxide assay. Expression of surface co-stimulatory molecules on dendritic cells treated with vaccine microparticles and other controls were determined by flow cytometer using different markers (CD40, CD80, MHCI and MHC II).

The yield of the microparticulate vaccine following spray drying was 80±5% w/w. Total protein content of whole cell lysate was 3.48±1.25 mg/mL. The particle size was 1 - 4µm. Zeta potential was -7±2 mV. There were significantly higher amount of nitric oxide released in the supernatant of cells exposed to vaccine microparticles compared to blank microparticles. CD40, MHC II and CD80, MHC I expression were significantly higher in the vaccine microparticles group compared to blank microparticles and vaccine suspension group.

The micro particulate metastatic breast cancer vaccine was effectively formulated via the spray drying technique and was evaluated for its in vitro profile.e