**Oral Presentation- Proteins & Cells at Interfaces**

Alison Douglas-PhD Candidate

Email: adouglas30@gatech.edu

Affiliation: Biomedical Engineering

Title of Abstract: Enhancing cell motility and angiogenesis in dense fibrin-based biomaterials

Presenting Author: Alison, M., Douglas

Affiliation: BME

Complete Author's List :

Alison M. Douglas (1), Laxminarayanan Krishnan (2), Ashley B. Allen (2), Alberto Fernandez-Nieves (3), L. Andrew Lyon (4), Robert E. Guldberg (2), and Thomas H. Barker (1,2,5)

All Author Affiliations :

Biomedical Engineering, Georgia Institute of Technology (1); Bioengineering, Georgia Institute of Technology and Emory University (2); Physics, Georgia Institute of Technology (3); College of Science and Technology, Chapman University (4); Chemistry and Biochemistry, Georgia Institute of Technology (5)

Abstract:

Fibrin, the natural hemostasis system, is a clinically attractive biomaterial for tissue engineering and regenerative medicine applications since its polymerization and degradation byproducts stimulate early wound repair events, such as endogenous cell infiltration and angiogenesis. However, in order to be used as a tissue sealant or for applications where gel stiffness is crucial (> 100s Pa), fibrin must be polymerized at higher than physiological concentrations. Although these supraphysiological gels provide more robust mechanical properties, their strength is linked to high concentrations where cell infiltration is inhibited. This migration/mechanical strength tradeoff is common to all polymer systems where strength of the polymer is proportional to the number of crosslinks, which determines the mesh/pore size, a critical limiting factor in cell invasion. We have engineered hybrid matrices through the addition of ultra soft microparticles (microgels), which maintain the mechanical properties of the bulk fibrin gel, but allow for enhanced cell spreading, motility, and angiogenesis in these dense polymer networks. When incorporated during polymerization, microgels partition and assemble into interconnected pocket domains. Additionally, we have found that the architecture of the microgel colloidal assembly within fibrin can be tuned through changing the concentration of microgels or modulating polymerization kinetics (i.e. altering thrombin concentration). Live cell migration data of fibroblasts and mesenchymal stem cells indicate that migration velocity is increased with increasing microgel concentration. Interestingly, these results have been confirmed both in the presence and absence of the protease inhibitor, aprotinin. Therefore, this system could serve as a potential new strategy for enhancing proteolysis-independent cell spreading and motility within dense extracellular matrices. Ex vivo angiogenesis assays utilizing microvessel fragments isolated from rat epididymal adipose tissue demonstrate enhanced sprouting and neovessel formation in constructs containing microgels compared to fibrin only controls. Additionally, preliminary data indicate that these constructs enhance vascular volume fraction in a rat subcutaneous implant model as measured with micro-CT imaging of perfused vasculature. These results taken together indicate that these constructs could be ideal engineered matrices for therapeutic angiogenesis.

**Oral Presentation- Rationally Designed Biomaterials**

Graham Temples-

Email: gtemple@g.clemson.edu

Affiliation: Clemson Bioengineering

Title of Abstract: Folate-functionalized polymeric micelle delivering combinatorial therapy to overcome drug resistant breast cancer

Presenting Author: Graham M. Temples

Affiliation: Clemson University Bioengineering

Complete Author's List :

Graham Temples, Jeoung Soo Lee

All Author Affiliations :

Department of Bioengineering, Clemson University, Clemson, South Carolina

Abstract:

Cancer continues to be one of the leading causes of death worldwide, with breast cancers accounting for nearly fourteen percent of all cancer related deaths in women. Prognosis is intimately tied to surface protein expressions; often growth promoting elements such as Human Epidermal Factor Receptor-2 or drug-resistance inducing P-glycoprotein are overexpressed. With breast cancers possessing high nucleic acid synthesis requirements, another marker often overexpressed is folate receptor alpha, a protein with a fortunately limited distribution elsewhere in the body. This potential expression differential was exploited as a means to selectively target breast cancer by conjugating a folate (FA) moiety to the surface of PgP (Poly (lactic-co-glycolic acid)-graft-polyethylenimine) micelle for targeted delivery of siRNA and chemotherapeutic agents. PgP was synthesized and characterized by 1H-NMR and GPC. To synthesize FA-PgP, FA was conjugated to the surface of PgP using Mal-PEG-SVA as a spacer. Following synthesis and purification, the structure of FA-PgP was characterized by 1H-NMR. FA-PgP exhibited selectivity when comparing the transfection efficiencies (GFP) against PgP in folate receptor alpha positive (MCF-7) and negative breast cancer lines (MDA-MB-468). Complexes constructed as mixed micelles comprised of FA-PgP and PgP demonstrate increased transfection efficiency compared to micelles constructed of FA-PgP alone, likely the result of the reduction of steric hindrance at the receptor site. Free folic acid performed as competitive inhibitor against FA-PgP, indicating pathway dependent sequestration. Future work includes co-administration of siRNAs targeting P-glycoprotein with chemotherapeutics such as Doxorubicin or Paclitaxel to overcome drug resistance in breast cancers.

**Oral Presentation- Biomaterials Design for Tissue Repair**

Juana Mendenhall-Assistant Professor

Email: juana.mendenhall@morehouse.edu

Affiliation: Morehouse College

Title of Abstract: The Effect of Hypoxia on Thermosensitive Poly(N-vinylcaprolactam) Hydrogels for Cartilage Tissue Engineering

Presenting Author: Juana Mendenhall

Affiliation: Morehouse College

Complete Author's List :

All Author Affiliations :

Abstract:

Injury and diseases that affect articular cartilage present a daunting challenge in orthopaedic medicine. During the onset of injury or disease, low oxygen environments decrease healthy cartilage cell growth reducing the efficacy of injectable hydrogels that maybe employed in a defective knee joint.1 Hence the use of injectable hydrogels with thermosensitive properties having therapeutic efficacy is needed for clinical applications in orthopeadic medicine. Poly(N-vinylcaprolactam)[PVCL] is a biocompatible temperature-responsive material that changes configuration in response to temperature it does not undergo hydrolysis overtime. PVCL displays a lower critical solution temperature (LCST) ranging from 32ºC-40ºC, that always it to form reversible gels. We have developed a therapeutic injectable composite hydrogel system that contains PVCL and hyaluronic acid (HA). In conjugation with hyaluronic acid, this thermosensitive hydrogel affords a robust hydrogel with tunable LCST parameters near physiological temperature. Hydrogels comprised of n-vinylcaprolactam, hyaluronic acid were prepared via free radical polymerization. Fetal bovine chondrocytes were harvested and seeded on various formulations of PVCL-HA under normoxia (21%) and hypoxia (1%) low oxygen conditions at 37°C. Cell viability was checked using fluorescent microscopy imaging of calcein AM and ethidium homodimer-1(EthD-1) kit according to manufactures protocols. hydrogel samples (n=4) were washed with PBS at 37°C then incubated with 2mM calcein AM and 4mM EthD-1 for 40 minutes at 37°C. Cell viability, collagen, DNA, and sulfated glycosaminglycans (GAG) was analyzed on Day 1, Day 3, Day 10 using fluorescent microscopy and cell viability percent was obtained. Chondrocyte cell viability and metabolism was investigated at 1% and 20% O2 level, respectively. Viability of cells was maintained for all samples throughout the 10 day time period. However, cell viability at 1% O2 levels remained higher than that of 20% O2 levels in PVCL-HA for each time point. PVCL-g-HA hydrogels reached a maximum of 89% on the third day of observance. Higher cell viability was also noted on meHA samples at 1% O2 levels than at 20% O2 levels, with the peak value at 74% on the first day of observance.

**Oral Presentation- Stem Cell-Biomaterial Interactions**

Jennifer Lei-Graduate Student

Email: jlei3@gatech.edu

Affiliation: School of Mechanical Engineering, Georgia Tech

Title of Abstract: Heparin Coating for Controlled Biomolecule Presentation to Mesenchymal Stem Cell Spheroids

Presenting Author: Jennifer Lei

Affiliation: Georgia Institute of Technology

Complete Author's List :

Jennifer Lei (1), Louis McLane (2), Jennifer E. Curtis (2), Johnna S. Temenoff (3)

All Author Affiliations :

School of Mechanical Engineering, Georgia Tech (1); School of Physics, Georgia Tech (2); Department of Biomedical Engineering, Georgia Tech/Emory University (3)

Abstract:

Mesenchymal stem cells (MSCs) have been used as cell therapies to aid in regeneration of a variety of injured tissue. Moreover, MSCs aggregated into small spheroids have enhanced anti-inflammatory properties over single MSCs grown in monolayer. However, MSC-based therapies may be rendered ineffective due to lack of control over cell fate post-injection. Therefore, we aim to develop a thin film coating for MSC spheroids to allow for simultaneous delivery of both cells and a soluble factor to direct cell differentiation. This system uses multilayer deposition of biotin and avidin to graft heparin onto cell surfaces. Heparin is a negatively charged glycosaminoglycan that can sequester and release positively charged proteins3. The objectives of this study were to characterize the heparin coating and its effects on MSC viability and function, as well as the bioactivity of the growth factor (TGF-β1) subsequently sequestered on spheroid surfaces. Biotin-heparin (heparin) and the model protein, histone, were fluorescently tagged for imaging. MSCs and mink lung epithelial cells (MLECs) were coated as single cells in suspension with sulfo-NHS-biotin (4mM), avidin (0.5mg/mL) and heparin (5μg/mL, 1mg/mL 5mg/mL). Each layer was incubated for 30 minutes with washing steps between layers. Once coated, 1000-cell MSC spheroids were formed using forced aggregation in AggreWells. Imaging was performed using a Zeiss LSM 700 confocal microscope and analyzed in ImageJ. For particle exclusion assays, cells were seeded in monolayer, coated with heparin and incubated with 3μm polystyrene beads. Separation of beads from cell membrane surface was quantified as the pericellular matrix thickness. To examine protein bioactivity, MLECs were used as a biological reporter of TGF-β1 activity. MLECs are transfected with a luciferase reporter gene that is expressed in response to TGF-β1. MLECs were coated and loaded with 0pg/mL, 3000pg/mL or 3μg/mL TGF-β1 and formed into 500-cell spheroids. Control aggregates were loaded with protein without heparin coating. After 24 hours, aggregates and supernatant was collected. Cells were assayed for luminescence using ONE-GloTM Luciferase assay buffer and supernatant was suspended over MLECs in a 96-well plate for 24 hours until luminescence was measured. Statistical significance was determined by one- or two-way ANOVA with Tukey’s post hoc test (p<0.05). MSCs were successfully coated using the multilayer technique to graft heparin onto the surface without affecting cell viability. Results of the particle exclusion assay demonstrated that the heparin coating prevented pericellular matrix accumulation over 1 day, suggesting that this is a viable method to control loading and surface presentation of growth factors in the given timescale. A higher initial coating concentration resulted in more heparin on cell surfaces. Over 14 days, fluorescent quantification showed a 40% loss of heparin for all three groups. These results indicate that coatings can be tuned by using different initial concentrations. MLECs were used to study the bioactivity of sequestered and released TGF-β1. Without heparin, there was minimal protein localized to cell surfaces, however, when heparin was present the model protein histone is observed on cell surfaces. Coated MLECs had significantly higher luminescence reported compared to noncoated samples at each concentration. Supernatants taken from coated aggregates with TGF-β1 loading of 3000pg/mL and 3μg/mL elicited a luminescence response significantly higher than their noncoated counterparts. These results suggest that a protein loaded onto these coatings remains bioactive after sequestration and release. Through these studies, we have developed a multilayer coating system to graft heparin and facilitate protein sequestration onto MSC spheroid surfaces without loss in cell viability and while maintaining growth factor activity over 24 hours. In the future, this simple and efficient method of presenting growth factors to stem cell aggregates may have significant implications in enabling local signaling between transplanted cells and surrounding tissue post-injection.

**Rapid Fire Presentations & Posters: Proteins & Cells**

Poster # 1

Tigran Abramyan-Graduate Research Assistant

Email: tabramy@clemson.edu

Affiliation: Clemson Bioengineering

Title of Abstract: Cluster Analysis of Ensembles of Conformational States of Adsorbed Proteins in Molecular Dynamics Simulations of Protein Adsorption

Presenting Author: Tigran Abramyan

Affiliation: Clemson Bioengineering

Complete Author's List :

Tigran Abramyan (1), Steven J. Stuart (2), Robert A. Latour (3)

All Author Affiliations :

Department of Bioengineering, Clemson University (1); Department of Chemistry, Clemson University (2); Department of Bioengineering, Clemson University (3)

Abstract:

All-atom molecular dynamics (MD) simulations hold a great promise as a valuable tool for understanding and predicting protein-surface interactions. However, experimental data is required in order to tune and validate these computational methods before they can be utilized with confidence. The main difficulty in direct comparison of experiments with simulations, however, is that experiments provide averaged properties of billions of adsorbed proteins over timeframes of seconds while conventional MD gives results for a single adsorbed protein over tens of nanoseconds. To address this issue, we use an advanced MD sampling algorithm, TIGER2, to generate an equilibrated Boltzmann-weighted ensemble of a molecular system (different adsorbed orientations and conformations of the protein on the surface of a material), from which equilibrated ensemble-average properties can be calculated for direct comparison with experiment. MD simulation methods produce trajectories of atomic positions (and optionally velocities and energies) as a function of time and provide a representation of the sampling of a given molecule’s energetically accessible conformational ensemble. Cluster analysis techniques can be used for the analysis of this generated ensemble, where individual conformations are clustered (grouped) into similar types based on their orientation and conformation relative to the surface. Application of the clustering algorithms can identify highly occurring conformations of the adsorbed state of the protein in the simulations. However, cluster analysis methods for analyzing MD trajectories have traditionally been developed for the analysis of protein structure in solution, but not for an ensemble of proteins in their adsorbed state. The objective of this research was, therefore, to develop and apply a cluster analysis method that can be used for the analysis of a large ensemble of states obtained from a simulation of protein adsorption behavior. The application of cluster analysis in the protein-adsorption simulations requires a different approach from regular protein-in-water simulations to structurally align adsorbed protein. For adsorbed-protein simulation, it is important to compare both tertiary conformation of the adsorbed state of the protein and its orientation over the material surface while tertiary conformational comparison alone is required for the analysis of solution behavior. We evaluate clustering analysis methods that addressed both orientation and conformation. Several well-known clustering algorithms, including hierarchical and partitional clustering techniques were examined to establish and validate an algorithm that was well-suited for the analysis of adsorbed protein configurations.

Poster # 2

Guillemro Alas-PhD Candidate

Email: galas3@gatech.edu

Affiliation: Chemistry, Georgia Institute of Technology

Title of Abstract: Protein and cell resistant brush polymer on medical grade stainless steel

Presenting Author: Guillermo R. Alas

Affiliation: Chemistry, Georgia Institute of Technology

Complete Author's List :

Guillermo R. Alas David M. Collard Andrés J. García

All Author Affiliations :

Chemistry, Georgia Institute of Technology; Chemistry, Georgia Institute of Technology; Mechanical Engineering, Georgia Institute of Technology

Abstract:

The limited lifespan of stainless steel implants as bone fixation devices (e.g., screws, pins and plates) arises from the failure of the implant to properly integrate into the host tissue. To enhance the interaction between steel and bone tissue, we have chemically modified the surface chemistry of 316L stainless steel with a high-density polymer brush that impedes non-specific protein adsorption and cell binding onto the surface. Stainless steel is modified with a monolayer of a silane that contains an initiator for surface-initiated atom transfer radical polymerizations (SI-ATRP). Polymerization of oligo(ethylene glycol) methacrylate (OEGMA) provides a hydrophilic brush on the surface that resists adhesion of proteins and cells. Peptide and protein ligands may be immobilized on the surface by coupling to the termini of the oligo(ethylene glycol) to promote host-implant interactions.

Poster # 3

Elizabeth Campbell-

Email: ecampbell3@gatech.edu

Affiliation: Biomedical Engineering, Georgia Institute of Technology

Title of Abstract:

Presenting Author: Elizabeth A. Campbell

Affiliation: Georgia Institute of Technology

Complete Author's List :

Campbell, E.A., Tang, J.L., Sulchek, T.

All Author Affiliations :

Biomedical Engineering, Georgia Institute of Technology(1); Mechanical Engineering, Georgia Institute of Technology (2,3)

Abstract:

T cells are cytotoxic lymphocytes that kill tumor cells by releasing granules containing perforin and granzymes that create pores in the target cell and trigger apoptosis. Our laboratory’s interest has been focused on the development of a new class of anticancer agents that locally amplify the immune response in a cancer-specific manner by artificially recapitulating the immunological recognition synapse. By activating cytotoxic immune cells with a janus particle, capable of binding to cancer cells and activating immune cells, apoptosis in cancer cells may occur through proximity, regardless of the tumor microenvironment. This general idea has already proven to have some degree of success with bispecific antibodies. However, the increased avidity associated with our particles suggests we can improve this response. Janus microparticles have been synthesized by sputtering gold onto a monolayer of carboxylated polystyrene particles. We specifically functionalized the respective surfaces using standard EDAC-NHS chemistry for the polystyrene component and thiol-PEG-biotin with strepavadin and NHS-PEG-biotin for the gold hemisphere. This fabrication method was validated by flow cytometry, confocal microscopy, and electron microscopy. Human anti-CD3 was conjugated to 3.0μm, 1.0μm, and 0.3μm carboxylated polystyrene microspheres using standard EDAC-NHS chemistry at different densities. The success of this conjugation was confirmed by labeling with fluorescent secondary antibody and measuring mean fluorescent intensity and fluorescent microscopy imaging. Bright field living cell imaging was used to visualize the interaction between the microparticles and immune cells so that an appropriate microparticle: cell ratio could be determined. Granzyme b analysis was performed on CD8+ T cells to determine the effects of anti-CD3 antibody presentation on the extent of cytolytic activity by the cells. Using confocal microscopy, two distinct areas of fluorescence indicated the successful bifunctionalization of the microparticle. This fluorescent signal was clearly confirmed using flow cytometry. The wholly-coated microparticles clearly interact with the Jurkat cells in a manner completely different than uncoated microparticles. ELISA analysis of granzyme b secretion demonstrated not only the ability of the microparticles with bound CD3 antibody to elicit cytolytic activity from the T cells, but that the response was dose-dependent and ligand-density dependent. These results indicate that microparticle-bound CD3 antibody is capable of modulating cytolytic activity in vitro. An increase in the secretion of granzyme b coupled with our existing expertise Janus particle formation suggest that delivering anti-CD3 via Janus particle treatment may be a viable therapy to kill cancer cells.

Poster # 4

Varun Chawla-Mr.

Email: VCHAWLA@CLEMSON.EDU

Affiliation: Department of Bioengineering, Clemson University

Title of Abstract: Effects of Clinically Relevant Mechanical Forces on Vascular Smooth Muscle Cells Under Hyperglycemia: An In Vitro Dynamic Disease Model

Presenting Author: Varun Chawla

Affiliation: CLEMSON UNIVERSITY

Complete Author's List :

Varun Chawla (1), Agneta Simionescu Ph.D. (1), Eugene M Langan III M.D. (2), Martine LaBerge, Ph.D. (1)

All Author Affiliations :

Department of Bioengineering, Clemson University (1); Department of Surgery, Greenville Health System (2)

Abstract:

Vascular complications are the leading cause of morbidity and mortality amongst diabetic patients, which represent nearly 25-30% population undergoing coronary artery revascularization procedures. In stent restenosis is a common occurrence in the current revascularization era with diabetic patients having accelerated neointimal hyperplasia and higher rates of restenosis. Dedifferentiation of SMCs to a synthetic/proliferative phenotype is associated with the development of restenosis. Clinically these cells experience cyclic strain from arterial distension and low wall shear stress due to disturbed flow post a stent strut or from transmural fluid flow. Effects of these mechanical forces SMCs when exposed to diabetes-associated complications remains untested. We hypothesize that exposure of VSCMs to clinically relevant mechanical forces under high glucose conditions would lead to a more ‘synthetic’ phenotype and may be contributive to exaggerated intimal hyperplasia in diabetic patients. P3-P5 rat aortic SMCs were seeded on type I collagen-coated silicone attached onto a 6-channel custom simulator connected to FlexCell 2000. Cells were stretched to 0-7% at 1Hz. Flow was provided to induce a wall shear stress of 0.25-0.5 dynes/cm2. Cells were maintained in DMEM with 10% FBS containing either 5.5 mM glucose for our low glucose concentration experimental group (LG) or 25 mM glucose for chronic hyperglycemia group (CG). In the acute hyperglycemic condition (AG), cells were cultured in 25 mM glucose for 84 hours. Media was replaced with DMEM containing 1% FBS and 1% Dextran 24 hr prior to a simulation to induce quiescence and adjust media viscosity respectively. Cell proliferation was quantified using CyQuant assay and Rhodamine-Phalloidin stain was used to image cells, which were analyzed by ImageJ software for cell area. SMCs under acute hyperglycemia showed the highest cell count (AG CT 44593 ± 5089) with nearly the same response shown by chronic hyperglycemic cells (CG CT 33026 ± 3455) when compared to low glucose stretched samples (LG CT 28641 ± 2861, p-value<0.05). Cells in the all the 3 groups showed a lower rate of proliferation when exposed to cyclic strain compared to controls (non-stretched or U): LG U 40213 ± 5009, AG U 60300 ± 3569, CG U 62473 ± 4594, p-value<0.05. Cellular hypertrophy decreased with application of stretch but was higher for acute (3928 ± 1200 μm2) and nearly the same for chronic samples (2501 ± 825 μm2) when compared to low glucose control (2331 ± 637 μm2 p-value<0.05). Non-stretched samples had higher cell area with both AG (5281 ± 2085 μm2) and CG samples (5702 ± 1806 μm2) demonstrating higher cellular hypertrophy compared to low glucose (2641 ± 1328 μm2, p-value<0.05). Results have shown that hyperglycemia would be contributive to the phenotypic modulation of RASMCs under mechanical stress and is associated with higher cellular hypertrophy and cell proliferation for both acute and chronic exposure. Current work involves comparing the combined effects of cyclic stretch and flow shear on SMCs under three different kinds of glycemic exposure.

Poster # 5

Jhilmil Dhulekar-Graduate Research Assistant

Email: jdhulek@clemson.edu

Affiliation: Clemson University

Title of Abstract: Delivery of Polymeric Nanoparticles Loaded with Non-Toxic Drug to Overcome Drug Resistance for the Treatment of Neuroblastoma

Presenting Author: Jhilmil Dhulekar

Affiliation: Clemson University

Complete Author's List :

Jhilmil Dhulekar, Olivia DeCroes, Stuart Grimes, Ian Hale, Paige Urig, Thomas Moore, Frank Alexis

All Author Affiliations :

Bioengineering, Clemson University

Abstract:

Neuroblastoma is a rare cancer of the sympathetic nervous system. A neuroblastoma tumor develops in the nerve tissue and is diagnosed in infants and children. Approximately 10.2 per million children under the age of 15 are affected in the United States and it is slightly more common in boys. Neuroblastoma constitutes 6% of all childhood cancers and has a long-term survival rate of only 15%. There are approximately 700 new cases of neuroblastoma each year in the United States. With such a low rate of survival, the development of a more effective treatment method is necessary. A number of therapies are available for the treatment of these tumors; however, clinicians and their patients face the challenges of systemic side effects and drug resistance of the tumor cells. The application of nanoparticles has the potential to provide a safer and more effective method of delivering drugs to tumors. The advantage of using nanoparticles for drug delivery is the ability to specifically or passively target tumors while reducing the harmful side effects of chemotherapeutics. Drug delivery via nanoparticles can also allow for lower dosage requirements with controlled release of the drugs, which can further reduce systemic toxicity. The aim of this research was to develop a polymeric nanoparticle drug delivery system for the treatment of high-risk neuroblastoma. Nanoparticles composed of a block copolymer were formulated to deliver a non-toxic drug in combination with a commonly used chemotherapeutic drug for the treatment of neuroblastoma. The non-toxic drug acts as an inhibitor to a factor present in neuroblastoma that is responsible for inducing drug resistance by the cells, which would potentially allow for enhanced activity of the therapeutic drug. A variety of studies were completed to prove the nanoparticles’ low toxicity, loading abilities, and uptake into cells. Additionally, studies were performed to determine the individual effect on cell toxicity of each drug and in combination. Finally, nanoparticles were loaded with the non-toxic drug and delivered with the free therapeutic drug to determine the overall efficacy of the drugs in reducing neuroblastoma cell viability.

Poster # 6

SUCHETA D'SA-Student

Email: sucheta.dsa@live.mercer.edu

Affiliation: Mercer University

Title of Abstract: In vitro immunogenicity assessment of whole cell lysate melanoma vaccine and adjuvant microparticles

Presenting Author: Sucheta D'Sa

Affiliation: Mercer University

Complete Author's List :

1. Sucheta D'Sa 2. Ashwin Chandrashekhar Parenky 3. Rikhav Praful Gala 4. Martin J. D'Souza

All Author Affiliations :

1. Sucheta D'Sa, Mercer University 2. Ashwin Parenky, Mercer University 3. Rikhav Praful Gala, Mercer University 3. Martin J. D'Souza, Mercer University

Abstract:

Objective: Induction of an immune responses following mucosal immunization is often dependent on co-administration of adjuvants that can initiate and support the transition from innate to adaptive immunity. In this study, melanoma whole cell lysate vaccine microparticles were combined with various adjuvants to evaluate the combination’s result on the immune response. This will enable the selection of a suitable adjuvant for future in vivo oral immunization studies based on CD40, CD80/86 and MHC I/II expression in antigen presenting cells. Methods: Whole cell lysate of B16F10 (murine metastatic melanoma cell line) was prepared using hypotonic lysis buffer. The microparticles were sprayed with a unique combination of cellulosic polymers for oral delivery using a Buchi 290 spray dryer. In this study, six different adjuvants were used viz. alum, CpG, cholera toxin, P4, Monophosphoryl Lipid A (MPL®), MF59®, Resiquimod (R848®). Adjuvant microparticles were formulated and sprayed in a similar manner as the whole cell lysate vaccine. Vaccine microparticles were characterized for size, charge and release pattern. Innate immune response elicited by dendritic cells on incubation with vaccine along with adjuvant microparticles was analyzed by measurement of nitric oxide using Greiss reagent. We evaluated the results of vaccine microparticles with and without adjuvant microparticles on expression levels of co-stimulatory molecules CD40, CD80/86 and MHC I/II (markers of antigen presentation) on dendritic cells using the flow cytometer. CD80/86 are markers necessary for T cell activation and survival. CD40 marker activates antigen presenting cells. MHCI/II present antigenic protein fragments to T cells. Blank microparticles, vaccine solution and adjuvant solution served as control groups for the study. Results: After spray-drying, the product yield was found to be in the range of 85-90 % w/w. The encapsulation efficiency was calculated as 65% w/w. The particle size ranged from 2-6 microns and average zeta potential was -11± 2.0 mV. Microparticles were non-toxic in nature and helped sustain the release of cancer antigens in simulated gastric and intestinal pH conditions. Vaccine and/or adjuvants given in microparticulate form induced significantly higher CD40, MHC II and CD86 expression compared with solution groups. Conclusion: Vaccine tested along with adjuvant microparticles induced a higher immune response when compared with vaccine alone.

Poster # 7

Rikhav Gala-Graduate Research Assistant

Email: 10924110@live.mercer.edu

Affiliation: Vaccine nanotechnology laboratory, College of Pharmacy, Mercer University

Title of Abstract: Formulation of oral particulate ovarian cancer vaccine

Presenting Author: Rikhav P Gala

Affiliation: Mercer University

Complete Author's List :

Rikhav P Gala, Nihal S Mulla, Sucheta D’Sa, Martin D’Souza

All Author Affiliations :

Vaccine nanotechnology laboratory, College of Pharmacy, Mercer University

Abstract:

Purpose: The aim of this project was to enhance the immunogenicity of ovarian cancer vaccine using a combination of multiple adjuvants by targeting different Toll-like receptors (TLR) pathways. The ability of vaccine microparticles to induce an immune response was evaluated in an in-vitro setting using antigen presenting cells. Vaccine microparticles were combined with different adjuvants acting upon different pathways is evaluated to induce a higher immune response. Methods: Ovarian cancer microparticulate vaccine was prepared by entrapping whole cell lysate of murine ovarian cancer ID8 cells in a polymer matrix of enteric polymers using a Buchi spray dryer (B-290). Adjuvants improve the immune response of the vaccine and its overall effectiveness. In this study we used 6 different adjuvants viz. alum, Monophosphoryl Lipid A (MPL®), MF59®, CpG, Resiquimod (R848®), Flagillin, P4. Adjuvants microparticles were also formulated in the similar way as the vaccine. Vaccine microparticles were characterized for size, charge and release. Innate immune response elicited by macrophages on incubation with vaccine particles and adjuvant particles was analyzed by measurement of nitric oxide using Greiss reagent. CD 80 and 86 are co-stimulatory molecules required for binding of antigen presenting cells to T-cell. Effect of vaccine microparticles on the level of MHC 1, MHC 2, CD80 and CD86 expression was measured (Accuri C6 flow cytometer) as its ability to enhance antigen presentation. Blank microparticles, vaccine solution and adjuvant solution served as the control groups for the aforementioned experiment. Results: Vaccine microparticles along with adjuvant induced significantly higher amount nitric oxide levels compared to control groups. CD 80 and CD 86 levels were elevated in groups receiving adjuvants (R848®, MF59® and alum) along with the vaccine as compared to only vaccine (p<0.001). A combination of Alum and MF59 induced a significantly higher response than individually (p<0.05). The higher response can be attributed to the different pathways of both alum and MF59. Conclusion: Microparticulate vaccines along with two adjuvants acting on different TLR, help inducing a more effective immune response and improved antigenicity of the ovarian cancer vaccine. For the success of such immune therapies, a combination of adjuvants would be imperative for further studies.

Poster # 8

Astha Khanna-Bioenginering department, Clemson University

Email: akhanna@clemson.edu

Affiliation: PhD candidate, Clemson University

Title of Abstract: Fabrication of Human Serum Albumin nanofilms for enhanced Hemocompatibility and Smooth Muscle Cell response

Presenting Author: Astha Khanna

Affiliation: PhD candidate

Complete Author's List :

Astha Khanna, Igor Luzinov, Ph.D., Fehime Vatansever, Ph.D , Eugene Langan III, MD, Martine LaBerge, Ph.D.

All Author Affiliations :

Astha Khanna(1), Igor Luzinov, Ph.D.(2), Fehime Vatansever, Ph.D (2), Eugene Langan III, MD(3), Martine LaBerge, Ph.D.(1) Department of Bioengineering, Clemson University(1); Department of Material Science & Engineering, Clemson University(2); Greenville Health System, Greenville, South Carolina(3)

Abstract:

Restenosis and thrombosis are two major clinical complications of endovascular stents. In this study, a novel bio-polymeric nanofilm of Human Serum Albumin (HSA) grafted on epoxy containing polymer poly (glycidyl methacrylate) (PGMA) is proposed as a drug delivery system reducing both neo-intimal hyperplasia by inhibiting smooth muscle cell (SMC) proliferation as well as shielding fibrinogen (Fg) adsorption and platelet adhesion thereby reducing thrombosis incidence. Thermal properties of freeze-dried HSA powder were measured by Differential Scanning Calorimetry(DSC) and Thermogravimetric Analysis(TGA). Plasma treated Nitinol (NiTi) discs were surface modified with 0.5% wt/vol PGMA (Mn=176000g/mol) in chloroform to produce an epoxy rich anchoring layer. PGMA functionalized discs were then dipped in 5% wt/vol HSA in deionized water for 2 hours at 37˚C for albumin deposition after which the solution was pumped out. Samples were then annealed at 155˚C vacuum oven for 1 hour. HSA grafted samples were kept overnight at 37˚C and cleaned and sterilized prior to experiments. HSA grafted samples were incubated in Rhodamine-NHS labeled Human Plasma Fibrinogen (1mg/ml, 0.5mg/ml, 0.25mg/mL and 0mg/mL) solution in PBS for 1 hour at 37˚C. PEG grafted discs and silicon membranes were used as negative and positive controls, respectively. Fluorescence was measured at 525nm and 575nm. Platelet-rich plasma isolated from fresh bovine blood was incubated on HSA nanofilms and controls for 1 hour at 37˚C. Platelet adhesion was measured by lactate dehydrogenase (LDH) assay. Sprague Dawley rat aortic SMCs were cultured on HSA nanofilms and controls to confluence for 48 hours in DMEM with 10% FBS and quantified by CyQuant cell proliferation assay at 480nm and 520nm. Thermal analysis shows that onset of degradation of HSA (200˚C) is unaffected by the annealing temperature (150˚C), also confirmed by FT-IR spectroscopy and CD spectroscopy. The thickness of the PGMA (12.5±0.4nm) and HSA layer (90±4.5 nm) were determined through ellipsometry on silicon wafers. The surface roughness of the coating (0.34±0.02nm) was studied by atomic force microscopy.There was significantly less human plasma fibrinogen adsorption on HSA nanofilms (90±9.8ng/mm2; p<0.05) as compared to bare SS. Adherent platelets from bovine platelet-rich-plasma on HSA nanofilms were quantified using the LDH assay. Results showed minimal platelet adhesion (2.73±1.2%; p<0.05) on HSA nanofilms. There was significantly less rat aortic SMC proliferation (37 ± 1.8%; data presented as mean±SD; n=5, p<0.05) measured on HSA nanofilms for 48 hours as compared to bare NiTi.

Poster # 9

Elliott Mappus-Effect of heparin-magnetite nanoparticles on vascular smooth muscle cell proliferation

Email: emappus@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: Effect of heparin-magnetite nanoparticles on vascular smooth muscle cell proliferation

Presenting Author: Elliott D. Mappus

Affiliation: Clemson University

Complete Author's List :

Elliott D. Mappus, O. Thompson Mefford, Delphine Dean

All Author Affiliations :

Bioengineering, Clemson University (1); Materials Science and Engineering, Clemson University (2); Bioengineering, Clemson University (3)

Abstract:

Vascular stents are widely used to treat atherosclerosis. Two of the complications with the procedure are an associated risk of thrombogenesis and intimal hyperplasia. One treatment option is systematic administering heparin treatment to activate antithrombin III leading to deactivation of thrombin and other proteases involved in blood clotting. This treatment is associated with high rates of bleed and other vascular complications. In addition to the widely known anti-coagulation effects, heparin has long been known to exhibit an anti-proliferative effect on the growth of cells. This study attempts to create a heparin magnetite nanoparticle in hope that the adhesion to the nanoparticle will moderate the anti-coagulation effect of heparin while inhibiting growth of vascular smooth muscle cells. We show the feasibility of this treatment option by first characterizing synthesized magnetite nanoparticles with zeta potential and dynamic light scatting. Cell cytotoxicity studies will confirm heparin nanoparticles are biocompatible with VSMCs at expected treatment concentrations. Differentiation of VSMCs will be noted by measuring the α-smooth muscle actin expression and atomic force microscopy measurement of cellular elastic modulus.

Poster # 10

Nihal Mulla-PhD, M.S

Email: nihal.shamsuddin.mulla@live.mercer.edu

Affiliation: Mercer University

Title of Abstract: Formulation development and characterization of microparticles as vaccine and adjuvant delivery systems

Presenting Author: Nihal S Mulla

Affiliation: mercer university

Complete Author's List :

Nihal S Mulla\*, Rikhav P Gala, Ashwin Parenky, Martin D’Souza

All Author Affiliations :

Mercer University

Abstract:

Purpose: The aim of this project is to prepare and characterize a formulation for delivering vaccine and adjuvant simultaneously. Since adjuvants enhance immunogenicity, effect of combining two adjuvants on cell surface expression of antigen presentation and co-stimulatory signal markers was also observed. Methods: Breast cancer vaccine was prepared by lysing 4TO7 murine breast cancer cell line. Microparticles containing lysate protein or adjuvant were prepared by spray drying along with enteric coating polymers using Buchi 290 spray dryer. Adjuvants used in combination with vaccine were Monophosphoryl Lipid A (MPL®), MF59®, Cholera Toxin, CpG, Resiquimod (R848®), Flagillin, P4. Microparticles were characterized for size, shape, charge and content. Vaccine microparticles (250µg/well) and adjuvant microparticles (250µg/well) were incubated with dendritic cells (300k/well) for 16 hrs. Antigenicity of the vaccine microparticles and its combination with adjuvant was evaluated by carrying out nitric oxide release assay. Dendritic cells were removed from the wells and processed for estimating CD 86, CD 40 and MHC II expression. CD 40 and MHC II are important for binding to CD4+ T cells, whereas CD86 expression is important for binding to CD8+ T cells. Results: Microparticles obtained after spray drying had a size range of 1-5µm. Simultaneous administration of vaccine and adjuvant induced a higher nitric oxide release, CD40, CD86 and MHC II expression on dendritic cells. Combination of MF 59 and P4 peptide induced higher expression of both CD 40 and MHC II expression. As a result, it will lead to higher binding to CD4+ T cells. Combination of P4 and R848 induced high CD86 expression. Higher CD 86 expression will cause better activation of CD8+ T cells. Vaccine and adjuvant given in microparticulate formulation induced significantly higher CD40, MHC II and CD86 expression compared to their solution groups. Conclusion: Vaccine and adjuvants microparticle administered together are more immunogenic than vaccine alone. Based on the obtained results we can select an adjuvant suitable for a cellular or humoral immune response.

Poster # 11

Nasim Nosoudi-Msc

Email: nnosoud@clemson.edu

Affiliation: Clemson university

Title of Abstract: Local inhibition of MMPs in abdominal aortic aneurysm rat model using anti-elastin decorated nanoparticles loaded with batimastat

Presenting Author: Nasim Nosoudi

Affiliation: Clemson university

Complete Author's List :

Nasim Nosoudi, Aditi Sinha, Pranjal Nahar-Gohad , Naren Vyavahare

All Author Affiliations :

Clemson university Clemson university Clemson university Clemson university

Abstract:

Ruptured abdominal aortic aneurysm (AAA) is the 10th leading cause of death in adults in the United States. Early detection of AAA is critical to decrease the mortality. AAA is associated with aortic elastin damage or degeneration due to the excessive activity of matrix metalloproteinases (MMPs). Systemic MMP inhibition has been shown to reduce growth of aneurysm in animals; however, MMPs are required for normal function of tissues and systemic inhibition showed limited success in clinical trials. We propose an approach to deliver MMP inhibitors to the site of AAA by using targeted nanoparticles loaded with batimastat (BB-94) as it is one of the most potent MMP inhibitors. Polylactic acid (PLA) NPs loaded with BB-94 were prepared using nanoprecipitation method. To study in vivo targeting to AAA, we created perivascular calcium chloride mediated abdominal aortic injury in rats (when early stage AAA develops); we intravenously injected BB-94 loaded or blank NPs (10 mg/kg body weight). The NPs were allowed to attach and deliver drug for 2 days. We then euthanized animals and extracted protein from aortas. After CaCl2 injury, MMP activity was 100% higher in group who received blank NPs compared to BB-94 NPs group, exhibiting complete reversal of increased MMP activity.

Poster # 12

Sharon Olang-

Email: olang@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: Investigation of Cysteine-rich Peptides from Herbal Plants

Presenting Author: Sharon Olang

Affiliation:

Complete Author's List :

Sharon Olang, Curt Harper, Phuong Nguyen

All Author Affiliations :

(1)Bioengineering, Clemson University, (2)Bioengineering Clemson University, Biology, (3)Nanyang Technological University

Abstract:

The main objective of this project was to discover cysteine-rich peptides (CRPs) from herbal plants that could be used as druggable biologics. We were interested in cysteine-rich peptides because it is known that they are chemically and enzymatically stable, and also stable when exposed to heat; this is due to the disulfide bonds present in these peptides. This project was split into two main parts: proteomics and genomics. Proteomics is the large-scale study of the structure and function of the peptides, which was conducted by extraction and purification. Genomics involves the sequencing, function, and structure of genomes, which was done by RNA extraction. RNA extraction that yielded a high concentration of pure RNA would be sent to a company for transcriptome sequencing.

Poster # 13

Timothy Olsen-Mr.

Email: trolsen@clemson.edu

Affiliation: Clemson University

Title of Abstract: Manipulation of Cellular Spheroid Composition and the Effects on Tissue Fusion

Presenting Author: Timothy R. Olsen

Affiliation: Clemson University

Complete Author's List :

Timothy R. Olsen (1) Megan Casco (1) Colby Williams (1) Dan Simionescu (1) Richard P. Visconti (2) Frank Alexis (1)

All Author Affiliations :

(1) Department of Bioengineering, Clemson University, Clemson, SC, 29634 (2) Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC, 29401

Abstract:

A critical process in the fabrication of complex tissue structures with cellular spheroids is related to their fusion. Tissue fusion is a self-assembly process in which two or more distinct cell populations, or tissues, make contact and form a single cohesive structure. Research has shown that some factors mediating tissue fusion include cell migration, cell-cell interactions, and cell-matrix interactions. Conventional tissue assembly and fabrication methods include cell printing, cell sheet techniques, and molds. These methods spatially orient the cells into a desired position through passive contact, but do not promote active contact to accelerate fusion of the tissue. The objective of this work was to determine what factors are critical to accelerate the fusion of Janus magnetic cellular spheroids (JMCSs) and to understand fusion mechanisms. The hypothesis driving this work was that by manipulating the composition of JMCSs, the fusion of JMCSs can be accelerated when exposed to magnetic forces. Primary rat aortic smooth muscle cells were cultured using DMEM:F12 cell culture media, 5% CO2 and maintained at 37˚C, unless otherwise noted. After filling glass chamber slides with media, 25 individual JMCSs were patterned around magnetic rings in a monolayer. Fusion was analyzed by tracking the change in inner diameter of patterned spheroid rings. A range of cell densities (5,000-100,000 cells per spheroid) and collagen contents (0 mg/mL – 0.3 mg/mL collagen type I) were used for tissue ring fabrication. Magnets were kept in place for 48 hours and then removed, followed by imaging with a Nikon AZ100 microscope. Capillary tubes were also used to quantify spheroid fusion and cell intermixing with varying cell densities and collagen contents when exposed to magnetic forces. In another study, primary rat aortic fibroblast, smooth muscle cell and endothelial cell solutions were fluorescently labeled prior to spheroid fabrication. Spheroids were patterned into tissue sheets and left to fuse for 24 hours. The tissue sheets were wrapped around a silicon tube, to mimic the orientation of a blood vessel, with a magnet in the lumen and left to fuse for 24 hours prior to analysis. The results of ring fusion studies showed that lower cell densities and collagen contents allowed for the most rapid and complete fusion over time when exposed to magnetic forces. Fusion studies in capillary tubes demonstrated similar results, and further confirmed that spheroid composition is important for the fusion process. A trilayer tube that mimics the structure of native blood vessels was successfully fabricated using JMCSs. This work demonstrated that the composition of JMCSs is critical for accelerating the fusion of spheroids when exposed to magnetic forces. This fundamental understanding is expected to provide a strong theoretical and methodological foundation for the development of new tissue engineering technologies.

Poster # 14

Shantanu Pradhan-

Email: szp0023@auburn.edu

Affiliation: Department of Chemical Engineering, Auburn University

Title of Abstract: PEG-fibrinogen hydrogel microspheres support tumorigenic phenotype of MCF7 breast cancer cells

Presenting Author: Shantanu Pradhan

Affiliation: Department of Chemical Engineering, Auburn University

Complete Author's List :

Shantanu Pradhan (1), Jacob M. Clary (1), Dror Seliktar (2), Elizabeth Lipke (1)

All Author Affiliations :

Department of Chemical Engineering, Auburn University (1), Department of Biomedical Engineering, Technion-Israel Institute of Technology (2)

Abstract:

Breast cancer has been a leading cause of cancer-related deaths worldwide. The field of cancer tissue engineering aims to provide in vitro platforms for the investigation of various disease mechanisms and drug-testing applications in order to mitigate the challenges posed by breast cancer-related incidences. The tumor microenvironment is known to play an influential role in the malignant progression of the disease. However, current two-dimensional (2D) monolayer culture and three-dimensional (3D) spheroid models are not able to capture key features of the complex tumor extracellular matrix (ECM), making them inaccurate in providing clinically-relevant data. In order to address this challenge, we have developed a novel 3D in vitro model for the long term culture of cancer cells and subsequent investigation of tumorigenic properties. Briefly, MCF7 breast cancer cells were encapsulated within poly(ethylene glycol)-fibrinogen (PEG-Fb) hydrogel microspheres using an innovative aqueous-oil emulsion technique and maintained in culture for 21 days. The ‘tumor microspheres’ (TM) so formed, were 100-300 µm in diameter and were larger, more spherical and more uniform compared to MCF7 cells aggregated as tumor spheroids (TS) using the hanging droplet method (50-100 µm in diameter). Live/Dead imaging revealed the high viability and consistent growth of cells within the TM through 21 days in culture. Scanning electron microscopy and fluorescence staining for cell nuclei and actin filaments revealed the 3D morphology and cellular organization within both the TS and TM. Cells within TM showed a loss of polarity, variation in cell distribution and loss of cell cell contact as compared to those within TS. Thus, cells grown within TM displayed a more disorganized and heterogeneous morphology as compared those within TS, which is reminiscent of the native tumor microenvironment. Overall, the tumor microsphere model provides a more physiologically relevant 3D culture platform for the growth and tumorigenic morphology of cancer cells as compared to the standard tumor spheroid model. The biochemical and mechanical properties of the PEG-Fb used for fabrication of the microspheres can be modulated to match that of native cancer tissue and the model can be potentially used for the investigation of tumorigenic mechanisms and in drug-testing applications.

Poster # 15

JORGE RODRIGUEZ-Dr.

Email: jorger@clemson.edu

Affiliation: Department of Bioengineering, Clemson University, 301 Rhodes Research Center, Clemson, SC 29634-0905, USA

Title of Abstract: Biomaterial Improves Compactness for Reproducible Cell-based Biological Targets for High Throughput Screening

Presenting Author: Jorge I. Rodríguez-Dévora

Affiliation: Clemson University

Complete Author's List :

Jorge I. Rodríguez-Dévora, Aesha Desai, Nasim Nosoudi, Delphine Dean

All Author Affiliations :

Department of Bioengineering, Clemson University, 301 Rhodes Research Center, Clemson, SC 29634-0905, USA

Abstract:

Methylcellulose (MC) supplemented media is known by its improvement in cell aggregation when fabricating three-dimensional spherical tissues. This study assesses the compactness and its effect on biomechanical properties of three-dimensional (3D) breast cancer (MCF-7) spheroids. Spheroids were culture by the hanging drop technique using appropriate media supplemented with MC. Droplets were manually pipetted at volumes of 30 µl, containing 2,000 cells/ droplet. At the third day, 3D spheroids were collected and studied for spheroid shape, cell proliferation, and elastic modulus. Indentation was performed on an Asylum Research MFP-3D atomic force microscope (AFM). Spheroids cultured in media supplemented with MC were created in > 90% of the hanging drops compared to the control group culture in media only that create spheroids in less than 10% of the drops. Suggesting that the addition of MC greatly improve the compactness of the cultured spheroids. The average spheroid volume was 275± 22 x 10^6 µm^3, the sphericity shape factor was 0.86 ± 0.06, and the diameter was 369.62 ± 18.3 µm (n = 18). In addition, the proliferation (LDH) assay confirmed that cells per spheroid were 1,874 ± 279 at Day 1, and proliferating to 2,554 ± 417 by Day 3. The biomechanical characteristics of MCF-7 spheroids resulted in an apparent elastic modulus of 7.39 ± 0.39 kPa. These results suggest that intercellular interactions presented in spheroids are 10-fold stronger that single cell studies, 600 ± 200 Pa. This study increases our understanding of how MC supplementation impact on the biomechanical behavior of 3D spheroid structures. In our aim to fabricate a more relevant in vitro drug screening assay, it is critical to develop more suitable biomaterials similar to MC.

Poster # 16

Nathan Rohner-Mr.

Email: nrohner@gatech.edu

Affiliation: George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology

Title of Abstract: Size-dependent molecular dissemination from tumors into regional and systemic tissues

Presenting Author: Nathan A. Rohner

Affiliation: George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology

Complete Author's List :

Nathan A. Rohner and Susan N. Thomas

All Author Affiliations :

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

Abstract:

Tumors secrete biomolecules (proteins, exosomes) of a broad range of physiochemical properties (chain-like biopolymers, membrane-enclosed particulates) and sizes (from 1-5 nanometer (nm) in hydrodynamic radius up to 30-100 nm and greater in diameter) that are involved in processes from immunosuppression to metastasis. Since normal blood capillaries are permeable only to low molecular weight (MW) solutes (<5 nm in diameter), lymphatic drainage supports the clearance of intermediate MW solutes (>40kDa or >5nm diameter but <100 nm) from the intercapillary tissue space, and cell-mediated trafficking clears large particulates (>100 nm), molecular size characteristics are hypothesized to crucially influence molecular clearance profiles and the capacity of tissue-produced molecular species to disseminate systemically. However, though the tumor vascular plexus is well described to be irregular and highly permeable, how it influences the extent and rate of molecular clearance as well as blood versus lymphatic partitioning of low versus high MW tumor-derived molecules remains ill-defined. To address this issue, the contribution of the developing tumor vasculature on molecular clearance and dissemination patterns was investigated by comparing the transport patterns of near infrared fluorescently labeled molecules from healthy versus malignant tissues that display well-documented changes in their complex neo-vascular plexus with tumor progression. We found that progression of B16F10 melanoma in C57Bl6 animals resulted in reduced clearance of 500 and 50 nm spheres from the tumor interstitium, while the clearance of 30 nm dextran was not inhibited relative to naïve peripheral clearance until the tumor progressed for seven days—correlating with a significant increase in tumor vascular surface area. Lymphatic-mediated transport to sentinel lymph nodes was also dramatically attenuated. Interestingly, tumor growth also resulted in increased blood permeability, enabling 500 and 50 nm spheres, but not 30 and 5 nm dextrans to disseminate to the lungs within 72 hours for late-stage tumors. This highlights the overlooked role of tumor vascular status and permeability in influencing not only tumor drug delivery but also the dissemination of tumor-derived molecules to regional versus systemic tissues. Ongoing studies focus on evaluating the impact of these altered molecular transport processes on anti-tumor immune response and metastasis.

Poster # 17

Apoorva Salimath-Ph.D Candidate

Email: akalasur@gatech.edu

Affiliation: Woodruff School of Mechanical Engineering; Petit Institute of Bioengineering and Bioscience; Georgia Institute of Technology

Title of Abstract: Biofunctionalized Hydrogels for Skeletal Muscle Force Actuators

Presenting Author: Apoorva S. Salimath

Affiliation: Woodruff School of Mechanical Engineering; Petit Institute of Bioengineering and Bioscience; Georgia Institute of Technology

Complete Author's List :

Andres J. Garcia

All Author Affiliations :

Woodruff School of Mechanical Engineering; Petit Institute of Bioengineering and Bioscience; Georgia Institute of Technology

Abstract:

A contractile muscle unit would play a significant role in ameliorating impaired morphologic and physiological function due to trauma, degenerative disease, aging or inactivity. Current therapeutics are limited to skeletal muscle grafts, as in-vitro engineered tissues are incapable of recapitulating natural muscle bundle architecture and function. Previous work involved 2D muscle strips, with naturally-occurring hydrogels, and incomplete examination of the effects of the scaffold on myogenic differentiation in a controllable manner. In this project, synthetic hydrogel scaffolds encapsulating C2C12 mouse skeletal muscle cells have been developed in vitro as a step towards regenerative medicine therapies for the enhancement or inducement of de novo functional skeletal muscle formation. The goal of this study is threefold: (i) to identify key properties in functionalized poly(ethylene glycol) (PEG)-maleimide hydrogels that promote cell attachment, proliferation and differentiation for the development of multinucleated myotubes and functional skeletal muscle tissue constructs. (ii) use specific geometries to promote uni-directional alignment of myotubes as an essential property to their function as effective contractile units, and (iii) to generate a force vs. time profile of constructs upon stimulation with contractile agents. The engineered bio-functionalized PEG matrix with maleimide cross-linking reaction chemistry gels rapidly with high cytocompatibility for ease of in situ delivery while still allowing “plug-and-play” design variation. Significant differences in myoblast viability were observed as a function of cell seeding density, polymer weight percentage, and bioadhesive ligands. Optimized conditions for cell survival, required for myotube development, were carried over for differentiation assays. 5%w/v PEG hydrogels functionalized with RGD peptides and cross-linked with protease-cleavable peptides incubated for 3 days before supplementation with 2% horse serum significantly increased expression of differentiated skeletal muscle protein and extent of multinucleation. Circumferential alignment is observed within toroidal hydrogel constructs cast on silanized PDMS molds. These gels are able to be attached to hooks connected to a micro-force transducer and measurement circuits for the elucidation of force vs. time profiles using MATLAB signal processing techniques. This work describes techniques to engineer a 3D microenvironment using synthetic hydrogels to promote the development of differentiated muscle tissue from skeletal muscle progenitor cells to form contractile units. These results show that synthetic hydrogel properties can be tuned to study the biophysical requirements of a cell from its environment, demonstrating the potential of engineered bio-functionalized PEG matrices for muscle regeneration.

Poster # 18

Atanu Sen-

Email: atanu@g.clemson.edu

Affiliation: Bioengineering, Clemson University

Title of Abstract: Fiber-based microcarriers enhance proliferation of hydrogel-encapsulated cells

Presenting Author: Atanu Sen

Affiliation: Bioengineering, Clemson University

Complete Author's List :

Atanu Sen, Jeoung Soo Lee, Thuy Thanh Le, Ho-Joon Lee, Nasim Nosoudi, Kathryn Stevens, Ken Webb

All Author Affiliations :

Bioengineering, Clemson University Bioengineering, Clemson University Material Science and Engineering, Clemson University Bioengineering, Clemson University Bioengineering, Clemson University Material Science and Engineering, Clemson University Bioengineering, Clemson University

Abstract:

Substrate topography plays an important role in the regulation of cell proliferation and differentiation. The goal of this project was to develop microcarriers consisting of short segments or staples of capillary channel polymer (CCP) fibers with micrometer-scale surface grooves that can be used to introduce topographic bioactivity into injectable, amorphous synthetic polymer networks as 3D matrices for cell transplantation. CCP fibers were prepared from poly-l-lactide by melt extrusion, embedded in OCT, cut on a cryostat, cleaned, sterilized, coated with fibronectin, and seeded with NIH 3T3 fibroblasts in a rotary reactor. After 48 hours in culture, the seeded microcarriers were incorporated into polyethylene glycol diacrylate / hyaluronic acid semi-interpenetrating networks during photopolymerization. Cell viability and proliferation were assessed by confocal microscopy over 3 weeks in culture. CCP microcarriers (185 ± 11 µm length) supported efficient cell adhesion and aligned morphology. Within 7 days, expanding clusters of dividing cells were visible around the microcarriers that grew into large aggregates by 14 days and began to join together. Semi-IPNs without microcarriers exhibited no such aggregates or apparent proliferation. Fibronectin-coated CCP microcarriers promoted directional contact guidance of fibroblasts and supported cell survival, sustained cell division, and the formation of expanding macroscopic clusters within degradable 3D hydrogel networks. This biodegradable and injectable staple-gel composite could be a feasible implantable model for tissue regeneration. Future studies will investigate the applicability of this model system for topography-mediated induction of neuronal differentiation from NSCs for the treatment of neurodegenerative disorders.

Poster # 19

Noel Vera-Gonzalez-

Email: nav6@duke.edu

Affiliation: Biomedical Engineering, Duke University

Title of Abstract: Oxygen Sensing Microparticles For Use In Tissue Engineering Scaffolds

Presenting Author: Noel A. Vera-Gonzalez

Affiliation: Biomedical Engineering, Duke University

Complete Author's List :

Noel A. Vera-Gonzalez, Nicole K. Virdone, Barbara A. Nsiah, Jennifer L. West

All Author Affiliations :

Biomedical Engineering, Duke University

Abstract:

A remaining hurdle in the field of tissue engineering is the creation of large scale tissue engineered constructs which have so far been limited by oxygen and nutrient diffusivity into the construct. Our lab aims to facilitate nutrient transport by incorporating self-assembling, pro-vasculogenic co-cultures of endothelial cells and pericytes in a pro-angiogenic hydrogel inside a microfluidic device, potentially maximizing cellular viability. The ability to measure oxygen within this system will facilitate optimal microchannel design in order to create large-scaled, implantable tissue. Leach et al. synthesized ruthenium (Ru)-based oxygen sensors that operate via reversible luminescence quenching by oxygen without needing to consume it. Currently, most metal-based oxygen sensors are coated with PDMS, polystyrene, PVC, or silica. Alternatively, polyethylene glycol (PEG) has superior biocompatibility and water solubility allowing better permeation of water-dissolved oxygen. Here, we describe new ruthenium-based oxygen sensing microparticles encapsulated within a cell laden, bioactive PEG hydrogel scaffold, which enable us to spatially measure oxygen throughout cell culture via fluorescent measurements over time. The oxygen sensing microparticles were synthesized by binding Ru(Ph2phen3)Cl2 to silica microparticles (d = 10-14µm), then coating them with PEG diacrylate (PEGDA) via an oil-in-water emulsion. The resulting particles were characterized using a light microscope and fluorescence plate reader to obtain their size and excitation/emission spectra, respectively. Additionally, we tested the functionality of these microparticles by repeatedly cycling between 0% and 20% oxygen and comparing the fluorescence for consistency. Next, we encapsulated the particles amongst 344SQ cells within PEG-based bioactive hydrogels developed by our lab [2]. The hydrogel system consists of 4% proteolytically degradable PEG-derivative, which incorporates a matrix metalloprotease (MMP)-sensitive peptide sequence within the polymer backbone, for cell-directed scaffold degradation, and 3.5mM PEG-tethered integrin binding peptide (PEG-RGDS) for cell adhesion. Particle fluorescence was measured using an Olympus VivaView® microscope every hour for 48 hrs total. After each study, a standard was created by manipulating atmospheric oxygen levels (monitored by a Sper Scientific Oxygen Pen), measuring the equilibrated fluorescence, then using the Stern-Volmer relationship, which describes the concentration-dependence exhibited by the dynamic quenching of oxygen to Ru(Ph2phen3)Cl2, in order to relate fluorescence back to oxygen concentration. We successfully synthesized oxygen sensing, PEGDA-coated microparticles of spherical morphology with diameters ranging from 25-35µm. The excitation and emission spectra revealed maximum excitation at 555nm and emission at 590-710nm. Fluorometer measurements confirmed that the particle fluorescence significantly decreased with increasing oxygen concentration. Additionally, the particle fluorescence at 0% and 20% oxygen remained consistent after several cycles, demonstrating their retention of functionality. After characterization, we were able to incorporate and image the microparticles within our bioactive hydrogels without any adverse effects on cells. Using MATLAB®, we developed an algorithm to extract normalized fluorescence intensity of individual particles from each image at every time point, then relate these intensities to partial oxygen pressure using the obtained standard and Stern-Volmer relationship. The resulting plots of local oxygen concentration per time showed decreasing oxygen levels beginning at 12 hrs and equilibrating to 5% oxygen after 20 hrs. An acellular control hydrogel showed no significant changes in fluorescence throughout the 48 hr period. We developed functional, Ru-based oxygen sensing microparticles that can be used in the presence of cells. This approach allowed us to non-invasively monitor local oxygen concentrations within a cell-laden, tissue engineered hydrogel by measuring fluorescence over time. In future studies, the particles will be used to measure differences in oxygen concentration throughout unknown environments, including in microfluidic-hydrogel devices, to study oxygen perfusion effects on self-assembled microvasculature networks.

Poster # 20

Trinh Vo-Graduate Student

Email: trinh.phuong.vo@live.mercer.edu

Affiliation: Mercer University

Title of Abstract: In Vitro and In Vivo Studies on Transdermal Particulate HPV Vaccine

Presenting Author: Trinh P. Vo

Affiliation: Mercer University

Complete Author's List :

Trinh Vo, Gitika Panicker, Ashwin Parenky, Mangalathu S. Rajeevan, Elizabeth R.Unger, Martin D’Souza

All Author Affiliations :

Trinh Vo1, Gitika Panicker2, Ashwin Parenky1, Mangalathu S. Rajeevan2, Elizabeth R.Unger2, Martin D’Souza1 1Mercer University College of Pharmacy and Health Sciences, Vaccine Nanotechnology, Laboratory, Atlanta GA 30341, USA; 2Chronic Viral Diseases Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA 30329

Abstract:

Purpose: Human Papillomavirus (HPV) vaccines are recommended by the World Health Organization for cervical cancer control programs world-wide. However, the cost of these vaccines and requirements for administration are significant barriers for vaccination in developing countries. Microparticulate vaccines have the potential to alleviate these problems. The purpose of this study is to develop an HPV16 microparticulate vaccine for transdermal administration and evaluate its efficacy in both in-vitro and in-vivo studies. Methods: HPV 16 virus-like particles (VLPs), containing L1 and L2 capsid protein, were produced in human embryonic kidney cells 293TT. VLPs were incorporated into a cellulosic polymer matrix and formulated into microparticles using a Buchi B-290 spray dryer in a single step. VLP encapsulation was determined using transmission electron microscopy (TEM) and Western blot analysis. For in –vitro study, antigen-presenting cells were exposed to vaccine and characterized for cell-surface expression sucha s CD40, CD80/86 and MHC II. For in-vivo study, AdminPatch® transdermal administration of VLPs as microparticles was compared to VLPs in solution. Female BALB/c mice (n=6 for each group) received 4 doses. Blood samples were collected and antibodies were detected with a direct HPV16 VLP IgG ELISA. Spleen and lymph node pools were prepared at week 40 to analyze CD4+, CD8+, memory T and B cells using FACS analysis of CD4, CD8, CD27, CD62L and CD45R markers. Results: The microparticle yield after spraying was 90% w/w, with average size 3.5+ 0.6 µm and average zeta potential -19.7 ¬+ 0.3 mV. VLP encapsulation efficiency was 85% based on Western blot detection of HPV16 L1 protein. TEM demonstrated intact conformation of the VLPs after spray drying. HPV 16 antibodies were detected more frequently in the microparticle group (3 of 6 mice by week 7 and 6 of 6 mice by week 12) than in the solution group (1 of 6 mice by week 12). Spleen and lymph node CD4+, CD27, CD62L and CD45R cell populations were significantly higher (p<0.02) for the microparticle cohort compared to the solution group. Conclusions: Transdermal administration of HPV VLP as microparticulate vaccine is more immunogenic than HPV VLP in solution.

Poster # 21

Aline Thomas-PostDoc

Email: aline.thomas@bme.gatech.edu

Affiliation: Georgia Institute of Technology

Title of Abstract: Immunomodulatory Materials for the Attenuation of Multiple Sclerosis

Presenting Author: Aline M. Thomas

Affiliation: BME

Complete Author's List :

Aline M. Thomas, J. Lori Blanchfield, Brian D. Evavold, Julia E. Babensee

All Author Affiliations :

Biomedical Engineering, Georgia Institute of Technology (1); Microbiology & Immunology, Emory University (2); Microbiology & Immunology, Emory University (2); Biomedical Engineering, Georgia Institute of Technology (1);

Abstract:

Autoimmune diseases affect nearly 5% of the United States population, with many patients suffering from multiple disorders. Central to their progression are dendritic cells, which have been shown to interact with and control the behavior of T-cells, macrophages, and other immune cells. In the Babensee laboratory, we manipulate dendritic cells in vitro using a combination cytokines that shift their behavior from an immunogenic one which exacerbates these disorders to a tolerogenic one which can delay and potentially halt disease progression. We then alter the immune response in vivo by transplanting these primed cells local to immunogenic sites using materials. Using our strategy of locally delivered tolerogenic dendritic cells, we have found that tolerizing superficial cervical lymph nodes, which have been shown to play a role in multiple sclerosis, can delay the progression of multiple sclerosis in the mouse EAE model.

**Rapid Fire Presentations & Posters: Materials**

Poster # 22

Samuel Bearden-Graduate Research Assistant

Email: samuel.l.bearden@gmail.com

Affiliation: Clemson University

Title of Abstract: A New Method for Molecular Detection and Identification in a Metallic Nanopore

Presenting Author: Samuel L. Bearden

Affiliation: Clemson University

Complete Author's List :

Samuel L. Bearden; Guigen Zhang

All Author Affiliations :

Department of Bioengineering, Clemson University;Department of Bioengineering, Clemson University

Abstract:

Nanopore sensors with single-molecule resolution will be the basis for the next generation of fast, long-read DNA sequencers. However, a reliable method of signal transduction is needed in order to extract useful information from molecular and sub-molecular analytes. With this goal, a new type of nanopore sensing technique was developed with sensitivity to changes in the electrical double layer. Transient alterations to the electrical double layer potential and the ionic current were collected as molecular analytes translocated the nanopore. The collected signals were correlated with the physical and electrical properties of the molecular analytes in a range of supporting electrolytes. A numerical model of the nanopore system was developed in order to characterize the mechanism of signal transduction. Modeling outputs were in good agreement to the experimental results and consistent with established electrochemical theory. The electrical double layer signal was found to be sensitive to the charge of the molecular analytes, as well as the analyte permittivity and/or size. The magnitude of the double layer signal was dependent on the concentration and type of supporting electrolyte. By developing this new molecular sensing technique, we demonstrate a new method of nanopore detection which may be implemented alone or in parallel with the well-known ionic current signal.

Poster # 23

Jayesh Betala -Graduate Student

Email: jbetala@clemson.edu

Affiliation: Clemson University

Title of Abstract: Inhibition of Smooth Muscle Cell Proliferation Using Drug -loaded Polymeric Micelles

Presenting Author: Jayesh V. Betala

Affiliation: Clemson University

Complete Author's List :

Jayesh V Betala, Sooneon Bae, JeoungSoo Lee Ph.D., Eugene M. Langan III M.D., Martine LaBerge Ph.D.

All Author Affiliations :

Bioengineering, Clemson University (1); Bioengineering, Clemson University (2); Bioengineering, Clemson University (3); Greenville Health System, Greenville (4); Bioengineering, Clemson University (5);

Abstract:

Stents have been successful in mitigating restenosis over the decades. However, stent thrombosis and restenosis is a major concern. It leads to at least 10% of deaths every year, while restenosis occurs in 13-36% of cases. Although DES have minimized in-stent restenosis (ISR), it has led to late-stent thrombosis that requires longer dual antiplatelet therapy. Antiproliferative agents such as paclitaxel and sirolimus have been successful in minimizing ISR, with heparin for reducing thrombosis. The objective of this study is to create a drug-eluting balloon to provide sustained and controlled release of heparin and paclitaxel to prevent thrombosis and restenosis. An amphiphilic polymer poly (lactide-co-clycolide)-graft-polyethyleneimine (PgP) has been synthesized in 4D Lab. We hypothesize that heparin, a negatively charged molecule, will bind to the positive charged PEI and paclitaxel, a non-charged molecule drug will be encapsulated in the hydrophobic core. We evaluated effect of antiproliferative agents loaded PgP on rat vascular smooth muscle cells (VSMCs) proliferation. To load heparin in PgP micelle, PgP and heparin (1:1 charge ratio) were dissolved in DI water and incubated overnight while stirring at room temperature to complete complexion and then freeze dried. The PgP-Hep complex was dissolved in PBS and then further diluted in DMEM to obtain final 50µg/ml heparin concentration. Paclitaxel and estrogen were dissolved in DMEM at20µM concentration. VSMCs were cultured at 37 °C in DMEM with 10% FBS and 1% Ab-Am. All the experiments were performed between passages 3 and 6. VMSCs were seeded at a density of 2×104 cells/ml in DMEM with 10% FBS and 1% Ab-Am for 24 hours. VSMCs were growth arrested in DMEM with 1% FBS and 1% Ab-Am for 24 hours. They were then treated with 1) PgP-hep- pac 2) Pgp-hep-estrogen 3) Pgp-hep 4) Estrogen 5) Heparin and 6) Paclitaxel, along with PgP and untreated cells served as control for 5 minutes and 24 hours. Proliferation was evaluated after 24 hours by performing MTT assay. Studies were repeated at least in triplicates. Statistical analysis were performed using one-way ANOVA, with a p-value < 0.05 considered to be significant. PgP-hep-pac proved to be the most effective treatment group for 5 minutes and 24 hours. Cell viability was 64% and 49% for 5 minutes and 24 hours respectively. PgP-hep-est and PgP-hep group showed viability of 72% and 76% for 5 minutes and 58% and 55% for 24 hours treatment. PgP showed viability of 84% and 75% for 5 minutes and 24 hours respectively, proving to be relatively less toxic at 5 minutes than 24 hours. Viability for est, hep and pac was 90%, 88% and 87% for 5 minutes and 94%, 83% and 72% for 24 hours respectively. One-way ANOVA showed significant difference in the treated groups with p-value <0.0001 as compared to control. Our preliminary data shows PgP can deliver heparin and paclitaxel simultaneously to inhibit VSMCs proliferation that can also minimize thrombosis events locally. In the future, we will coat the balloon with drug loaded PgP and evaluate the inhibition of rat VSMC proliferation in vitro.

Poster # 24

Jeffery Borden-

Email: jeffery.borden@louisville.edu

Affiliation: University of Louisville

Title of Abstract: Toward Determining the Time Course of the Mechanical Properties of a Bone Graft Substitute used to Fill a Drill-Hole Defect: a Micro-CT and Micro-FEA Study

Presenting Author: Jeffery W. Borden

Affiliation: University of Louisville

Complete Author's List :

Jeffery W. Borden 1,4 Michael J. Voor, PhD 1,2,3,4

All Author Affiliations :

1 - Department of Mechanical Engineering 2 - Department of Bioengineering 3 - Department of Orthopaedic Surgery 4 - Orthopaedic Bioengineering Laboratory

Abstract:

There are significant costs in researching, developing, and clinically approving the use of new bone grafting technology. The goal of this research is to develop a methodology that addresses this expensive process by utilizing experimental and micro-CT data to generate complex three-dimensional parametric finite element models to represent the mechanical quality of healing trabecular architecture. A standardized test method has been developed to evaluate the healing micro-architecture of cancellous bone tissue. The model uses a drill-hole defect in the rabbit distal femur filled with a bone graft or bone graft substitute and followed over time. A pilot study was conducted to replicate a healing animal defect study. Two separate bench test procedures were evaluated. The first stage of the pilot study used the distal femoral condyles of eight mature New Zealand white rabbits. The bone samples were extracted post mortem. The samples were drilled from the lateral portion of the condyle. In a randomized fashion, the defect was either filled with synthetic bone graft or left empty. The synthetic material used for this part of the study was calcium sulfate cement (Plaster of Paris). The condyles were mounted into a sectioning jig and cut into cubic test specimens. The samples were sectioned in cubic shapes dimensioned 7.5mm x 7.5mm x 5mm with a centrally located 5mm thru-hole in the 7.5mm square face. The specimen samples were platen compression tested using an MTS load frame (MTS, Bionix 858 Test System) and a load/displacement controlled test program. A second part of the pilot study evaluated a standardized foam material (Pacific Research Laboratories) with known cancellous-like material properties to be used during model correlation. The foam cube samples were shaped and tested in compression as previously described. For the live animal study, the distal lateral femoral condyle of twelve mature New Zealand white rabbits provided the trabecular bone tissue for modeling the healing bone quality in a non-destructive manner [1]. The femurs were drilled to create a 5mm diameter critical defect and filled with either synthetic bone graft substitute or left empty. The pilot experiment cubes were scanned at 7μm voxel resolution ex vivo. The live rabbit femurs were scanned at time periods of 3, 7, and 12 weeks at 28μm in vivo and 14μm ex vivo voxel resolution. For each phase of the study, the micro-CT scanned images were imported into ImageJ, rotated and cropped to provide the correct orientation and to isolate the cubic test cross-sectional area. The resulting image stacks were exported to the volume rendering, element generating, materials defining softwares MIMICS and 3MATIC to create voxel-based and tetrahedral-based three-dimensional finite element models [2]. The three-dimensional finite element models (FEM) were imported into finite element analysis (FEA) software ABAQUS. The models were defined by material properties, fixed boundary conditions, and specified displacement controls. These idealized models are to be correlated with the experimental results. The cubic shape surrounding the defect provides a good differentiation between the stiffness of different materials within the drill-hole defect (Fig 1,2). The stress distribution of the empty defects were consistent with the failure of cancellous bone. The interaction of the different materials of the filled defects indicate that the stiffness of the healing defect material plays an important role in the overall strength of the cancellous bone in the condyle region (Fig 3). The correlation of the models and assessment of the healing bone quality is still ongoing. The preliminary results provide a feasible path in which to study the structural integrity of in vivo healing bone tissue.

Poster # 25

Colin Burns-Heffner-Student (MS)

Email: cburnsh@clemson.edu

Affiliation: Clemson University

Title of Abstract: Tissue Fixation and Digestion Chemicals Impact the Mechanical Properties of Surgical Mesh

Presenting Author: Colin T. Burns-Heffner

Affiliation: Clemson University

Complete Author's List :

Colin T. Burns-Heffner, Melinda K. Harman

All Author Affiliations :

Clemson University

Abstract:

Surgical mesh is a medical device consisting of implantable polymer materials that is commonly used to augment surgical repair of hernias and to reinforce weakened tissues. Various polymer materials and composites are used, such as expanded polytetrafluoroethylene (ePTFE), polypropylene (PP) of various weights, and polyester. Mesh degradation, contraction, migration, and oxidation can alter mechanical properties ultimately decreasing functionality of the implanted mesh. This suggests that measuring changes in mesh compliance, flexural rigidity, and other mechanical properties could provide clinically relevant insight. Previous studies have characterized material properties of explanted surgical mesh and compared it to unused mesh. However, because explanted mesh is commonly exposed to processing chemicals before testing, it is essential to determine whether the reported changes in material properties are due to ex vivo exposure to chemicals or some physiological factor. The purpose of this study is to measure the mechanical properties of two types of pristine hernia meshes after treatment with formalin and bleach solutions to simulate the tissue fixation and tissue digestion procedures commonly used on explanted mesh. Two different unused surgical meshes were tested: Composix E/X (CR Bard / Davol Inc), and Ultrapro (Ethicon Inc. All mesh samples were cut into 25 mm x 75 mm strips and placed in 1X phosphate buffered saline (PBS) at 37°C for 18 hours to equilibrate at physiological conditions. Three strips of each type of mesh were then set aside as a control, while the rest were fixated in 10% formalin for 24 hours. Half of these fixed meshes were then soaked in a bleach solution (0.525% NaOCl) for 24 hours. The tensile stiffness of the mesh was then measured with an MTS load frame using a uniaxial tensile test (0.2 mm/sec) . Results from the tensile test show fixation in 10% formalin for 24 hours had no significant effect on tensile stiffness (t-test, p<0.05). However, soaking in the bleach solution resulted in an increase in stiffness compared to the PBS control strips. The Ultrapro experienced a 14% increase in stiffness while the Composix E/X experienced a 19% increase in stiffness. Due to the limitation of a small sample size, this increase was not statistically significant. Based on results in the current study, fixation in 10% formalin for 24 hours does not alter stiffness. Therefore, when testing explanted mesh after short exposure to formalin, any measured changes in stiffness are likely due to physiological impact rather than chemical exposure. In contrast, use of bleach for chemical tissue digestion increased mesh stiffness. Therefore, it is not recommended that bleach be used for tissue digestion for a duration of 24 hours. Shorter digestion times or alternative chemicals such as trypsin and sodium dodecyl sulfate (SDS) should be explored.

Poster # 26

Erin Casey-Tissue Digestion Method Suitable for Explanted Hernia Mesh

Email: ecasey@clemson.edu

Affiliation: Clemson University

Title of Abstract: Tissue Digestion Method Suitable for Explanted Hernia Mesh

Presenting Author: Erin Casey

Affiliation: Clemson University

Complete Author's List :

Erin Casey, Cassie Gregory, Amy Phillips, Melinda K. Harman

All Author Affiliations :

Bioengineering, Clemson University (1); School of Medicine, University of South Carolina (2); Bioengineering, Clemson University (3)

Abstract:

Surgical meshes are polymer-based materials used to augment surgical repair of abdominal hernias. While highly successful, some patients with hernia repairs experience pain and discomfort that can require mesh removal. It is believed that the main cause of this pain is stiffening of the mesh due to in vivo oxidation. The overall goal of this project is to establish a registry of explanted (surgically removed) mesh in order to characterize their material properties. The first aim is to determine an appropriate tissue digestion method to remove excess tissue before testing. Previous studies of explanted mesh have used sodium hypochlorite (bleach, or 13% NaClO) to remove adherent tissue, claiming the agent did not induce changes in the mesh. However, replication of their cleaning process and mechanical testing showed as much as a 19% increase in mesh stiffness. Five tissue digestion methods will be compared: 24 h bleach soak at 37 °C; 24 h bleach soak at 25 °C; 2 h bleach soak at 25 °C; 24 h trypsin soak followed by a two week SDS soak at 25 °C; and 24 h PBS soak at 25°C as a control. Large samples (> 5"x9") of both unused and explanted mesh will be chosen so that each of five protocols can be performed on 1"x3" sections of the same mesh and then replicated using three separate meshes. Complete tissue removal will be confirmed on a subset of mesh using an assay imaged with confocal microscopy. Following tissue digestion, the samples will undergo testing on an MTS load frame to quantify mesh stiffness. The load will be applied at a rate of 25 mm/min with a 1-inch gauge. Stiffness will be calculated as the applied force divided by the change in length. Stiffness will be compared between pristine and explanted samples that have undergone the same digestion method. To date, an IRB protocol to acquire explanted surgical mesh has been approved. Unused and explanted mesh samples have been obtained. Statistical comparison of the five tissue digestion protocols will be used to identify the most effective protocol (i.e. the method resulting in the smallest change in mesh stiffness between pristine and explanted samples and complete tissue removal. Successful completion of this project will define a tissue digestion protocol for explanted surgical meshes. Furthermore, the effect of tissue digestion solutions on mesh weight and pore size will be investigated. The mesh registry will undergo the tissue digestion procedure en route to mechanical testing and chemical analysis.

Poster # 27

Thripthy Chandran-Student

Email: chthripthy@gmail.com

Affiliation: Mercer University

Title of Abstract: Trastuzumab functionalized Poly-Ɛ-Caprolactone/Pluronic based Nanoparticles for Targeted Delivery Of Docetaxel

Presenting Author: Thripthy Chandran

Affiliation: Mercer University

Complete Author's List :

Martin D'Souza

All Author Affiliations :

Department of Pharmaceutical Sciences, Mercer University

Abstract:

Docetaxel is a semi-synthetic, taxane analog which is used in the treatment of several cancers. Commercially available docetaxel formulation, Taxotere manifests several undesirable effects both due to the drug and the excipient Tween 80. Hence, development of targeted delivery system has a great potential in overcoming the dose limiting toxicities and low oral bioavailability associated with poorly soluble docetaxel. In our lab, we aimed to design novel trastuzumab conjugated poly-ɛ-caprolactone (PCL)/pluronic F108 based nanoparticles for docetaxel that can accumulate at the tumor site and enhance the cellular internalization of the nanoparticles. Docetaxel was loaded into the (PCL)/pluronic F108 nanoparticles by solvent displacement method. Briefly, poly-ɛ-caprolactone (15mg/ml) and 10%w/w docetaxel were dissolved in acetone. This solution was added dropwise to F108 solution containing a mixture of F108 and F108 –COOH to form nanoparticles. The R- OH end groups of pluronic polymer F108 were chemically modified to form F108 –COOH groups. The nanoparticles with F108 –COOH were then bioconjugated to NH2 groups of trastuzumab using EDC and sulfo –NHS. The trastuzumab modified nanoparticles were then characterized for their size and zeta potential. Cellular uptake and internalization of the nanoparticles was studied using coumarin -6 loaded trastuzumab modified nanoparticles in BT-474 cells. Balb/c mice were injected with 4TO7 murine breast cancer cells and the passive targeting ability of nanoparticles was studied using near infrared carbocyanaine dye DiR. The size distribution of the tastuzumab modified nanoparticles was around 190 nm – 450 nm and zeta potential was -1.89 ± 4.89 mV respectively. Cell uptake of trastuzumab modified coumarin -6 loaded nanoparticles was studied in HER2 positive BT474 breast cancer cells and was found to be higher than coumarin -6 loaded nanoparticles alone. Time dependent uptake of the ligand modified particles was similar to nanoparticles alone and was highest at 2hours. The PCL/F108 nanocarrier systems could passively accumulate at the tumor site in the mice as early as 0.5 hours and exhibited sustained release of the DiR dye. The preliminary results suggest that the poly-ɛ-caprolactone/pluronic nanoparticles can be a promising delivery system and can be effectively employed as targeted drug delivery systems for the treatment of solid tumors.

Poster # 29

Michael DiBalsi-Master Student

Email: mdibals@g.clemson.edu

Affiliation: Drug Design, Development and Delivery Laboratory, Department of Bioengineering, Clemson University, Clemson, SC 29634, USA

Title of Abstract: Heparin-immobilized Electrospun Nanofibers for Vascular Sutures

Presenting Author: Michael DiBalsi

Affiliation: CLEMSON UNIVERSITY

Complete Author's List :

Michael DiBalsi†, Sooneon Bae†, Guzeliya Korneva†, Konstantin G. Kornev‡, and Jeoung Soo Lee†,\*

All Author Affiliations :

†Drug Design, Development and Delivery Laboratory, Department of Bioengineering; ‡School of Materials Science & Engineering, Clemson University, Clemson, SC 29634, USA

Abstract:

Free tissue transfer is a widely used technique for soft tissue reconstruction in which surgical sutures are used to connect the graft and implant site vasculature. One of the most common complications that can lead to graft failure is anastomotic thrombosis. Heparin is the most commonly used anti-coagulant to prevent anastomotic thrombosis. Various studies have shown that heparinized surfaces provide improved thrombo-resistance. Specifically, immobilized heparin on the surface of biomedical devices binds plasma anti-thrombin III (AT-III), concentrating and increasing its activity to reduce platelet adhesion, increase plasma re-calcification time, and (increase/reduce) activated partial thromboplastin time. The objective of this study was to develop heparin-immobilized electrospun nanofibers composed of PLGA, PEO, and a proprietary positively charged amphiphilic copolymer (P-AC) as a microvascular suture. We hypothesize the positively charged electrospun nanofiber may interact with negatively charged heparin via electrostatic interactions and provide sustained and controlled release of heparin from the suture. P-AC/PLGA nanofiber sutures, made of a different composition of PLGA, PEO, and P-AC were fabricated by the electrospinning technique, and then the resultant nanofibers were heparinized by incubation in 1% heparin solution in PBS, then lyophilized. The morphology and diameter of the resulant fibers were characterized by FE-SEM, and the mechanical properties were evaluated by tensile testing (MTS Synergie 100). The amount of immobilized heparin on the P-AC/PLGA nanofibers was measured using toluidine blue (TB) colorimetric assay. SEM images of the nanofibers showed a twisted fibrous yarn without significant difference between nanofibers with or without P-AC. After incubation with heparin solution, TB assay demonstrated that 2.13 ± 0.43 µg of heparin was present per 10 mm length of nanofibers. In summary, various types of P-AC/PLGA/PEO electrospun nanofibers were successfully fabricated using electrospinning technique. Heparin was immobilized on the positively charged P-AC/PLGA/PEO nanofibers via ionic interaction. Currently, we are preparing electrospun nanofibers with various compositions to improve the heparin loading efficiency, and evaluating the feasibility of heparinized nanofibers as surgical sutures for vascular anastomosis.

Poster # 30

Melissa Gaillard-Polymeric Polylactide Beads as Microcarriers in Targeted Cell Therapy

Email: mgailla@clemson.edu

Affiliation: Clemson University

Title of Abstract: Polymeric Polylactide Beads as Microcarriers in Targeted Cell Therapy

Presenting Author: Melissa D Gaillard

Affiliation: Clemson University

Complete Author's List :

Melissa D Gaillard

All Author Affiliations :

Melissa D. Gaillard, Erin J. McCave, Karen J.L. Burg

Abstract:

Over 12% of women will develop invasive breast cancer at some point in their lifetime. Many treatment options are available; however, a mastectomy is the only option that allows for reconstructive surgery of the breast. As such, there is a need to develop new, minimally invasive reconstruction options. One such option is by delivering the patient’s own healthy cells to the defect site in order to generate new adipose tissue. The short-term goal of this project is to develop a novel breast tissue platform and to maximize cell growth on and biocompatibility with polylactide microcarriers by adjusting polymer processing methods. Polylactide beads were produced by 3 different processing methods by varying vessel size, paddle size, and PVA solution concentration. The first study assessed cell growth on each polylactide bead batch. Cells were seeded onto each type of microcarrier in a tilt-suspension culture system. Cells were visualized using IHC staining and fluorescence microscopy. The goal of the second study was to create a breast tissue platform in which the functionality of the microcarriers could be evaluated. Mechanical properties of the soft gel scaffolds with and without polylactide beads were quantified using Atomic Force Microscopy. The long-term goal of this project is to create a tunable, porous polymeric microcarrier with controlled degradation profiles onto which assorted cells, including adult stem cells, could be loaded. This would result in a tissue-engineered device that could heal or rebuild a defect site in a minimally invasive manner.

Poster # 31

Kayla Gainey-

Email: kgainey@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: GlucoSense: Design of a Low Cost Diabetes Glucometer System

Presenting Author: Kayla E. Gainey

Affiliation: Clemson University

Complete Author's List :

Kayla Gainey(1), Tyler Ovington(1), Dr. John DesJardins(1), and Dr. Delphine Dean(1)

All Author Affiliations :

(1)Bioengineering, Clemson University

Abstract:

More than 347 million people in the world have diabetes and require daily blood glucose monitoring. As glucometers have evolved, they have become more accurate (~3% variance), but test strips can be expensive for patients, especially those without health insurance. In addition, in low-resource settings that rely on donated medical supplies, matching meters and strips are not always available to patients. The goal of our project is to design a low-cost meter and strip system that can be used in resource poor settings when standard meters or strips are not available. Our strategy is to create test strips that may be printed on-demand by a standard inkjet printer. This system would serve as a means for resource-poor settings to manage diabetes. To print the enzyme, we used emptied color-ink cartridges. Glucose oxidase, horseradish peroxidase, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) are inserted in the printing wells of separate cartridges. These enzymes catalyze a glucose reaction whose final products elicit a color change. The enzymes are printed using a template in Microsoft Word. By varying the color in the templates, we can select the amount of each enzyme applied to the paper. Filter paper is used due to the high viscosity of blood to provide adequate flow. Once the paper has the solutions printed, it is covered with contact paper for structure and to prevent bleed-through. For proof of concept, the strips were tested using glucose solutions of varying concentrations (0-450 mg/dl). To read the strips, we designed a low-cost glucometer using LED lights, a photodetector, and an amplifier that outputs the absorbance, which is processed by an Arduino microcontroller to determine the glucose concentration based on a standard curve. We found that the absorption measurement was able to distinguish between glucose solutions with 25 mg/dl accuracy. This provided us with a standard curve to relate absorbance to glucose concentration; it also showed that the enzyme concentrations were capable of accurately processing the glucose in the range of 0-350 mg/dL. The glucometer has shown a difference in outputs when strips of varying darkness are used. However, the code is not yet in place to provide a calculated reading. After trying several test strip designs, we have a strip that will control the amount of blood the enzymes are exposed to while allowing for use of the control photodetector. Our preliminary testing has shown a definitive color change on the strips that were printed using an inkjet printer when a 450 mg/dl glucose solution was applied. Currently, work is being done to provide the necessary stability to the enzymes and dyes to increase test strip shelf life. The research thus far confirms that a printable test strip is feasible as a means for monitoring glucose levels. While these strips are not as fast as current commercially available strips, they meet the ISO standard for accuracy in a glucometer. In addition, the current design of the measurement device is inexpensive and easy to build. Improvements can be made and more testing is planned. While photodetectors have been used successfully in other applications to measure absorbance, a completed circuit needs to be tested to be certain they will work in this system. Acknowledgements: Clemson Creative Inquiry, NIH K25 HL092228

Poster # 32

Dmitry Gil-

Email: dgil@clemson.edu

Affiliation: Clemson University

Title of Abstract: Polypropylene hernia meshes: in vitro modeling of degradation

Presenting Author: Dmitry GIl

Affiliation:

Complete Author's List :

Dmitry Gil, Alexey Vertegel

All Author Affiliations :

Clemson University

Abstract:

Nowadays hernia repair is one of the most frequently performed surgical procedures in the United States, with an estimated 800 000 new cases annually. However, it has been reported that approximately 30% of patients experience pain or discomfort following inguinal or abdominal hernia repair. It was proposed that the cause of these complications might be chemical and structural alterations of prosthetic materials. However, degradation processes of hernia repair meshes, which occur after implantation, are studied insufficiently. There is a common theory that prosthetic materials degrade due to the high concentration of reactive oxygen species at the surgical site. At the same time it was presumed that implants may be more susceptible to the enzymes such as myeloperoxidase that are produced in acute inflammatory phase. Hence, the major goal of this work is to reveal actual factors that cause polypropylene (PP) hernia meshes failure. In order to achieve this aim we treated two types of PP hernia meshes (Composix™ and UltraPro™) in H2O2/CoCl2 and NaOCl solutions for 40 days and then analyzed the samples using FTIR, DSC, TGA and SEM techniques. Moreover, tensile strength testing was performed in order to evaluate mechanical properties of treated samples. We have shown that UltraPro™ meshes are less vulnerable to ROS and OCl- anions than Composix™ implants. Also we have demonstrated that oxidation of the implants changes their mechanical properties.

Poster # 33

Ben Green-

Email: bmgreen@clemson.edu

Affiliation: Department of Bioengineering. Clemson University

Title of Abstract: Polymeric Micelle as a Drug and Gene Delivery Carrier for Brain Tumor

Presenting Author: Benjamin M. Green

Affiliation: Department of Bioengineering Clemson University

Complete Author's List :

Benjamin Green, So-Jung Gwak, Graham Temples, and Jeoung Soo Lee

All Author Affiliations :

Department of Bioengineering. Clemson University

Abstract:

Primary tumors centralized to the brain and spinal cord are among the most difficult to treat due to the fragile nature of the surrounding tissue. This issue is compounded by the introduction of drug resistant lines, such as glioblastoma, and leads to low survival amongst the diagnosed. Temzolomide (TMZ), a DNA alkylating drug, is commonly used to treat glioblastomas, but is rendered ineffective against the drug resistant lines by the overexpression of O-6-methlygunaine-DNA methyl transferase (MGMT), a DNA repair protein. Small interfering RNAs (siRNAs) have been investigated as a precursor treatment for drug resistant cancers because of their ability to down regulate their target protein by preventing translation. Our approach is based upon amphiphilic copolymers poly(lactide-co-glycolide)–g-polyethylenimine (PgP) that spontaneously form polymeric micelles in aqueous solution. This material offers three important capabilities: 1) loading of hydrophobic drugs in the PLGA hydrophobic core, 2) complexation of nucleic acids with the PEI hydrophilic shell, and 3) cell-type specific targeting through surface conjugation of cell-type specific ligands or antibodies. Poly(lactic-co-glycolic acid)-graft-poly(ethylenimine) (PgP) was synthesized as a nucleic acid and drug delivery vehicle and was first evaluated as a gene carrier using pGFP as a reporter gene in B35 neuroblastoma cells and T98G glioblastoma cells. The transfection efficiency and cytotoxicity of PGP/pGFP complexes at various N/P ratio were evaluated at 48hrs post-transfection of PGP/pGFP complexes in non-serum and 10% serum condition. Transfection efficiency was measured by flow cytometry and cytotoxicity of PGP/pGFP complexes was evaluated by MTT assay. In both cells, transfection efficiency was higher in serum conditions than non-serum condition and the % transfected cells was approximately 75 and 60 % in serum condition without significant cytotoxicity in B35 and T98G cells, respectively. Currently, we are testing the silencing effect of PgP/MGMT siRNA polyplexes in T98G cells. In the future, we will evaluate TMZ loaing efficiency in PgP micelle and the cytotoxicity of TMZ-loaded PgP/MGMT siRNA polyplexes in T98G cells. Acknowledgements: This study was partly supported by NIH grant # P20GM103444

Poster # 34

Mohammad Mahdi Hasani-Sadrabadi-

Email: mahdi.hasani@gatech.edu

Affiliation: Bioengineering Program, G. W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta 30332, GA, USA.

Title of Abstract: Microfluidic Fabrication of pH-Responsive Core-Shell Nanoparticles for Oral Delivery of Cancer Therapeutics

Presenting Author: Mohammad Mahdi, Hasani-Sadrabadi

Affiliation: Bioengineering program, G. W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta 30332, GA, USA.

Complete Author's List :

Mohammad Mahdi Hasani-Sadrabadi†,‡, Fatemeh Sadat Majedi‡, Philippe Renaud‡, Karl I. Jacob†,\*

All Author Affiliations :

† G. W. Woodruff School of Mechanical Engineering and School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta 30332-0295, GA, USA. ‡ Laboratoire de Microsystemes (LMIS4), Institute of Microengineering and Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

Abstract:

Conventional chemotherapy systems are normally administered by injection, and unfortunately involve circulating significant concentrations of these toxic drugs systemically. Here we demonstrate an efficient tumor targeting nanoparticulate drug delivery system, which is designed for the oral administration of cancer therapy. For the first time, a solvent-free, dual microfluidic platform has been employed to synthesize nanocarriers with highly pH-tunable core-shell structure. These nanocarriers consisted of a fine-tuned self-assembled polymeric core precisely coated with a sacrificial pH-responsive layer of defined thickness, and were fabricated via a microfluidic device with two discrete mixing steps. The sacrificial shell protects the core layer drugs and prevents their release in the severe pH conditions of the gastrointestinal tract, while allowing for drug release in the proximity of a tumor. Quantum mechanics and molecular dynamics simulations were performed to investigate the molecular interactions governing behavior of the core-shell system. The release profile and cytotoxicity of the nanoparticles (loaded with paclitaxel as a model chemotherapeutic drug, PTX) against Caco-2 cell line were studied using sequential incubation in different pH conditions. These results revealed a PTX release profile highly dependent environmental on pH. This microfluidic approach is a new way to design efficient chemotherapeutic delivery systems that take advantage of pH conditions in the digestive tract to deliver more potent therapeutics to the target site. Such advancements in oral administration routes promise ‘chemotherapy at home’ for future cancer care.

Poster # 35

Devon Headen-Microfluidic Cell and Cell-Cluster Encapsulation in PEG-4MAL Hydrogel Microspheres

Email: dheaden@gatech.edu

Affiliation: Petit Institute for Bioscience and Biotechnology, Georgia Tech

Title of Abstract: Microfluidic Cell and Cell-Cluster Encapsulation in PEG-4MAL Hydrogel Microspheres

Presenting Author: Devon M. Headen

Affiliation: Petit Institute for Bioscience and Biotechnology, Georgia Tech

Complete Author's List :

Devon M. Headen, Andrés J. García

All Author Affiliations :

Petit Institute for Bioscience and Biotechnology, Georgia Tech; Petit Institute for Bioscience and Biotechnology, Georgia Tech

Abstract:

We have engineered a microfluidic platform on which cells or cell-clusters can be encapsulated within synthetic hydrogel microcapsules, providing potential immunomodulatory effects for transplanted cells, while simultaneously providing a precisely controlled, biofunctional cellular microenvironment. These microcapsules can be scaled from approximately 20 μm to 800+ μm diameter by altering device geometry and flow rates, providing flexibility required for many applications in cell or drug delivery. The microfluidic platform is paired with a synthetic hydrogel system based on 4-arm PEG (PEG-4MAL) with maleimide functional units, which enables incorporation of protease degradable or non-degradable crosslinks, as well as ‘plug-and-play’ functionalization with bio-active ligands and growth factors for precise microenvironment control. In this system, peptides or small molecules are used to crosslink hydrogels using cyto-compatible maleimide Michael-type addition reactions, and cysteine-containing peptides can be tethered to the polymer. Additionally, pore size of hydrogel capsule, and therefore its permeability to critical immune molecules, can easily be tuned when using PEG-4MAL, by varying critical hydrogel parameters such as polymer weight percent, and macromer molecular weight. In vitro studies show that microcapsules functionalized with the peptide adhesive ligand GRGDSPC (‘RGD’) support viability of clinically relevant cell types including human mesenchymal stem cells and human pancreatic islets. The function of encapsulated pancreatic islets is not grossly effected, but there is some delay in insulin responsiveness when compared to unencapsulated islets. To decrease this time lag, as well as to mitigate risk of hypoxic microcapsule cores, capsules containing islets have been shrunk to 200 μm – 300 μm without increasing the likelihood of microfluidic device failure due to clogging. Limits of small–scale cell encapsulation have also been investigated, with mesenchymal stem cells being encapsulated in ~20-40 μm capsules, well below size limits of electrostatic droplet generators traditionally used for cell encapsulation. This microfluidic platform, when combined with cytocompatible, synthetic PEG-4MAL hydrogel chemistry, provides a necessary step forward in cell encapsulation technologies. PEG-4MAL improves on current, natural hydrogel schemes by enabling precisely-controlled, well defined cellular microenvironment, and microfluidics allow for reduction in microcapsule size beyond what is possible with current encapsulation approaches.

Poster # 36

Devante Horne-Undergraduate

Email: devanth@clemson.edu

Affiliation: Clemson University

Title of Abstract: Effects of Industrially Processed PLGA Thin Films on Drug Delivery and Material Properties

Presenting Author: Devante, A., Horne

Affiliation: Clemson University

Complete Author's List :

Devante Horne, Yubin Zhou, Kumar Vedantham, Ph.D., Terry Steele, Ph.D.

All Author Affiliations :

Devante Horne (1), Yubin Zhou (2), Kumar Vedantham, Ph.D. (2), Terry Steele, Ph.D. (2) Bioengineering, Clemson University (1); Materials Science and Engineering, Nanyang Technological University (2)

Abstract:

Thin films are widely used in pharmaceutical applications as conduits for localized drug delivery. Currently, thin film processing includes methods such as knife casting and spin coating. However, these solvent based processing methods have drawbacks such as the presence of residual organic solvent post-processing; the removal of which is costly and labor intensive. Previously our group has investigated the tuning of drug release from PLGA thin films controlled by terminal end group modification. Now, we are interested in developing a “solvent-free” processing method to produce PLGA thin films controlled by terminal end group modification. We are proposing an extrusion method that can be industrially scaled-up for thin film production. We will report the effects of processing parameters on extruded PLGA film’s physicochemical properties as well as its drug release properties. Various formulations of PLGA thin films were produced using the following materials: Methyl-ester terminated PLGA (PLGA5004, 50kDa), Carboxylic Acid terminated PLGA (PLGA5004A, 40kDa), and fluorescein diacetate (FDAc, 416.39g/mol). PLGA5004:PLGA5004A were mixed at different ratios [1:0 (S1), 0.5:0.5 (S2), 0:1(S3)]), each loaded with 3 different weight ratios of FDAc (0.5%, 1%, 2%). The polymer-drug mixtures were melt extruded using the counter-rotating twin screw (1x4mm die) laboratory extruder. Each formulation was extruded for 1, 5, and 10 minutes residence time at 120°C and 100°C. Each extruded strip was then pressed with a 2ton force (60°C) into a thin film using a melt press. A metal punching tool and a hammer was used to form 4.8mm diameter punchouts from the pressed films. These punch-outs were used for drug release and other characterization. GPC was used to analyze the effect of extrusion processing parameters on the degradation of PLGA. A high-throughput screening method was used to quantify the amount of FDAc released from each film. The drug release media used was a PBS-Tween solution. Samples collected at predetermined time intervals were analyzed for FDAc release using a microplate reader. Controlled release of FDAc was observed for all three PLGA formulations (S1, S2, S3). Formulations processed with lower residence time showed higher release rate for all three formulations. Preliminary results indicate PLGA 5004 (S1) showed a higher release of FDAc compared to PLGA5004A (S3) and S2 formulations. Processing temperature did not have much effect on the drug releasing properties of different formulations. Protocols for thin film preparation (260µm) using melt extrusion and melt pressing techniques were established. We conclude that extrusion processing did not induce degradation of PLGA, as confirmed from GPC analysis. We did observe a controlled release of FDAc from extruded PLGA films that showed dependence on extrusion processing time and modification of PLGA with different terminal group ratios.

Poster # 37

Olukayode Karunwi-

Email: okarunw@clemson.edu

Affiliation: Department of Bioengineering, Clemson University

Title of Abstract: Biofabrication of a Dual Responsive Glucose and Lactate Implantable Biosensor

Presenting Author: Olukayode Karunwi

Affiliation: Bioengineering, Clemson University

Complete Author's List :

Olukayode Karunwi (1,2), Fouzan Alam (1,3) and Anthony Guiseppi-Elie, Sc.D. (1,2,3,4)

All Author Affiliations :

Center for Bioelectronics, Biosensors and Biochips (C3B), Clemson University Advanced Materials Center (1); Bioengineering, Clemson University (2); Chemical and Biomolecular Engineering, Clemson University (3); Electrical and Computer Engineering, Clemson University (4)

Abstract:

With the current state of progress in trauma management by first responders, there is a need for development of an electrochemical biotransducer that can be used with a wireless implantable biosensor system for continual measurement of interstitial glucose and lactate; beginning from the site of the accident and en-route to a trauma center. Fabrication of oxidoreductase enzyme-rich biorecognition membranes deposited via pyrrole electropolymerization at microfabricated electrodes has been achieved. This construct was then electrochemically overoxidzed to create a non-conductive enzyme-hosting polymer film and finally the entire biotransducer was coated with a phosphorylcholine-containing biomimetic hydrogel to mitigate biofouling and reduce the foreign body response. The role of a catalytic layer of Fe/Ni-hexacyanoferrate placed at the electrode-enzyme interface for enhanced and stable peroxide response was examined for its influence on biosensor performance. The catalytic layer produced a 20-fold increase (14.19 nA compared to 0.7 nA) in buffered H2O2 response at 650 mV vs. Ag/AgCl. In vitro characterization showed a sensitivity of 0.68 mA/cm2/mM and a limit of detection of 0.05 mM for glucose and a sensitivity of 0.36 mA/cm2/mM, limit of detection of 7.9 mM for lactate. The catalytic layer conferred a more stable and consistent response but a threefold reduction in sensitivity (0.32 nA/mM) compared to the control (0.94 nA/mM).

Poster # 38

Amanda Macaluso-Graduate Student

Email: amacalu@clemson.edu

Affiliation: Department of Bioengineering, Clemson University

Title of Abstract: A Simple Assay for Detecting Biofilm Accumulation on Commonly Used Medical Device Materials

Presenting Author: Amanda G. Macaluso

Affiliation: Department of Bioengineering, Clemson University

Complete Author's List :

Amanda G. Macaluso Donna R. Weinbrenner, Ph.D Melinda K. Harman, Ph.D

All Author Affiliations :

Department of Bioengineering, Clemson University (1,3); Department of Biological Sciences, Clemson University (2)

Abstract:

Biofilm formation on reusable medical devices poses a high risk for infection. Current materials and design characteristics of many reusable devices create opportunity for greater biofilm accumulation and pose challenges for proper cleaning methods. High throughput screening methods based on colorimetric assays have been proposed for quantifying biofilm adherence to polystyrene tissue culture plates. The purpose of this study is to apply this method to detect biofilms that have been grown under static and dynamic conditions on a variety of materials commonly used in reusable medical devices. Staphylococcus epidermidis biofilms were grown under both static and dynamic conditions. For dynamic growth a Drip Flow Biofilm Reactor (DFR) (Biosurfaces Technologies Corp., Bozeman, MT) was implemented according to an ASTM method (ASTM 2647-08) for low shear continuous flow conditions modified for use with staphylococci by Buckingham-Meyer, et.al (2009). Biofilms were grown on coupons (25mm x 75mm) representative of commonly used medical device materials: 316 L stainless steel (n=3), polycarbonate (n=3), polypropylene (n=3), and silicone rubber (n=3). Biofilms were grown for 48 hours. Coupons were washed to remove planktonic bacteria and then heat fixed for 1 hour at 60°C. Fixed biofilms were stained with 0.1% Harleco crystal violet gram stain for 10 minutes. Excess stain was removed by washing until wash solution was clear. Coupons were then sonicated in 20mL of 33% acetic acid for 20 minutes to elute the stain from the adhered biofilm. The eluted stain solution (200μl) was transferred to the appropriate wells of a sterile, polystyrene 96 well tissue culture plate and read in a microplate spectrophotometer (Epoch™, BioTek® Instruments, Inc.,Winooksi, VT) at a wavelength of 492nm. The amount of crystal violet bound to each coupon was then quantified by measuring optical density in each well. Based on preliminary results, biofilm accumulation varied for material type and growth conditions. In static conditions, biofilm accumulation was greatest on stainless steel based on it having the highest optical density value. In dynamic conditions, biofilm accumulation was greatest on polypropylene based on it having the highest optical density value. Biofilm accumulation on polycarbonate was the least in both static and dynamic conditions based on it having the lowest optical density value. Ongoing work will determine the relationship between optical density and biofilm concentration (CFU/mL). Colorimetric assays provide a simple, yet quantitative method for detecting accumulated biofilm on reusable medical devices, providing an important tool for validating cleaning methods. Future work will compare these findings with more advanced fluorescent assays commonly used in confocal imaging of biofilms.

Poster # 39

Nicholas Marais-

Email: nmarais@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: Cited Causes of TKR Failure in the United States and the Associated Financial Burden

Presenting Author: Nicholas C. Marais

Affiliation: Clemson University

Complete Author's List :

Nicholas C. Marais Eric M. Lucas, PhD Taylor Gambon John D. DesJardins, PhD

All Author Affiliations :

Bioengineering, Clemson University (1); Bioengineering, Clemson University (2); Bioengineering, Clemson University (3); Bioengineering, Clemson University (4)

Abstract:

Current healthcare outcomes require a more comprehensive review of the cited causes of failure of primary and revision TKR procedures. The goal of this study was to conduct a long-term analysis of failure trends and to include the associated financial burden caused by each mode of failure. The Nationwide Inpatient Sample (NIS) was used to assess over 250,000 revision TKR procedures between the years 2005-2011. A processing algorithm developed in Statistical Analysis Software (SAS) was used to extract and process the data from each procedure per year. The most frequent and most costly revision TKR procedure was the all component replacement (00.80). Mechanical loosening (996.41) was the most common diagnosis overall for all revision procedures (28.54%). Implant failure (996.43) showed an inverse relationship to other mechanical complication of prosthetic joint implant (996.46) over time. Patients over the age of 75 had the highest risk of periprosthetic fracture (996.44) and bearing surface wear (996.46). Mechanical loosening caused the greatest financial burden each year, costing approximately $250 million dollars in 2011. While the rate of each cause of failure remained constant, mechanical loosening increased. Because there is a positive trend in the number of new implants each year, it can be hypothesized that mechanical loosening will be a higher risk factor and financial burden long term. So although rates of revision due to each failure mode are an acute healthcare cost, the health care system will face an increasing burden most likely due to mechanical loosening.

Poster # 40

VeeAnder Mealing-

Email: vmealin@clemson.edu

Affiliation: Bioengineering, Clemson University

Title of Abstract: Bone Decomposition After Death: Developing a Forensic Bioreactor to Mimic Burial Settings

Presenting Author: VeeAnder Mealing

Affiliation: Clemson University

Complete Author's List :

VeeAnder Mealing, Matthew Pysh, Katherine Weisensee, Elena Mikhailova, Melinda Harman

All Author Affiliations :

Bioengineering, Clemson University (1); Bioengineering, Clemson University (2); Sociology and Anthropology, Clemson University (3); Agricultural, Forest and Environmental Sciences, Clemson University (4); Bioengineering, Clemson University (5)

Abstract:

This research introduces a new technological system, namely a forensic bioreactor, to simulate decomposition of the human skeleton as relevant to forensic science. The proposed forensic bioreactor mimics environmental conditions for a burial setting in an outdoor context, providing for control of environmental variables known to impact bone decomposition and potential use in estimating the postmortem interval (PMI). Skeletonization of human remains is estimated to occur within approximately 6 months of death under average weather conditions. In an outdoor context, a number of environmental conditions (physical, biological, and chemical) alter skeletal remains through degradation and weathering. The remaining bone consists of an organic and inorganic matrix, primarily composed of Type I collagen proteins and calcium hydroxyapatite, respectively. The chemical citrate {C6H8O7} is bound to the apatite crystal in bone, and studies suggest that citrate can be used to determine the PMI because it has known expected concentrations in skeletal material and it is believed to have a predictable decomposition rate with time. Currently, studies evaluating the impact of environmental conditions on citrate degradation in a controlled setting have not been reported. Eight closed forensic bioreactors with biosystems controls capable of monitoring and controlling environmental conditions will be created. Each will house four individual plastic soil chambers that are open to the atmospheric conditions within the bioreactor. These conditions will be defined using the following variables: temperature, soil moisture, and soil type. The bioreactors will be programmed to maintain an average temperature of four different climates ranging from Frigid (0°-7°C) to Hyperthermic (>22°C), two soil moisture types, Udic and Aridic, and two soil orders, Ultisol and Mollisol. All of these target ranges are relevant to body decomposition based on previous forensic research. Previous studies in our lab demonstrated that air temperature could be highly variable when monitored manually using a simple thermometer and does not sufficiently capture the various environmental variables known to impact decomposition. Therefore, the environmental conditions in each bioreactor will be monitored using air and soil temperature sensors, humidistats, and soil moisture sensors within each bioreactor and soil chamber. All sensors and environmental control systems will be interfaced with LabView software programmed to provide for continuous monitoring of environmental conditions and routine recording of outputs. Environmental conditions will be checked daily so that variations in the controlled parameters (temperature, humidity, soil moisture) are minimized. Soil chemistry (e.g. pH, mineral content, etc.) will be measured through soil laboratory analysis prior to its introduction into each bioreactor.

Poster # 41

Olanrewaju Oludipe-

Email: olanrewaju.oludips@gmail.com

Affiliation: Morehouse College

Title of Abstract: Preparation and Characterization of Thermo-sensitive Nanofibers with Neuroprotective Nanoparticles

Presenting Author: Olanrewaju Oludipe

Affiliation: Morehouse College

Complete Author's List :

Dr. Juana Mendenhall

All Author Affiliations :

1. Chemistry Department, Morehouse College. 2. Smart Bio materials Lab, Morehouse College.

Abstract:

Electrospinning is a process that applies electric charge to a polymeric solution to overcome the solution’s surface tension and draw out fibers from the solution which upon, by means of evaporation of a solvent, produces randomly oriented fibers (mostly in the micro or nano range) on a grounded substrate. This project entails using electrospinning to prepare thermo-sensitive poly(n-vinylcaprolactam)[PVCL] nanofibers filled with cerium oxide (CeONP) nanoparticles. PVCL is defined as a thermo-sensitive polymer due to its ability to change its molecular confirmation upon temperature change exhibiting a lower critical solution temperature called a cloudy point. The rationale for preparing PVCL fibers incorporated with CeONP is to design a nueroprotective 3D tissue scaffold for tissue engineering applications. In this study, PVCL polymers were synthesized followed by preparation of highly concentrated and viscous solutions of PVCL-CeONP at various concentrations. These solutions wereelectrospun at various parameters to produced nanofibers with various size dimensions and 3D architecture. Fiber size dimensioned were analyzed using scanning electron microscopy (SEM). Future studies consist of determining the porosity and morphology of the fiber mat samples using Image J® as well as of determining cell viability and cytoprotection of PVCL-CeONP on cartilage cells under low oxygen environments.

Poster # 42

Sean Patterson-

Email: thepatterson16@gmail.com

Affiliation: Morehouse College

Title of Abstract: Investigating Thermal & Gelation Properties of Poly(N-vinyl caprolactam) Crosslinked hydrogels

Presenting Author: Sean Patterson

Affiliation:

Complete Author's List :

Sean Patterson, Chang Y. Ryu, Juana Mendenhall, Matthew Ravalli

All Author Affiliations :

Rensselaer Polytechnic Institute, Morehouse College

Abstract:

Injectable hydrogels are considered smart materials due to their ability to change gelling properties upon temperature change. Recently, our research lab has poly(n-vinyl)-crosslink-PEO hydrogels that show promise as injectable hydrogels for tissue engineering applications. For the development of injectable hydrogels, rheological characterizations of these materials have been performed to optimize the gelation temperature and elastic moduli of the materials. Specifically, we have prepared a series of PVCL-PEO samples to study how the physical gelation depends upon heating the polymers using rheological measurements. Based on the differential scanning calorimetery (DSC) results, our PVCL-PEO samples did not show the PEO melting peaks, suggesting the PEO did not formed crystalline domains in the samples. This allows us to focus on the rheological studies for the gelation of the copolymers without the potential complications of PEO melting transitions. By graphing the elastic and viscous moduli (G’ and G”) against angular frequency and temperature, the physical changes in the PVCL-PEO samples have been monitored. Dimethyl formamide (DMF) was used as solvent first, but this not produce a good homogeneous solution for rheological experiments. Therefore, Tetrahydrofuran (THF) was chosen as a solvent to prepare sample solutions. Rheological data was collected for a heat/cool temperature cycle between 25°C and 50°C at a rate of 2°C/min. Frequency sweeps have been compared at 25°C before and after the temperature ramp to show that the PVCL-PEO developed gel-like rheological response (G’ ~ ω°). A sharp rise in the elastic modulus (G’) is observed at ~ 41°C for the PVCL homopolymer solution, which corresponds to the lower critical solution transition (LCST) temperature of PVCL in THF. In contrast, the rheological changes upon heating for the PVCL-PEO-7 and PVCL-PEO-8 copolymer solutions had been more or less gradual over the temperature range from 30°C to 50°C. PVCL-PEO-7 shows a significant drop in G’ at low frequencies to indicate there are defects in the network structures at 25 °C. However, after heating the sample to 50 °C the sample shows rheological response for well-developed networks, where G’ ~ ω0. PVCL-PEO-8 shows the G’ ~ ω0 response even before heating to 50 °C, to suggest that the sample already develop “defect-free” network structures. After heating, there has been significant increase in the elastic modulus to indicate that the network density have greatly increased in this case. We have studied how the thermal history of the gel-forming polymers affects the rheological responses. Specifically, the temperature–induced gelation of PVCL-PEO samples have been studied using rheological measurement under dynamic shear using parallel plates. PVCL-PEO-7 had defective network structure at room temperature before the temperature cycle. Those defects in the network structures had been healed by heating the samples to 50 °C. In contrast, PVCL-PEO-8 did not have defects in the network at room temperature before the cycle. However, the network density greatly increased upon the application of the temperature cycle to 50 °C to increase the elastic modulus by about 2 orders of magnitude.

Poster # 43

Matthew Pysh-Graduate Student

Email: mpysh@g.clemson.edu

Affiliation: Bioengineering, Clemson University

Title of Abstract: Analytical Methods for Assessing Bone Biochemistry to Determine Citrate Concentration and Mineral Content: Applications for Forensic Anthropology

Presenting Author: Matthew K. Pysh

Affiliation: Clemson University

Complete Author's List :

Matthew Pysh, Katherine Weisensee, Mark Schlautman, Melinda Harman

All Author Affiliations :

Bioengineering, Clemson University (1); Sociology and Anthropology, Clemson University (2); Environmental Engineering & Earth Sciences, Clemson University (3); Bioengineering, Clemson University (4);

Abstract:

This research aims to develop and verify analytical methods to assess bone biochemistry and changes that occur with bone decomposition. This has application in forensic anthropology for determining the post-mortem interval (PMI), or time elapsed since a person’s death, based on analysis of bone tissue. PMI currently is measured by methods that are difficult to apply to skeletal remains, namely evaluating soft tissue decomposition. Bone is formed by osteoblasts and mineralized by calcium and phosphate in a ratio (Ca/P) of approximately 1.67. It consists of an organic and inorganic matrix primarily composed of Type I collagen proteins and calcium hydroxyapatite, respectively. The chemical citrate {C6H8O7} is bound to the apatite crystal in bone. When exposed to outdoor environmental conditions, weathering (physical, biological, chemical) and microorganisms alter and degrade skeletal remains. Studies of bone recovered from burial settings suggest that soil composition can impact Ca/P and that bone citrate degrades at a predictable rate with time. However, when applied to human bone specimens with known PMI, predicted PMI varied from days to decades. Therefore, better analytical methods are needed to detect small changes in Ca/P and citrate weight percent (wt%) in skeletal remains. Biochemical techniques will be applied to known controls of purified bovine bone mineral (NuOss™, Collagen Matrix) having consistent biochemical properties (known Ca/P and citrate wt %) and porcine rib bones acquired from a local slaughterhouse. Citrate concentration and mineral content will be evaluated using select analytical techniques, namely a colorimetric assay combined with absorption spectroscopy, High-performance Liquid Chromatography (HPLC), and other spectroscopy instruments (Raman, etc.) available for broad-spectrum analysis of chemical components in biological tissues. Standard curves will be generated to evaluate detection of known citrate concentrations, and later to determine effects of the bone prepation method on the detected concentrations. Preliminary studies have shown that absorption spectroscopy and HPLC can detect citrate at different concentrations, with greater reliability for higher concentrations. Assessment of bone mineral content is ongoing. In order for assessment of bone biochemistry to be viable options in forensic anthropology, these analytical techniques must be easily executed and rigorously proven to generate accurate results.

Poster # 44

Sarah Rowlinson-Graduate Research Assistant

Email: sarcorow@gmail.com

Affiliation: Department of Bioengineering, Clemson University

Title of Abstract: Clemson Bioengineering Society's Biomaterials Education and Outreach

Presenting Author: Sarah C. Rowlinson

Affiliation: Clemson University

Complete Author's List :

Sarah C. Rowlinson and Zahra Ronaghi

All Author Affiliations :

Department of Bioengineering, Clemson University

Abstract:

The ability of the U.S. to remain competitive in the global economy depends on increasing the number of qualified STEM graduates. There is a significant discrepancy between students interested in STEM fields in middle and high school and those graduating with STEM degrees. This discrepancy is commonly referred to as the “Leaking STEM Pipeline”. This issue is especially true in the southeast. Clemson Bioengineering Society (CBS) is doing its part in “Plugging the Leaks in the STEM Pipeline” by leading a number of activities with the purpose of engaging and retaining students in STEM fields. For a topic as misunderstood as bioengineering, it is crucial that researchers remove the perceived walls between academia and the local community by communicating with the public. CBS members have designed and lead activities for large (+100) and small (<20) groups of students ranging from 2nd graders though incoming freshmen. These activities have involved a wide demographic of students including women and underrepresented minorities in the immediate Clemson area as well as those traveling from other South Carolina counties and Georgia. Bioengineering topics covered in these activities include orthopaedic implants, image guided surgery, tissue engineering, balloon angioplasty demonstrations and hydrogels. In order to better retain students in the Bioengineering Department and matriculate students of exceptional caliber, CBS has a mentoring program in which graduate students are paired with undergraduates to help guide mentees through internship and co-op searching and other career development opportunities. During the presentation, the authors will discuss in-depth how to execute various bioengineering activities and properly engage students depending on age, group size and education background.

Poster # 45

Alex Schudel-S-Nitrosated Poly(propylene sulfide) Nanoparticles for Enhanced Nitric Oxide Delivey

Email: aschudel@gatech.edu

Affiliation: School of Materials Science and Engineering, Parker H. Petit Institute for Bioengineering and Biosciences

Title of Abstract: S-Nitrosated Poly(propylene sulfide) Nanoparticles for Enhanced Nitric Oxide Delivey

Presenting Author: Alex Schudel

Affiliation: School of Materials Science and Engineering, Parker H. Petit Institute for Bioengineering and Biosciences

Complete Author's List :

Alex Schudel (1,2) and Susan Thomas (2,3)

All Author Affiliations :

(1)School of Materials Science and Engineering, (2)Parker H. Petit Institute for Bioengineering and Biosciences, (3)George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology,

Abstract:

Nitric oxide (NO) is one of the most important small molecules in physiology. Its large diffusivity and high reactivity allow it to contribute to a wide spectrum of physiological functions including vasodilation, immune-cytotoxic defenses, lymphatic contractions, and tumor dynamics. NO therefore represents an exciting putative drug candidate for the treatment of a myriad of vascular and lymphatic related pathologies. However, as the result of scavengers such as haemoglobin and its short half-life, NO produced in vivo largely mediates its effects proximal to its synthesis source. Delivery of exogenous NO to deep tissue and/or cellular targets therefore remains a challenge and requires a delivery vehicle capable of stabilizing and transporting NO amidst these physiological pressures. While several NO donors and NO-loaded synthetic polymer platforms have been previously developed, few biocompatible formulations have been explored for NO delivery that can exploit the improved tissue and cell targeting activity of colloidal formulations. To address this we have synthesize a unique NO donor platform based on a previously reported Pluronic-stabilized, poly(propylene sulfide) (PPS)-core nanoparticle (NP) technology. Here, we modify this technology and exploit the chemical reactivity of NO to form stable adducts capable of efficient donation under physiological conditions. We characterized the physical properties of S-nitrosated NP (SNO-NP), determined the chemical reaction of S-nitrosation with the free thiols of the NP core, and demonstrated mechanistic behavior of SNO-NP as a putative therapeutic for the lymphatic resident filarial worm B. malayi.

Poster # 46

Kevin Schwartzman-Undergraduate Research Assistant

Email: kgschwa@clemson.edu

Affiliation: Department of Bioengineering, Clemson University

Title of Abstract: Metrology of Explanted Joint Replacements with Modular Tapers: Validation of Non-Destructive Profilometry using PVS Impression Molds

Presenting Author: Kevin G. Schwartzman

Affiliation: Department of Bioengineering, Clemson University

Complete Author's List :

Kevin G. Schwartzman Pooja Panigrahi, M.S. Melinda K. Harman, PhD

All Author Affiliations :

Department of Bioengineering, Clemson University

Abstract:

Bore and cone taper junctions are incorporated into many joint prosthesis designs in order to achieve modularity of different device components. Surface profilometry analysis of roughness at the taper interfaces is a non-destructive technique useful in failure evaluation of explanted joint prostheses. However, the bore surface is particularly difficult to assess microscopically without taper destruction. Out-of-round metrology, another non-destructive analytic method, provides 2-D data radial to the taper axis (ISO 1101), but is less applicable for 3-D scans on an isolated area on the taper surface. Polyvinyl Siloxane (PVS) has been successfully used as a microscopic impression material in the forensic and dental fields, but its application to bore taper junctions of joint prostheses has not been validated. The purpose of this study is to evaluate the suitability of PVS impression molds as a means for quantifying surface roughness of bore taper junctions. Light viscosity PVS (Doje’s Forensic Supplies) was used to take molds of bore tapers from 17 explanted knee prostheses. Non-contact profilometry (Bruker NPFlex Light Interferometer) was used to evaluate both the bore tapers and PVS molds by taking 10 measurements in 3mm x 3mm regions for each explant. Measurements were recorded for 3 parameters: average roughness (Ra), lateral distance between machine lines, and peak-to-valley height of machine lines, with the latter two measured from linear profile scans. PVS molds of the bore taper surfaces achieved a high degree of conformity to the machined taper surface. Upon separation of the PVS molds from the bore taper surface, no tearing, cracks, or air bubbles were visually evident. There were no significant differences (T-test>>0.1) between the molds and tapers when comparing mean values of Ra (0.463 ±0.044 μm, 0.476 ± 0.052 μm), lateral distance between machine lines (530.53 ± 108.16 μm, 548.72 ± 84.16 μm), and peak-to-valley height of machine lines (2.636 ± 0.428 μm, 2.732 ± 0.415 μm). The ability to replicate features at the sub-micron scale validates the use of PVS impressions for profilometric measurements in place of destructive analysis. In addition to superior elastic recovery, tear strength, and stability over time, PVS molds are useful for explant analysis since average roughness, peak-to-valley difference, and lateral difference between surface features are relevant for characterizing surfaces of bore and cone taper junctions after in vivo function.

Poster # 47

Justin Shaw-

Email: jeshaw@g.clemson.edu

Affiliation: Bioengineering with a concentration in materials, Clemson University, SC, USA.

Title of Abstract: Finding the Ideal Nonthermal Plasma Treatment Settings for Maximum PLGA Bioadhesion

Presenting Author: Justin E. Shaw

Affiliation:

Complete Author's List :

Kaitlyn Hackathorn Justin Shaw

All Author Affiliations :

Bioengineering with a concentration in materials, Clemson University, SC, USA. Bioengineering with a concentration in materials, Clemson University, SC, USA.

Abstract:

This review presents the effects of plasma treatment on the adhesion of poly(lactic-co-glycolic acid) (PLGA) to porcine aorta endothelium. PLGA is currently used for drug delivery thin films but increasing the polymer’s adhesion to biological surfaces, such as vascular endothelium, would diversify applications of drug delivery. Plasma treatment introduces functional groups to the surface of polymers. As such, it is a potential method for increasing thin film bioadhesion. Proof that plasma treatment improves the bioadhesion of thin films post-production can lead to the clinical use of plasma treatment for direct application of drug delivery thin films in more environments. First, this review analyses various settings of plasma treatment to determine the ideal parameters for optimizing the bioadhesion of PLGA. The bioadhesion results for each variable are compared to find which variables have the greatest impact on bioadhesion. GPC of plasma treated films is then examined to determine the effect of plasma treatment on the surface and bulk compositions of PLGA thin films. Ultimately, an ideal parameter is determined for enhancing the bioadhesion of PLGA thin films designed for drug delivery.

Poster # 48

Kyle Snethen-Graduate Assistant

Email: ksnethe@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: Manufacturing Tolerance Impacts Stresses in Bore-Cone Taper Junctions of Modular Total Knee Replacements: A Finite Element Analysis

Presenting Author: Kyle Snethen

Affiliation: Clemson University

Complete Author's List :

Kyle Snethen, Jorge Hernandez, Melinda Harman

All Author Affiliations :

Bioengineering, Clemson University

Abstract:

Revision total knee replacements (TKR) often exhibit modular stem extensions which are assembled via bore-cone taper-lock junctions providing surgeons versatility intra-operatively to match patient-specific needs. Studies of modular hip replacements suggest that manufacturing tolerances of the bore-cone tapers can impact the stability of these modular junctions during in vivo function. However, analysis specific to TKR modularity is lacking. The purpose of this study is to use finite element (FE) modeling to compare stresses in bore-cone tapers of varying manufacturing tolerance within modular TKR. A 3D geometric model of the bore-cone taper junction was developed from gross measurements on a retrieved modular TKR tibial baseplate to establish the domain for the FE analysis. The angle mismatch, defined as the bore taper angle minus cone taper angle, served as a measure of manufacturing tolerance and was varied by ±0.25° from a perfect fitting taper junction (angle mismatch = 0). Ti6Al4V alloy materials properties were assigned to both tapers and a coefficient of friction (μ=0.6) was defined at the taper interface. The FE model consisted of an initial step representing an impact load to assemble the modular component onto the baseplate followed by a subsequent step characteristic of physiological off-axis loading experienced during gait. Boundary conditions were representative of a worst-case scenario where stem fixation exists without proximal support of the tibial baseplate, consistent with ASTM standard F1800-2007 used for mechanical testing of tibial baseplates. Results indicated a perfect fit between the bore-cone taper leads to a relatively uniform stress distribution compared to negative and positive angle mismatches. Negative mismatch resulted in higher stress concentrations near the base of the cone taper and at the rim of the bore taper, while positive mismatch resulted in higher stress concentrations near the end of the cone taper within the closed bore. This behavior can be attributed to the respective regions where contact initially occurs during assembly for positive and negative angle mismatches. This preliminary study shows the stress distribution within taper junctions of modular TKRs can be influenced by the angle mismatch. While the maximum stress magnitude (527 MPa) is below the yield strength (896 MPa) of Ti6Al4V alloy, high stress concentrations can impact the fatigue behavior of the taper junction. It is essential that modular taper designs for TKR are robust to manufacturing variations and off-axis loading conditions that occur in vivo. Future work will aim to further validate this model and expand the study to a more in-depth parametric analysis.

Poster # 49

Christine Stamer-

Email: cstamer@clemson.edu

Affiliation: Clemson University

Title of Abstract: Quantifying Variations in the Femoral Head-Neck Moment Arm and Associated Surface Changes on Retrieved Modular Total Hip Replacements

Presenting Author: Christine, M., Stamer

Affiliation: Clemson University

Complete Author's List :

Christine Stamer, Ryan Taylor, Pooja Panigrahi, Melinda K. Harman

All Author Affiliations :

Department of Bioengineering, Clemson University

Abstract:

Prosthesis designs for total hip replacement routinely have modular femoral heads to give surgeons intraoperative flexibility. These heads attach to the neck trunnion on a femoral stem using a bore-cone taper junction. Different bore depths alter the position of the head center on the neck trunnion. This affects the moment arm at the bore-cone modular junction since the joint reaction force at the hip passes through the center of the femoral head. There are concerns that variations in this head-neck moment arm can negatively impact micromotion at the modular junction, leading to corrosion. Therefore it is important to understand if there is a correlation between the calculated moment arm and the amount of corrosion observed on explanted modular hip prostheses. This research aims to characterize the in vivo performance of modular junctions in modern hip prostheses. The purpose is to understand how changes in component geometry impact the bore-cone junction between the femoral head and neck of the prosthesis. This will be achieved by: 1) quantifying variations in the head-neck moment arm; 2) characterizing corrosion and other changes on the surfaces of these modular bore-cone tapers; and 3) comparing the moment arm calculations with observed changes at the bore-cone modular junctions. Explanted hip prostheses (n=64) were collected through an IRB-approved implant retrieval program. All prostheses had modular heads attached to femoral stems using a bore-cone taper junction. From this group of hip prostheses, 17 require further disassembly and are excluded from this study. The remaining prostheses were measured using calipers, including the parameters of femoral head height, femoral head diameter, bore depth, contact length, and trunnion depth, which were used to calculate the head-neck moment arm. Corrosion and other changes to the surfaces of the modular bore-cone junctions will be assessed using qualitatively and quantitatively. The Goldberg method will be used to qualitatively score the degree of corrosion on both the bore and cone interfaces. For statistical significance, three researchers will independently score each modular bore-cone junction. Quantitative image analysis will be completed using calibrated high resolution photographs of the cone trunnions and ImageJ software to measure the percent area of trunnion corrosion. Finally, surface roughness will be measured in a grid of 40 points on each cone trunnion using a non-contact interferometer. To date, all prostheses were dimensioned, the head-neck moment arms determined, and the moments acting about the proximal end of the neck trunnion calculated. The assessments of surfaces are ongoing. Ultimately, the moment arm data and the observed changes at the bore-cone modular junctions will be compared to understand the impact of component geometry.

Poster # 50

Qining Sun-Mr.

Email: qsun32@gatech.edu

Affiliation: Renewable Bioproducts Institute (RBI), School of Chemistry and Biochemistry, Georgia Institute of Technology

Title of Abstract: Xylan Reinforcement on Cellulose bionanocomposite film

Presenting Author: Qining Sun

Affiliation: Renewable Bioproducts Institute (RBI), School of Chemistry and Biochemistry, Georgia Institute of Technology

Complete Author's List :

Qining Sun (1);Anurag Mandalika (2);Thomas Elder (3);Sandeep S. Nair (1);Xianzhi Meng (1);Fang Huang(1) and Art J. Ragauskas(1)

All Author Affiliations :

(1)School of Chemistry and Biochemistry, Institute of Paper Science and Technology, Georgia Institute of Technology, 500 10th St., Atlanta, GA 30332, USA (2)Louisiana Forest Products Development Center, LSU Agricultural Center,Louisiana State University, Baton Rouge, LA 70894, USA (3)USDA-Forest Service, Southern Research Station, 2500 Shreveport Highway,Pineville, LA 71360, USA

Abstract:

The ever-increasing global demand for materials and international dependency on conventional petroleum resources plus the environmental concern call for alternative sustainable sources and greener technologies. Novel bionanocomposite films have been prepared by depositing xylan onto cellulose nanowhiskers through a pH adjustment. Analysis of strength properties, water vapour transmission, transparency, surface morphology and thermal decomposition showed the enhancement of film performance. This provides a new green route to the utilization of biomass for sustainable biomaterials production.

Poster # 51

Daniel Bowers-Grad Student / Lab and Research Spec.

Email: bowers.daniel.t@gmail.com

Affiliation: University of Virginia

Title of Abstract: Spatiotemporal Oxygen Sensing Nanofibers for the Study of Tissue Engineering Constructs

Presenting Author: Michael L. Tanes

Affiliation: University of Virginia

Complete Author's List :

Daniel T. Bowers(1), Michael L. Tanes(2), Anusuya Das(1,3), Yong Lin(1), Nicole A. Keane(1), Rebekah A. Neal(1), Kenneth L. Brayman(4), Cassandra L. Fraser(5), Edward A. Botchwey(1,2)

All Author Affiliations :

Department of Biomedical Engineering, University of Virginia(1); Department of Biomedical Engineering, Georgia Institute of Technology and Emory University(2); Orthopaedic Surgery, University of Virginia(3); Department of Surgery, University of Virginia(4); Department of Chemistry, University of Virginia (5)

Abstract:

Tissue engineering techniques have the potential to stimulate regeneration of damaged or diseased tissues, however a major challenge is preventing cell death within thick constructs due to low oxygen tension. Unfortunately, many established methods to measure oxygen concentration, such as using electrodes, require mechanical disturbance of the tissue structure. To address the need for scaffold-based oxygen concentration monitoring, a single-component, self-referenced oxygen sensor was made into nanofibers. BF2dbm(I)PLA nanofibers were electrospun in a base solvent of methylene chloride. Electrospinning process parameters were tuned to produce a biomaterial scaffold that is an excellent substrate for cell attachment and growth. The ratio of an oxygen sensitive phosphorescence signal to an oxygen insensitive fluorescence signal was calculated at each image pixel to determine an oxygenation value. Using a custom set up, standardization curves show that in fully supplemented media, the fibers are responsive to dissolved oxygen concentrations less than 15 parts per million. NIH3T3 cells were used for biocompatibility studies while D1 cells were used for oxygen measurements. Cell viability was confirmed using propidium iodide and fluorescein diacetate. Appropriate oxygen sensitivity of the scaffold was confirmed in vitro by cell-derived hypoxia correlating to the location and density of an adherent cell monolayer. Sensor activation in ischemia and cell transplant models in vivo show oxygenation decreases on the scale of minutes. The first 2-3 weeks after a tissue engineering scaffold is implanted are crucial for successful implant integration, including vascularization. Increases in oxygenation as the tissue healed following dorsal skinfold chamber placement were shown, with measurements out to 14 days. In conclusion, the internal standard of the dye fluorescence reduces the number of compounds required in the scaffold. These fibers are sensitive to oxygen concentrations which occur in monolayer cell culture. Therefore, this boron dye based nanofiber scaffold is capable of providing real time spatial oxygen concentrations which can be used as a platform to study tissue engineering scaffolds.

Poster # 52

Cheyenne Rhodes-

Email: crhodes6@memphis.edu

Affiliation: The University of Memphis

Title of Abstract: Extended In Vitro and In Vivo Degradation Evaluation of Sodium Acetate Buffered Chitosan Sponges

Presenting Author: Marsalas Whitaker

Affiliation: The University of Memphis

Complete Author's List :

Cheyenne S. Rhodes; Marsalas Whitaker; Ashley C. Parker; Heather Doty; Jessica A. Jennings; Warren O. Haggard

All Author Affiliations :

Biomedical Engineering, The University of Memphis (1)

Abstract:

Chitosan sponges (CS) have been developed and used as a local antibiotic delivery device to reduce bacteria in wounds. The purpose of these studies was to determine and compare extended degradation of CS in vitro and in vivo. Biocompatibility of CS in vivo was also evaluated. In the in vitro study, 8mm sodium acetate buffered Chitopharm S (BCS) CS of varying pHs including 5.6, 6, 6.3, and 7 pH (control) were weighed then covered with 35mL of 1mg/mL lysozyme, 1% antimicrobial additive, and a 1x PBS solution. The samples were placed in an incubator on a shaker at 37°C, solution replaced every 2 days, and time points taken at 2, 14, and 28 days. After each time point, the CS was dried, weighed, and percent chitosan remaining was assessed. In the in vivo compatibility and degradation study with an established, IACUC approved intramuscular rat model, 8mm sterile CS including neutral Primex (NP), 5.6 pH buffered Primex (BP), neutral Sentrex (NS), and 5.6 pH BCS CS were placed into 4 separate back muscle pouches on each rat. After 14 and 28 days, implant sites were excised for comparison using histological analysis. In vitro degradation analysis (n=3; results in avg±dev) resulted in the percent chitosan remaining shown below. Percent Remaining(%) Sponge 2 day 14 day 28 day 5.6 pH 65.3±2.3 71.3±2.4 75.8±4.2 6.0 pH 62.7±3.6 75.4±3.0 71.7±2.1 6.3 pH 62.8±4.2 75.9±6.3 74.9±1.9 7.0 pH 93.1±1.9 109.7±3.3 110.8±5.0 Histology analysis of the rat model (n=10; results in avg±dev) resulted in the chitosan per defect area (pda), the fibrous tissue pda, and the average tissue response grade (3 reviewers; on a scale of 0=negligible to 5=severe inflammatory response) shown below. 14 day 28 day Sponge Chitosan(%) Fibrous(%) Grade Chitosan(%) Fibrous(%) Grade NP 5.3±2.2 19.0±6.7 2.8±1.0 5.5±3.1 16.0±6.5 2.5±1.3 BP 5.5±1.9 17.9±4.6 3.0±0.8 10.1±5.1 18.0±5.8 2.8±0.9 NS 8.1±3.2 14.1±6.6 2.3±0.9 5.8±3.0 16.1±5.7 1.9±0.7 BCS 3.4±1.7 18.5±10.6 3.0±1.0 7.5±3.1 20.5±5.5 2.8±0.9 Degradation occurred primarily within the first 2 days of in vitro testing, significantly in the buffered CS, minimally in the neutral, and negligible degradation thereafter. The in vivo tested sponges all exhibited similar levels of fibrous tissue pda and mild inflammatory response. All CS experienced significant in vivo degradation; however, the buffered CS had the most degradation through 14 days, with little additional degradation between 14 and 28 days. The 5.6 BCS sponge tested in vivo degraded significantly more than those tested in vitro after 14 and 28 days.

Poster # 53

Joseph Wortkoetter-A Self-Assembly Approach on Perylene Monoimide Dye

Email: jwortko@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: A Self-Assembly Approach on Perylene Monoimide Dye

Presenting Author: Joseph L. Wortkoetter

Affiliation: Clemson University

Complete Author's List :

Joseph Wortkoetter Dr. Avijit Jana Dr. Yanli Zhao

All Author Affiliations :

Joseph Wortkoetter: Department of Bioengineering, Clemson University Dr. Avijit Jana: Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Dr. Yanli Zhao: Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University,

Abstract:

One of the biggest factors to ensure a successful cancer therapy is the early detection of the localized and disseminated tumor cells. However, with the lack of specificity and sensitivity of the current, predominant methods for detection (X-ray, magnetic resonance imaging, positron emission tomography, radiography, ultrasound, computerized tomography) a need for a non-invasive, high-resolution modality is imperative. Among new optical imaging technologies being explored, near-infrared (NIR) fluorescence has begun to garner much attention. Because of the deep tissue penetration of the NIR spectral range (700-2500 nm), NIR fluorescence has promising implications for non-invasive, deep-tissue imaging. Furthermore, the low autofluorescence from tissues in the NIR spectral range suggests that background interference will be reduced when using NIR fluorescence. When looking for a NIR organic fluorescent dye, several factors must be taken into consideration. Obviously, the dye must function within the NIR spectral range and have a high quantum yield, indication strong fluorescence. In order to be successful as a dye, the compound must also have a large stokes shift between absorption and emission spectral to minimize interference. Stability of the compound in solvents and the biological system is also important. Finally, NIR probes should be able to target desired cells; therefore, available bioconjugation with specific ligands is desired. Conventional dyes have limitations such as poor stability, low quantum yield, and poor hydrophilicity, so only a few NIR dyes are readily available. Previous work by has explored the excimer formation of perylene derivatives and the possible use for a targeted dye. The purpose of this project was to explore one of the derivatives of perylene and attempt to create a tunable fluorescent dye. The dye in question, perylene-3,4-dicarbozimide with functional group: 2,5-di-tert-butylphenyl has showed promising results in formation of excimer configuration in aqueous solution as well as the ability to maintain the compound in monomer state when isolated in a pluronic-127 micelle. The compound has also proven to be very stable and future studies regarding the possible bioconjugation of the compound to target cancer cells is underway.

Poster #54

Jeoung Soo Lee-professor

Email: ljspia@clemson.edu

Affiliation: Department of Bioengineering, 4D Lab, Clemson University, Clemson, SC 29634, USA

Title of Abstract: Polymeric nanotherapeutics as combinatorial therapy for spinal cord tumor

Presenting Author: So-Jung, Gwak

Affiliation: Department of Bioengineering, 4D Lab, Clemson University, Clemson, SC 29634, USA

Complete Author's List :

So-Jung Gwak, Justin Nice, Benjamin Green, Christian Mack, Graham Temples, Jeoung Soo Lee

All Author Affiliations :

Department of Bioengineering, 4D Lab, Clemson University, Clemson, SC 29634, USA

Abstract:

Spinal cord tumors are neoplasms of the central nervous system. These tumors exert non-mechanical back pain, especially middle or lower back pain. The current treatment can vary depending on the type of tumor and chemotherapy, radiation therapy, and surgery are most common. However, there is no effective clinical treatment for spinal cord tumors. In this study, we developed that polymeric micelles as an efficient delivery carrier for the combinatorial therapy of nucleic acid and anticancer drug for spinal cord tumor. Poly (lactide-co-glycolide)-g-polyethylenimine (PgP) were synthesized and characterized by 1H-NMR and GPC.The size distribution and surface charge of polyplex were evaluated using dynamic laser light scattering by Zeta PALS. The transfection efficiency and cytotoxicity of PgP/pGFP complex were evaluated in C6 cells and B35 cells in the presence of serum. We evaluated the feasibility of PgP as a gene carrier using plasmid DNA encoding beta-galactosidase gene (pβ-gal) in rat spinal cord tumor model. C6 were injected in the T5 of spinal cord and then PgP/pβ-gal polyplexes were injected at 5 days post-injection of tumor cells. Beta-gal expression was evaluated by X-gal staining kit. The molecular weight of PgP was calculated approximately 48,791. Particle size of PgP/pDNA was approximately 130 nm at all N/P ratio and surface charge of polyplexes were positive above N/P ratio of 64/1. In both cells, transfection efficiency was approximately 65 and 75.1% in serum condition without significant cytotoxicity in C6 and B35 cells, respectively. To evaluate of gene expression, naked pβ-gal, PEI/pβ-gal and PgP/pβ-gal were injected into spinal cord tumor. Seven days after injection, β-gal expression was higher in the injection of complex of PgP/p

**Rapid Fire Presentations & Posters: Tissue Repair**

Poster # 55

Christopher deBorde-Graduate Assistant

Email: cdebord@g.clemson.edu

Affiliation: Clemson University

Title of Abstract: Development of a Tissue Engineered Construct for Mitral Valve Regeneration

Presenting Author: Christopher, P. deBorde

Affiliation: Clemson University

Complete Author's List :

Christopher deBorde, Lee Sierad, Jun Liao, Agenta Simionescu

All Author Affiliations :

Bioengineering, Clemson University (1;)Bioengineering, Clemson University (2) Agricultural and Biological Engineering, Mississippi State University (3); Bioengineering, Clemson University (4)

Abstract:

Pathologies of the mitral valve are alarmingly common. Stenosis, regurgitation, and prolapse are affecting valve function resulting in atrial fibrillation, arterial thromboembolism, pulmonary edema, pulmonary hypertension, cardiac hypertrophy and heart failure. Valvular repair and replacement such as using band annuloplasty and glutaraldehyde-fixed valves are current treatments, but their use if often associated with thromboembolic complications, calcification, and the risk of additional reoperations. A tissue engineered option is feasible and holds great potential. The aim of this study is to develop an acellular, noncytotoxic mitral valve scaffold, which can be recellularized with human adipose tissue-derived stem cells (ASCs). We will investigate how the scaffold’s niche microstructure together with biomechanical and biochemical cues provided by bioreactor conditioning encourage the differentiation of seeded stem cells into valve specific cells. For our approach, porcine mitral valves were treated with detergent-based solutions to remove cells, while leaving a well-preserved extracellular matrix scaffolds. Scaffold characterization included biaxial mechanical testing of the annulus and leaflets, thermal denaturation evaluation, histological analysis, as well as resistance to collagenase and elastase. Scaffolds were treated with penta-galloyl glucose (PGG) for stabilization and injected with human adipose derived stem cells. The annulus and leaflet structures were also covered with ASCs. The constructs were mounted in a bioreactor for three weeks and their conditioning was analyzed using immunohistochemistry. Scaffold characterization results indicate a complete removal of cellular and nucleic acids. Overall, extracellular matrix integrity was not lost, as we were able to maintain the collagen and elastin microstructure (Figure 1). Some basal lamina components were also present in our acellular scaffolds. Biaxial mechanical testing of the scaffold indicates a decrease of mechanical strength, due to decellularization; however, after treatment with PGG, mechanical properties were restored to native capabilities. Internal and external seeding of our scaffolds were successful. A cytotoxicity assay indicates living cells after seeding indicating our scaffold as noncytotoxic. Our bioreactor allows for coaptation of the leaflets when physiological pressures and pulsatile flow were applied. Our PGG-treated mitral valve scaffolds are void of any xenogeneic cellular components and have ideal mechanical properties. We have retained basal lamina proteins essential for cellular attachment and preserved the integrity of our extracellular matrix. Our scaffold is noncytotoxic, as seeded cells were alive after three weeks in a bioreactor study.

Poster # 56

Jose Garcia-

Email: jgarcia34@gatech.edu

Affiliation: Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta GA, U.S.A

Title of Abstract: PEG hydrogels functionalized with a Collagen-mimetic peptide and vascular endothelial growth factor for regeneration of critically-sized bone defects

Presenting Author: Jose R. Garcia

Affiliation: Graduate Research Assistant

Complete Author's List :

José R. García, Andrés J. García

All Author Affiliations :

Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta GA, U.S.A (1); Petit Institute of Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta GA, U.S.A (2)

Abstract:

Non-healing bone defects and fractures represent a serious clinical problem with over 600,000 bone replacement procedures performed each year in the U.S. and costing over 5 billion dollars annually. Bone tissue engineering and especially those involving cell therapy offer an exciting alternative to traditional auto- and allograft techniques which are limited by tissue supply, morbidity, poor bioactivity, and the possibility of disease transmission. Current approaches to cell therapy however suffer from poor engraftment and survival of delivered cells due to surrounding ischemic tissue and improper biological cues. Our group has previously shown that coating of a scaffold with the collagen-mimetic peptide GFOGER enhances bone regeneration and bridging of a critical-sized defect when incorporated onto a PCL scaffold. The purpose of this study was to incorporate vascular endothelial growth factor (VEGF) in a hydrogel vehicle to enhance vascularization in a murine segmental bone defect model. We hypothesize that the incorporation of VEGF into our constructs will improve the therapeutic value of GFOGER scaffolds. PEG-MAL precursor was reacted with rhVEGF165, GGYGGGPG(GPP)5GFOGER(GPP)5GPC, and cross-linked with GCRDVPMSMRGGDRCG. To study VEGF release, VEGF was tagged with AlexaFluor 488 and hydrogels placed in either a collagenase solution or a PBS solution and samples analyzed for fluorescence. To assess the biological functionality of VEGF in hydrogels, a 3D network formation assay was performed with a co-culture of GFP-expressing endothelial cells (HUVECs) and a pericyte-like cell (10T1/2). To assess the bone regenerative capacity of the constructs, mice underwent surgery to remove a critically-sized segment of radial bone followed by implantation of different constructs. Bone regeneration was monitored through microCT. Upon exposure to collagenase, VEGF-containing gels released 100% of cross-linked VEGF as opposed to approximately 40 and 60% of VEGF that was retained when hydrogels were placed with PBS. Co-culturing of HUVECs with 10T1/2 cells with VEGF available in either the hydrogels or in the media showed significantly greater network formation than present in no VEGF conditions indicating the biological activity of the PEG-lyated VEGF. When placed in the animal model, constructs containing VEGF seemed to exhibit higher amounts of blood vessel formation as well as marginal increases in bone formation.

Poster # 57

Ian Hale-Mr.

Email: ihale@clemson.edu

Affiliation: Clemson University

Title of Abstract: Non-Invasive Deep Tissue Imaging of Polymer Degradation Using X-Ray

Presenting Author: Ian T. Hale

Affiliation: Clemson University

Complete Author's List :

Ian T. Hale (1) Timothy R. Olsen (1) Lundy L. Davis (3) Samantha E. Nicolau (3) Caroline C. Duncan (3) Daniel Whitehead (2) Brooke A. Van Horn (3) Frank Alexis (1)

All Author Affiliations :

Department of Bioengineering, Clemson University (1) Department of Chemistry, Clemson University (2) Department of Chemistry and Biochemistry, College of Charleston (3)

Abstract:

Trends predict that there will be a transition from permanent implantable devices used for temporary therapeutic treatment to biodegradable devices that help repair the body and regenerate damaged tissues. Permanent implantable devices have issues with long term biocompatibility, mechanical failure, and secondary surgeries for revision/removal. Here we report our recent findings on non-invasive deep tissue imaging of polymer degradation using x-ray. It is challenging to monitor degradation and the physical state of biodegradable polymeric implants in deep tissue. The hypothesis is that by using a novel imaging contrast agent with an FDA approved polymer (polycaprolactone, PCL), it will allow for quantification of in–situ polymer degradation, detection of defects, and characterization of in vivo degradation via x-ray. Small discs made of PCL functionalized with contrast agent (i-PCL) were submerged in phosphate buffered saline (PBS) and imaged weekly using x-ray (Tingle, 325MVET), and then compared to control discs made of polylactic acid (PLA). Molecular weight of i-PCL (16.5kDa) was determined using GPC. In vitro results suggest minimal degradation over 15 weeks. Presto Blue viability assays were performed using primary rat aortic smooth muscle cells seeded on polymeric films to quantify cell viability. Small defects were made in i-PCL discs and the x-ray image intensities were compared to controls without defects. For in vivo analysis, 3 Sprague Dawley rats (male, 8 wks) were subcutaneously implanted with both an i-PCL disc and a control PLA disc and imaged weekly. ImageJ was used for all image analyses. Retrieved implants were processed, sectioned, and stained with Hematoxylin and Eosin and Masson’s Trichrome stains. Films made from i-PCL had no adverse effects on cell viability through 72 hours, when compared to PLA. Image intensities of defected samples were 18% lower than controls and defects were visualized through bone in rabbits. The in vivo imaging results show that the i-PCL remains clearly visible throughout the duration of the study (8 wks), while control PLA discs could not be visualized once implanted. Imaging analyses demonstrated that the relative x-ray image intensity of the i-PCL discs decreased 30%, suggesting degradation in physiological conditions in vivo. We have demonstrated that a functionalized FDA approved biodegradable polymer can be imaged using x-ray and that its degradation and changes in morphology can be monitored over time in vitro and in vivo. Results demonstrated that x-ray image intensity decreased minimally over time in vitro, while in vivo studies showed degradation. Changes in image intensity of small defects in the polymer were able to be quantified, while also being visualized through bone. In future work, other FDA approved biodegradable polymers will be functionalized and the degradation characterized using x-ray imaging.

Poster # 58

Marian Hettiaratchi-Graduate Student

Email: mhettiaratchi3@gatech.edu

Affiliation: Georgia Institute of Technology & Emory University

Title of Abstract: Heparin Microparticle Delivery of Bone Morphogenetic Protein-2 (BMP-2) for Bone Regeneration

Presenting Author: Marian H. Hettiaratchi

Affiliation: Georgia Institute of Technology & Emory University

Complete Author's List :

Marian H. Hettiaratchi (1), Johnna S. Temenoff (1,2), Robert E. Guldberg (2,3), Todd C. McDevitt (1,2)

All Author Affiliations :

The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology & Emory University (1); The Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology (2); The George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology (3)

Abstract:

Bone morphogenetic protein-2 (BMP-2) offers a promising means of stimulating endogenous repair mechanisms to heal injured bone. However, current biomaterials have limited ability to locally deliver bioactive BMP-2 to stimulate repair. Glycosaminoglycans, such as heparin, strongly interact with BMP-2, and thus have the potential to overcome this limitation. This study was undertaken to characterize heparin microparticles as an effective BMP-2 delivery vehicle. Heparin microparticles were fabricated from methacrylamide-modified heparin using a water-in-oil emulsion, followed by thermal cross-linking at 55°C. Microparticles were loaded with BMP-2 by overnight incubation at 4°C. BMP-2-loaded microparticles induced alkaline phosphatase activity in myoblasts (C2C12) that was comparable to soluble BMP-2 at multiple BMP-2 doses (10 and 30 ng/well) and microparticle doses (0.02, 0.1, and 0.5 mg/well), indicating that microparticle-bound BMP-2 retained bioactivity. Constructs for in vivo delivery were made by mixing microparticles (1 mg) into 2% RGD-functionalized alginate (150 µL) within a polycaprolactone mesh tube. In vitro BMP-2 release from the constructs was monitored over three weeks (37°C); constructs containing 500ng of soluble, alginate-bound BMP-2 exhibited significantly higher release (>20%) than constructs containing equivalent microparticle-bound BMP-2 (<15%). To evaluate ectopic bone formation, constructs containing fluorescently labeled microparticles (1 mg) and 5 µg of BMP-2 (soluble or microparticle-bound) were implanted subcutaneously in rats (n=6/group) for six weeks. Fluorescent imaging of constructs indicated persistence of ~55% of the microparticles after six weeks in vivo. Preliminary micro-computed tomography results suggest that microparticle-loaded BMP-2 constructs induced higher mineralized bone formation than constructs containing equivalent soluble BMP-2. These results indicate that heparin microparticles are an effective delivery vehicle that can elicit BMP-2-mediated responses in vitro and in vivo. Thus, heparin microparticles may improve in vivo BMP-2 localization for bone repair and serve as a versatile platform for tissue regeneration.

Poster # 59

Michael Jaeggli-

Email: mjaeggl@clemson.edu

Affiliation: Clemson University

Title of Abstract: Using Patient-Specific Geometry to Develop Scaffolds for Aortic Heart Valve Tissue Engineering

Presenting Author: Michael P. Jaeggli

Affiliation: Clemson University

Complete Author's List :

Michael Jaeggli (1), Dan Simionescu (1), Jun Liao (2), Agneta Simionescu (1)

All Author Affiliations :

Department of Bioengineering, Clemson University (1); Department of Agricultural & Biological Engineering, Mississippi State University (2);

Abstract:

Valve reconstruction or regeneration with living tissue is a daunting project for biomedical engineers and surgeons alike. Our translational approach to the development of living valve replacements includes the accurate replication of each patient’s 3D aortic valve architecture for optimal functionality. To create anatomically correct constructs, we used digital image processing of patient chest CT images and generated solid aortic valve root 3D structures using a stereo-lithography printer. Collagenous scaffolds were prepared from acellular porcine pericardium with a layer of collagen/GAG hydrogel on the cusp interior. Layered scaffolds were attached to the molds, dried, stabilized with a non-toxic polyphenolic agent, rehydrated, the spongiosa seeded with human mesenchymal stem cells and valves subjected to functionality tests in a physiological heart valve bioreactor in sterile culture medium. Engineered valves exhibited excellent functional characteristics; most cells were alive, elongated significantly and stained positive for vimentin and actin, among other markers, suggestive of mechanical stimuli-induced stem cell differentiation into VICs. Ongoing studies are focused on endothelialization of the novel valve surfaces using stem cell-derived endothelial cells and rotational 3D seeding devices. In conclusion, autologous stem cell-seeded collagenous scaffolds shaped to recapitulate the aortic heart valve shape and mechanics may provide future foundations for patient-tailored heart valve tissue engineering.

Poster # 60

Christopher Johnson-Bacteriophage Therapy to Reduce Bacterial Burden in Infected Bone Regenerative Implants

Email: c.t.johnson@gatech.edu

Affiliation: Coulter Department of Biomedical Engineering, Georgia Institute of Technology

Title of Abstract: Bacteriophage Therapy to Reduce Bacterial Burden in Infected Bone Regenerative Implants

Presenting Author: Christopher T. Johnson

Affiliation: Georgia Institute of Technology

Complete Author's List :

Christopher T. Johnson, Maria Diaz Ortiz, Andres J. Garcia

All Author Affiliations :

Christopher T. Johnson (1)(2)(3) Maria Diaz Ortiz (1) Andres J. Garcia (3)(4) Coulter Department of Biomedical Engineering, Georgia Institute of Technology Emory University School of Medicine (2) Petit Institute for Bioengineering and Bioscience (3) Woodruff School of Mechanical Engineering

Abstract:

Implant-associated infections are a significant clinical problem accounting for over 1 million infections per year. Infection typically leads to device failure and complete removal of the implant. Bone fractures and non-union defects are injuries that often require surgical intervention where biomaterials, bone grafting, and protein therapeutics (BMP-2) are used to correct the defect. Pseudomonas aeruginosa is a significant clinical pathogen capable of developing widespread antibiotic resistance and is the most common gram-negative cause of implant associated orthopedic infection. This motivates the development of bone regenerative implants that eliminate infection and regenerate bone using antimicrobial strategies other than antibiotics. Bacteriophages are viruses specific to bacteria. They infect the target pathogen, replicate, lyse the microbe, and propagate their progeny to infect other pathogens. This provides an amplification response to contaminants, but is self-limited in that propagation ceases once the pathogen is eliminated, making them a desirable antimicrobial. Our lab has recently developed a poly (ethylene glycol) based hydrogel to deliver BMP-2 and integrin specific peptides to facilitate bone regeneration in a critically-sized radial segmental defect in a mouse. The main objective of this work is to develop and validate a therapeutic hydrogel infection model to serve as a platform to evaluate antimicrobial regenerative medicine implants. Here we develop a murine model of regenerative implant associated infection using bioluminescent, pathogenic bacteria to inhibit bone formation in vivo using a critically-sized radial segment defect. The animals develop osteomyelitis, characterized by bone resporption and recovered bacteria 8 weeks post-implantation. We further show that the addition of bacteriophage to the infected hydrogels significantly reduced the viable bacteria 8 weeks after implantation. The bacteriophage delivered in the hydrogel system is active in vivo, and present seven days post implantation. In conclusion, bacteriophage delivery in a regenerative medicine implant is a viable option to prevent contamination by Pseudomonas aeruginosa.

Poster # 61

Vaideesh Parasaram-Enhanced matrix elastin production and organization using pentagalloyl glucose in pulmonary fibroblast cultures

Email: vparasa@clemson.edu

Affiliation: Clemson University

Title of Abstract: Enhanced matrix elastin production and organization using pentagalloyl glucose in pulmonary fibroblast cultures

Presenting Author: Vaideesh Parasaram

Affiliation: Clemson University

Complete Author's List :

Vaideesh Parasaram, Nasim Nosoudi, Naren Vyavahare

All Author Affiliations :

(1) Department of Bioengineering, Clemson University; (2) Department of Bioengineering, Clemson Univeristy; (3) Department of Bioengineering, Clemson Univeristy.

Abstract:

Pulmonary emphysema is one of the two pathological conditions encompassed by the term Chronic Obstructive Pulmonary Disease (COPD). COPD is mainly caused by cigarette smoking with a death toll of 18 million Americans. It has been established that destruction of elastin in the alveolar walls is one of the major mechanisms involved in the progression of the emphysema. Pentagalloyl glucose (PGG), a derivative of tannic acid (TA) has been shown to protect matrix elastin from elastase activity and also aid in elastin deposition in the extracellular matrix (ECM). Here we tested if elastin deposition of rat pulmonary fibroblasts can be increased by the use of PGG either in normal conditions or under inflammatory conditions (addition of TNF-α). At day 21 PGG treated group had significantly higher elastin than the control group. TNF-α combined with PGG treated group had higher elastin than compared to TNF-α group. The results obtained are promising in the way that PGG allowed fibrous elastin deposition as compared to the cells experiencing inflammatory conditions. We hope that PGG can be used to regenerate the damaged elastin alveolar wall attachments and help restore the elasticity of emphysematous lungs.

Poster # 62

Ashwin Parenky-Graduate Student

Email: 10909990@live.mercer.edu

Affiliation: Department of Pharmaceutical Sciences, Mercer University

Title of Abstract: A detailed mechanistic study on adjuvants and optimizing antigenicity of particulate cancer vaccines

Presenting Author: Ashwin Parenky

Affiliation: Department of Pharmaceutical Sciences, Mercer University

Complete Author's List :

Ashwin Parenky (1), Trinh Vo (1), Maurizio Chiriva-Internati (2), Martin D'Souza (1)

All Author Affiliations :

(1)Department of Pharmaceutical Sciences, Mercer University (2)School of Medicine, Texas Tech University Health Sciences Center

Abstract:

Purpose: Sperm protein 17 (Sp17) is a cancer/testis antigen which is expressed aberrantly in several cancers like prostate, ovarian cancer, hepatocellular carcinoma and multiple myeloma. Its restricted expression in normal tissues and aberrant expression in cancers makes it an attractive target for cancer immunotherapy. Enhancing delivery of Sp17 may significantly improve clinical outcome by eliciting a specific and sustained anti-tumor response. The two main goals of this project were 1) to formulate Sp17 microparticles (MP) and investigate its efficacy in vitro. 2) Conduct a detailed mechanistic study on adjuvants that may augment anti-tumor efficacy of Sp17 MP. 3) To test combination of two adjuvants in conjunction with Sp17 MP for synergetic effect Methods: Recombinant Sp17 was expressed in M15 cells, isolated and purified using the Ni-NTA fast start kit (Qiagen). Sp17 and adjuvants were encapsulated separately in MP using the Buchi B-290 spray drier. Particle size, zeta potential and SEM imaging was performed on microparticles. SDS-PAGE was performed to confirm the stability of Sp17 in MP. Release of Sp17 from MP was performed in gastric and intestinal pH conditions. Eight adjuvant MP were screened on DC 2.4 cells by studying several innate and adaptive immune markers like nitric oxide, CD40, CD80, CD86, CD54 and MHC-II. Results: Sp17 MP had an average particle size of 3.59 ± 0.5µm and zeta potential of +9.36mV. Encapsulation efficiency of Sp17 was found to be 78%. SEM images confirmed particles were irregular in shaped with surface indentations. SDS-PAGE confirmed the presence of Sp17 encapsulated in its native form. Cumulative release of Sp17 was approximately 15% in simulated murine gastric and intestinal pH conditions. Nitric oxide release was significantly (p<0.05) higher in adjuvant treated groups compared to Sp17 MP. CD 40 and CD 80 expression was elevated in R848, MPL and MF59 treated groups (p<0.05) compared to Sp17 MP. Combination of R848 and Alum, R848 and MF59 and R848 and P4 showed enhanced expression of CD80. CD40 elevation was highest in MPL and R848 combination. Conclusion: Sp17 MP in combination with R848, MPL and MF59 MP significantly improve innate and adaptive immune response to cancer antigens.

Poster # 63

Akia Parks-Cathepsin activity in supraspinatus tendinopathy: Identification in human chronic tears and temporal induction in a rat overuse model

Email: aparks14@gatech.edu

Affiliation: W.H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University

Title of Abstract: Cathepsin activity in supraspinatus tendinopathy: Identification in human chronic tears and temporal induction in a rat overuse model

Presenting Author: Akia N. Parks

Affiliation: W.H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University

Complete Author's List :

Parks, Akia; Seto, Song; McFaline-Figueroa, Jennifer; Soslowsky, Louis J.; Karas, Spero; Ghattas, Timothy; Sloan, Harris; Tayrose, Gregory; Platt, Manu; Temenoff, Johnna

All Author Affiliations :

W.H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University; McKay Orthopaedic Research Laboratory, University of Pennsylvania; Department of Orthopaedics, Emory University; Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology.

Abstract:

Supraspinatus tendinopathy is common among athletes and laborers and is generally attributed to overuse. Tendon degeneration results from extracellular matrix degradation (ECM) and leading to full tendon tears with high reinjury rates even after surgery. Though many factors have been implicated in the etiology of supraspinatus tendinopathy, exact mechanisms are not well understood. Cysteine cathepsins are a family of proteases that include powerful human collagenases shown to be involved in tissue-destructive diseases. The objective here is to analyze cathepsin activity in tendinopathic supraspinatus tendons in humans and rats. Our hypothesis is that cathepsins are upregulated in damaged supraspinatus tendon tissue, degrading the collagen ECM and deteriorating tendon structure. Multiplex cathepsin zymography was used to quantify cathepsin activity in tissues from human patients and a rat tendon overuse model after 4 and 8 weeks of overuse. Histological images of damaged and control rat supraspinatus tendons were also acquired to assess structural damage. Human tissue samples were isolated from patients with chronic tears undergoing surgery for repair of supraspinatus tendon rupture. Male Dahl Salt Resistant rats (330±20 g initial weight, Harlan Labs) were trained and subjected to a daily downhill running regime for 4 or 8 weeks (n=6/group). Age-matched rats were allowed cage activity and served as controls (n=6/timepoint). At each endpoint, supraspinatus tendons (n=6/group) were dissected, detached from the bone, and frozen until analysis. For histology, tendons from the 4 and 8 week running groups and the 8 week control group (n=3/group/timepoint) were cryosectioned into 10µm sections and stained with hematoxylin and eosin (H&E). For cathepsin zymography, the rat supraspinatus tendons were systematically divided into the insertion (17% of the total tendon length starting from the bone attachment point) and midsubstance (the remainder of the tendon) regions. Human tendon samples were processed without division into regions. Equal amounts of protein from samples (n=4) were run on each gel, with cathepsin V as a positive control. Densitometry analysis was performed on images using ImageJ (NIH) and values were normalized to the cathepsin V band within the same gel. Data (rodent samples only) were Box-Cox transformed and analyzed by t-tests on a per zymogram basis to assess statistical differences (p<0.05). Values are reported as mean ± standard deviation. In 5 of 6 samples, multiplex cathepsin zymography of the human tendon samples demonstrated bands at 75, 37, 28, and 20 kDa, indicating active cathepsins K, V, S, and L respectively. Zymography of the rat tendon showed active bands between 25-35 kDa and at 75 kDa, indicative of cathepsins L and K, respectively. The overuse samples increased 1.8-fold in active cathepsin L and 4.2-fold in cathepsin K only in the insertion region. The zymography from midsubstance regions did not reveal any significant change in active cathepsins. From histology, comparisons between the control and experimental groups within each timepoint by a semi-quantitative scoring system measured significant differences only at the insertion region. In the 4-week group, scores for cell shape and regional variations in cellularity were significantly different from the controls, while in the 8-week group, cell shape and fiber orientation were significant compared to controls. This study has demonstrated the effects of overuse on cysteine cathepsin activity both in the supraspinatus tendon tissue of human patients with chronic tears and in overused supraspinatus tendons of rats. The demonstration of cathepsin activity suggests that these proteases are playing a role in the progressive degeneration of the tissue. Because we have shown that similar cathepsins are active in both a rat supraspinatus overuse model and in end-stage human tendinopathy, we can use this animal model in the future to further elucidate proteolytic mechanisms of early tendon damage and explore treatment options for chronic tendinopathy in a way that cannot be accomplished in humans.

Poster # 64

Lindsey Sanders-Characterization of a Multi-Functional Tetronic Surgical Adhesive for Soft Tissue Applications

Email: lindses@clemson.edu

Affiliation: Clemson University

Title of Abstract: Characterization of a Multi-Functional Tetronic Surgical Adhesive for Soft Tissue Applications

Presenting Author: Lindsey K. Sanders

Affiliation: Clemson University

Complete Author's List :

Lindsey K Sanders (1), Thompson Mefford, Ph.D. (2), Ken Webb, Ph.D. (1), Jiro Nagatomi, Ph.D. (1)

All Author Affiliations :

Department of Bioengineering at Clemson University, Clemson, SC (1); School of Material Science and Engineering at Clemson University, Anderson, SC (2)

Abstract:

The objectives of this study are to synthesize and characterize a multi-functional Tetronic adhesive comprising acrylate groups for crosslinking with dithiothreitol (DTT), N-hydroxysuccinimide (NHS) ends for strong covalent bonding with tissue, and chitosan as a hemostatic agent. The hydrogel adhesive was chemically cross-linked by addition reaction. Hemostatic properties of the adhesive were examined with coagulation tests using sheep blood and in vivo blood loss studies using rat liver. Briefly, 1 mL of sheep blood was mixed with either 50mg solid or 100µL (30%) solution of modified Tetronic polymers (no DTT) in glass test tubes at 37°C and and tubes were inclined to observe blood flow every 15 sec. Female Sprague-Dawley rats were anesthetized and secured to a surgical board inclined at 45°. After abdominal incision, a pre-weighed filter paper and parafilm were placed under the liver and the main artery was clamped using a surgical hemostat. After puncturing with an 18G needle, modified Tetronic adhesive (100µL) was immediately applied on the surface of the liver wound and the clamp was removed. The amount of blood absorbed on the filter paper was weighed after 3 min (final wet weight) and reweighed after 3 days (final dry weight). No adhesive treatment was used on the control rats. Coagulation tests demonstrated that the modified Tetronic polymer solid clotted blood significantly faster than the solutions. In vivo studies confirmed the addition of chitosan into the adhesive advanced the ability of bi-functional Tetronic adhesive to stop blood loss from a puncture on the liver.

Poster # 65

Jessica D. Weaver-Postdoctoral Fellow

Email: jessica.weaver@me.gatech.edu

Affiliation: Georgia Institute of Technology

Title of Abstract: Microfluidic-based islet encapsulation and transplantation

Presenting Author: Jessica D. Weaver

Affiliation: Georgia Institute of Technology

Complete Author's List :

Jessica D. Weaver(1), Devon M. Headen(2), Andres J. Garcia(1,2)

All Author Affiliations :

Woodruff School of Mechanical Engineering, Georgia Institute of Technology (1); Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology (2)

Abstract:

Islet transplantation is a promising therapy for the treatment of Type I Diabetes, but its widespread application is hindered by immune and autoimmune responses to the graft, which necessitate administration of potent immunosuppressive drugs. Systemic immunosuppression has demonstrated limited effectiveness in islet transplantation, resulting in typical graft lives of 18-60 months, while the immunosuppressive regimen itself conveys a host of adverse side effects. Islet encapsulation has demonstrated potential for reducing the need for systemic immunosuppressive drugs by mitigating the direct antigen recognition pathway of immune activation. However, traditional encapsulation techniques have been hindered by multiple challenges; in particular, large capsule diameters result in limitations in passive diffusion of nutrients and insulin through the capsule, as well as large graft volumes that limit transplant site availability to large, poorly vascularized spaces such as the peritoneal cavity. Within this study, we optimized capsule size and composition for islet viability and function in vitro, where reduced capsule diameter resulted in improved function in encapsulated islets. Due to reduced capsule volumes generated with our microfluidic-based encapsulation technique, a selection of two transplant sites with high potential for clinical translation, the epididymal fat pad (EFP) and the small bowel mesentery (SBM), were selected for transplant site optimization. Encapsulated and free islets were delivered to the transplant sites using our previously developed vasculogenic, degradable hydrogels as a delivery vehicle, and each site was evaluated for capsule retention, site vascularization, and integration with host tissue. Finally, we demonstrate diabetes reversal in a diabetic mouse model using a syngeneic marginal islet mass transplant within the EFP, where encapsulated islets performed comparably to controls. Future studies in allogeneic diabetic mouse models will explore the potential of our encapsulation technology to prevent or delay graft immune rejection.

**Rapid Fire Presentations & Posters: Stem Cells**

Poster # 67

SOONEON BAE-Ph.D. Student

Email: sbae@g.clemson.edu

Affiliation: MicroEnvironmental Engineering Laboratory, Department of Bioengineering, Clemson University, Clemson, SC 29634, USA

Title of Abstract: Osteogenesis of Mesenchymal Stem Cells in Dexamethasone-releasing Semi-IPN Hydrogels

Presenting Author: Sooneon Bae

Affiliation: CLEMSON UNIVERSITY

Complete Author's List :

Sooneon Bae, Ho-Joon Lee, Jeoung Soo Lee, Ken Webb

All Author Affiliations :

MicroEnvironmental Engineering Laboratory, Department of Bioengineering, Clemson University, Clemson, SC 29634, USA

Abstract:

Scaffold-based delivery of bioactive molecules capable of directing stem cell differentiation is critical to the development of point-of-care cell therapy. Although bone morphogenetic proteins (BMPs) are the most well-established and potent growth factors in engineered bone regeneration, the clinical use of BMPs is limited by significant side effects and exorbitant costs. Dexamethasone (DX) is a synthetic glucocorticoid that stimulates osteogenic differentiation of MSCs by initiating and regulating the expression of osteoblast-specific transcription factors such as runt-related transcription factor 2 (RUNX2), and osterix (SP7). While a number of systems have been developed that provide chemically-controlled release of DX, there are few release systems designed to be cell-responsive. Previously, we have described semi-IPN networks composed of hydrolytically degradable PEG-diacrylates and hyaluronic acid (HA) that support hyaluronidase-dependent cellular remodeling. The objective of this study was to synthesize dexamethasone-conjugated hyaluronate (HA-DXM) and evaluate its ability to promote osteogenic differentiation of MSCs encapsulated within PEG-based semi-IPNs. HA-DXM was prepared by reaction of HA and DX-monosuccinate (DXM) in DMSO containing 1,1'-carbonyldiimidazole (CDI) and characterized by ATR-FTIR and HPLC. hMSCs (12.5 × 106 cells/ml) were photopolymerized in 6% PEG-bis-(2-chloropropanoate) [PEG-bis-AP] with 0.36% HA-DXM or native HA (control), 0.1% I2959, and 1 mM acrylate-PEG-GRGDS. Semi-IPNs were maintained in culture for 21 days in the presence (gels with native HA) or absence (HA-DXM) of soluble dexamethasone. hMSC differentiation was evaluated by RT-PCR, ALP activity, calcium deposition, and histological staining. HA-DXM was successfully synthesized and incorporated in semi-IPNs for 3D culture of hMSCs. Encapsulated hMSC with HA-DXM exhibited levels of osteoblast-specific gene expression, ALP activity, and matrix mineralization comparable to controls with conventional supplementation. These studies demonstrate that DX conjugated to HA within semi-IPNs can be effectively released in bioactive form and stimulate hMSC osteogenic differentiation. This approach offers an efficient system for local delivery of osteogenic molecules empowering point of care applications.

Poster # 68

Amy Clark-Graduate Student

Email: acheng3@gatech.edu

Affiliation: Mechanical Engineering, Georgia Institute of Technology

Title of Abstract: Integrin-Specific Hydrogels for the Delivery of Human Mesenchymal Stem Cells in Bone Repair

Presenting Author: Amy Y. Clark

Affiliation: Georgia Tech

Complete Author's List :

Amy Y. Clark, Andres J. Garcia

All Author Affiliations :

Mechanical Engineering, Georgia Institute of Technology

Abstract:

Cell-based strategies have emerged as promising therapies for the treatment of diseased organs. Adult human mesenchymal stem cells (hMSC) constitute a critical component of the hematopoietic stem cell niche in the bone marrow, and although hMSCs have shown promising results in clinical trials, inadequate control of cell fate and cell engraftment in host tissues limits the success of this cell-based therapy. Integrin-mediated cell adhesion plays a central role in tissue formation, maintenance, and repair by providing anchorage forces and triggering signals that regulate cell function. We hypothesize that biomaterials presenting integrin-specific adhesive motifs will direct hMSC signaling and specification. The objective of this project is to engineer bioartificial hydrogels presenting integrin-specific ligands to create biomimetic niches for hMSC differentiation as well as cell delivery vehicles for enhanced in vivo engraftment and function. The following research is innovative because it focuses on engineering specificity to integrin receptors to promote stem cell differentiation and survival without the use of exogenous growth factors, integrates novel in vivo imaging, and utilizes novel hydrogel chemistry. Our results show that hydrogels functionalized with collagen I-derived GFOGER adhesive peptide significantly enhance transplanted hMSC viability and bone formation in a critically-sized bone defect model compared to the fibronectin-derived RGD-functionalized hydrogels.

Poster # 69

Elizabeth Duncan-Undergraduate Biomedical Engineering Student

Email: ecduncan@memphis.edu

Affiliation: University of Memphis

Title of Abstract: The Effect of Adenosine on the Proliferation and Osteogenic Differentiation of Rat Mesenchymal Stem Cells

Presenting Author: Elizabeth C. Duncan

Affiliation: University of Memphis

Complete Author's List :

Elizabeth Duncan Amy Abell Ph.D. Warren Haggard Ph.D. J. Amber Jennings Ph.D.

All Author Affiliations :

Biomedical Engineering, University of Memphis; Biology, University of Memphis

Abstract:

Each year, approximately six million fractures occur in the United States, many with accompanying bone loss. Among these fractures, up to 10% suffer from delayed union or nonunion. Current treatment for bone fractures or non-union of bone can include expensive growth factors or an additional painful surgical procedure such as an autograft to facilitate bone healing. Adenosine has been shown to increase the proliferation rate of fibroblasts and osteoblast precursor cells. If adenosine can promote similar growth and mineralization as growth factors, it may also be useful as a therapeutic to promote healing in bone defects. Because mesenchymal stem cells are important in the healing process, adenosine may act to improve mesenchymal stem cell proliferation and differentiation at the wound site. We hypothesize that adenosine increases mesenchymal stem cell (MSC) proliferation and differentiation into bone. Rat mesenchymal passage 8 stem cells were subjected to media containing adenosine ranging in concentration from 125 µg/ml to 1,500 µg/ml (n=8 in each group). Cell viability was determined using Cell Titer Glo (Promega) assays. After 48 hours, proliferation of MSCs was significantly higher than 5% serum controls for cells with adenosine added in concentrations from 250 uM to 1000 uM. For osteogenic differentiation evaluation, passage 8 rat MSCs were seeded in 24 well plates. Three variable media were tested: osteogenic differentiation media, 1mM adenosine in osteogenic differentiation media, and 1mM adenosine without osteogenic additives. A negative non-osteogenic control media used was α-MEM with 10% fetal bovine serum and Normocin. Cells were harvested at the following time points: Day 3, Day 7, and Day 14. Two assays were performed to quantify osteogenic differentiation with n=4. A picogreen assay was performed to determine cell proliferation. An alkaline phosphatase (ALP) assay was performed as a marker of osteogenic differentiation measured with a 5mM phosphatase substrate assay and spectrophotometric detection. ALP was normalized to DNA quantity for each well. In this study the hypothesis that osteogenic differentiation is enhanced by adenosine was not confirmed. It is possible that ALP peaked before or after the limited time points in this study and was not detected. While increased markers of differentiation were not observed for groups containing adenosine, this may be due to increased proliferation activity delaying differentiation. Morphological changes in cells were observed in response to osteogenic media with and without adenosine, but not in cells exposed to adenosine in the absence of osteogenic factors. We have found evidence that adenosine does increase cell migration. We are incorporating adenosine into phosphatidylcholine, calcium sulfate, and chitosan sponges to measure the concentration released.

Poster # 70

Petra Kerscher-Production of 3D engineered cardiac tissues using a highly reproducible one-step encapsulation procedure of human induced pluripotent stem cells​

Email: pzk0011@auburn.edu

Affiliation: Chemical Engineering, Auburn University

Title of Abstract:

Presenting Author: Petra Kerscher

Affiliation: Chemical Engineering, Auburn University

Complete Author's List :

Petra Kerscher (1), Irene C Turnbull (2), Joonyul Kim (3), Alexander J Hodge (1), Dror Seliktar (4), Christopher J Easley (3), Kevin D Costa (2), Elizabeth A Lipke (1)

All Author Affiliations :

Chemical Engineering, Auburn University (1) Icahn School of Medicine at Mount Sinai, Mount Sinai (2) Chemistry, Auburn University (3) Biomedical Engineering, Technion-Israel Institute of Technology (4)

Abstract:

The aim of this study is to create a highly efficient, reproducible one-step approach to directly encapsulate and differentiate human induced pluripotent stem cells (hiPSCs) into mature, synchronously contracting 3D engineered cardiac tissues. Current methods to create 3D human cardiac tissues employ a multi-step approach, where stem cells are differentiated into contracting cardiomyocytes (CMs), dissociated into single cells, and re-combined with a biomaterial to provide a 3D architecture necessary for more accurate drug-screening applications and CM maturation. This approach not only involves multiple cell handling steps, but also disrupts important cell-cell junctions and causes high degree of cell loss during the dissociation step. Here we describe a new, direct approach to produce 3D engineered cardiac tissues using a one-step hiPSC encapsulation procedure to provide important 3D architecture throughout cardiac differentiation. PEG-fibrinogen (PEG-Fb), a hybrid biomaterial that provides cell adhesion sites and degrades in response to cell-secreted factors, in combination with a photocrosslinking process are employed to form 3D engineered tissues. Initial tissue stiffness for successful stem cell survival and 3D cardiac differentiation (24 hours post-encapsulation) was determined to range between 90 – 370 Pa. Post-encapsulation, hiPSCs were cultured in their pluripotent state for three days prior to cardiac initiation. First areas of contractions were observed on day 7 of differentiation, which resulted into uniform contracting cardiac tissues over time that retained their spontaneous contractility for over three months. Sarcomeric α-actinin and gap junction protein connexin 43 were expressed by day 10; aligned sarcomeres (day 30) and mature gap junctions developed over time. CM maturation increased significantly from day 10 to day 30, as quantified by the ratio of β over α myosin heavy chain gene expression; Cx43 expression increased from 0.5±0.3 (day 20) to 1.0±0.1 (day 30). Additionally, day 15 CMs showed 1:1 correspondence to electrical stimuli of up to 2 Hz. Finally, TEM of day 130 tissues showed structural features of mature CMs, including numerous mitochondria, highly aligned Z-bands, T-tubules, and intercalated discs. These results proof our ability to directly encapsulate, culture, and differentiation hiPSCs into 3D engineered cardiac tissues in a highly reproducible, time-effective approach that mimics the native myocardium. Due to its tunable mechanical properties and photocrosslinking abilities, PEG-Fb is a suitable biomaterial that provides a 3D microenvironment for hiPSC culture and differentiation. HiPSCs can be successfully incorporated into PEG-Fb where they differentiate into contracting CMs with aligned sarcomeres and mature features that have not been observed in stem cell-derived CMs yet. Tissues stained positive for cardiac-specific markers and responded to outside pacing frequencies up to 2 Hz. This one-step encapsulation approach is highly reproducible, time-efficient, and provides the ability to produced mature CMs, important for drug-screening applications.

Poster # 71

Katy Lassahn-Engineering the Microenvironment of Embryoid Bodies via Heparin-modified Gelatin Microparticle Incorporation

Email: khammersmith3@gatech.edu

Affiliation: The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology

Title of Abstract: Engineering the Microenvironment of Embryoid Bodies via Heparin-modified Gelatin Microparticle Incorporation

Presenting Author: Katy A. Lassahn

Affiliation: Georgia Institute of Technology

Complete Author's List :

Katy A. Lassahn, Andres M. Bratt-Leal,Christian Mandrycky, Todd C. McDevitt

All Author Affiliations :

(1)The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology (2)The Parker H. Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology

Abstract:

Embryonic stem cells (ESCs) are capable of differentiation to cells of all three germ lineages and are therefore an attractive cell source for tissue engineering applications. ESC differentiation is commonly initiated through the formation of 3D cell spheroids termed embryoid bodies (EBs). EB differentiation is difficult to control by media manipulation alone due to complex 3D cell-cell and cell-matrix interactions; therefore biomaterial incorporation within EBs is used as a method to improve control of the multicellular microenvironment. Previous work has demonstrated that incorporation of morphogen-releasing, biomaterial microparticles (MPs) can be used for directed differentiation within EBs. In this study, the use of gelatin and heparin-modified gelatin MPs to engineer the EB microenvironment was investigated. Heparin was chosen for its intrinsic ability to bind growth factors within native tissues. The potential of heparin-modified MPs was assessed through analysis of growth factor (GF) binding capacity, sequestration of GFs secreted by EBs, and phenotypic effects on EB differentiation and morphogenesis. The average diameter of the gelatin MPs and heparin-gelatin MPs was 8.1 μm +/- 2.5 and 8.9 +/- 2.7 respectively. A toluidine blue assay indicated the presence of 5.53 ± 1.3 µg heparin per mg of MPs. Depletion assay results using both recombinant growth factor and conditioned media samples suggested that heparin modification of gelatin MPs augmented IGF-II, VEGF, and BMP-4 binding capacity and this binding changes if additional factors are present in solution. Conditioned media collected at days 5, 7, and 9 showed differences among groups, but proved to be variable among trials (n=3). Analysis of EB morphology in histological sections revealed the presence of organized structures within heparin-gelatin MP treated EBs which resembled structures of the gut that were absent in untreated EBs. VE-Cadherin immunostaining showed expression localized around heparin-gelatin MPs that was not observed in gelatin MPs. Heparin modification of gelatin MPs incorporated within EBs resulted in altered GF secretion and phenotypic changes within the EBs. GF binding experiments suggest this could be due to GF availability to cells within EBs and that heparin presentation may affect subsequent cell growth and differentiation. Utilizing heparin or other molecules with intrinsic GF binding capabilities for the engineering of biomaterials could be a useful tool in tissue engineering, particularly when used to sequester endogenously expressed morphogens in order to direct stem cell differentiation more efficiently than current conventional methods.

Poster # 72

Torri Rinker-Graduate Student Researcher

Email: trinker3@gatech.edu

Affiliation: Biomedical Engineering, Georgia Institute of Technology and Emory University

Title of Abstract: Heparin Biomaterials for Modulation of Endochondral Differentiation

Presenting Author: Torri E. Rinker

Affiliation: Temenoff Lab, Georgia Tech

Complete Author's List :

Torri E. Rinker and Johnna S. Temenoff

All Author Affiliations :

Biomedical Engineering, Georgia Institute of Technology and Emory University

Abstract:

Extracellular matrix (ECM)-inspired biomaterials, such as highly sulfated heparin glycosaminoglycans (GAGs), can modulate cell microenvironments through inherent protein binding properties. Modified heparin, which can be crosslinked into microparticles (MPs), has potential to sequester endogenously-produced proteins, providing a novel approach to perturb biological systems solely through introduction of an ECM component. However, use of heparin MPs alone as a tool to modulate cell differentiation has not been evaluated. Thus, we compared the ability of heparin-based MPs vs. poly(ethylene-glycol)-based (PEG) MPs (with low protein-binding capacity) to modulate ATDC5 cell differentiation in an in vitro model of endochondral ossification. Spheroid aggregates (700 cells/spheroid) were formed by centrifuging known ratios of MPs and suspended cells in agarose wells, followed by culture in non-stick dishes. Experimental groups included spheroids with heparin or PEG MPs at 3:1 (high MP) and 1:3 (low MP) MP:cell ratios, and samples with no MPs. Spheroids were cultured on orbital culture at 37°C and 5% CO2. Culture medium consisted of DMEM/F-12 with L-glutamine, antibiotics, ITS mix, 10 mM β-glycerophosphate, and 50 μg/mL ascorbic acid. Timepoints were taken at days 1, 6, 12, and 18. Over 18 days, Safranin-O staining indicated reduced GAG production in high heparin MP groups, while PEG MPs groups showed similar GAG deposition to no MP control. Gene expression for chondrogenic markers collagen II and aggrecan was down-regulated in a dose-dependent manner in high heparin MP groups compared to no MP controls (7.4+1.8- and 16.5+6.18-fold decrease for collagen II and aggrecan, respectively, at day 6), unlike high PEG MP groups, which showed less down-regulation (1.8+0.15 and 2.85+0.21-fold decrease for collagen II and aggrecan). Immunostaining for collagen II showed a similar delay in deposition in the high heparin MP group while no delay was observed in the high PEG MP group. Overall, in a model of endochondral ossification, heparin MPs successfully modulated timing of cell differentiation in a dose-dependent manner, likely through the mechanism of protein sequestration. Thus, this highly potent ECM-inspired technology may be utilized to manipulate the cell microenvironment through innate sequestering properties, providing a unique avenue for cellular control in tissue regeneration and repair.

Poster # 73

Denise Sullivan-Graduate Student

Email: denise.sullivan@gatech.edu

Affiliation: Biomedical Engineering, Georgia Institute of Technology and Emory University

Title of Abstract: p(N-isopropylmethacrylamide) Microparticles for Controlled BMP4 Delivery within Embryonic Stem Cell Aggregates

Presenting Author: Denise, D., Sullivan

Affiliation: Georgia Tech

Complete Author's List :

(1) Denise D. Sullivan (2) Shalini Saxena, (3)Jeff C. Gaulding, (4)Marian Hettiaratchi, (5) L. Andrew Lyon,(6) Todd C. McDevitt

All Author Affiliations :

(1)Biomedical Engineering, Georgia Institute of Technology and Emory University (2) Materials Science and Engineering, Georgia Institute of Technology, (3) Chemistry, Georgia Institute of Technology, (4) Biomedical Engineering, Georgia Institute of Technology and Emory University, (5) Chemistry, Georgia Institute of Technology, (6) Biomedical Engineering, Georgia Institute of Technology and Emory University

Abstract:

Strategies to direct differentiation of embryonic stem cell (ESC) aggregates, or embryoid bodies (EBs), often employ an “outside-in” approach by addition of soluble factors to culture medium. However, diffusion of soluble factors into EBs is limited resulting in nonhomogenous differentiation. One method to direct differentiation and potentially overcome diffusion limitations is via incorporation of engineered microparticles (MPs), that can be used to control molecular delivery locally, within 3D multicellular aggregates. Coupling of pNIPMAm microgels to core particles combines the hydrophilic and morphogen-releasing properties of hydrogels with a dense core material that enhances incorporation within EBs. The objective of this study was to characterize pNIPMAm MPs for controlled delivery of bioactive BMP4 within EBs to direct ESC differentiation. Core-shell MPs were constructed with the shell consisting of microgels composed of 68% pNIPMAm, 2% N,N’ methylene-bisacrylamide, 30% acrylic acid and synthesized by precipitation polymerization. Microgels were coupled to functionalized polystyrene cores by UV excitation. The growth factor binding capacity of MPs was examined with BMP4 loading (10 and 100 ng/mg MP) for 18 hours at 4ºC. An ELISA was used to quantify passive release of BMP4 at 37ºC from MPs. Bioactivity of BMP4-laden MPs was evaluated using an in vitro alkaline phosphatase (ALP) assay to quantify ALP activity of skeletal myoblasts (C2C12s). MPs were incorporated within EBs by forced aggregation in micro-well inserts at MP:cell seeding ratios of 1:3, 1:1 and 3:1. BMP4 was delivered solubly and via MPs to ESC aggregates to evaluate gene expression of pluripotency marker, Oct-4, and mesoderm marker, Brachyury-T. BMP4-laden MPs released approximately 60% of BMP4 over 14 days. MPs maintained BMP4 bioactivity as treatment with BMP4-laden MPs induced ALP activity similar to delivery of soluble BMP4. Maximum MP incorporation within EBs was achieved with ~80 MPs per EB. Oct-4 expression decreased over 7 days of differentiation in all groups. BMP4-laden MPs demonstrated comparable expression of Brachyury-T to soluble BMP4, despite the delivery of 20-fold less total protein from the MPs over the same period of time. These results suggest that pNIPMAm MPs can be used as a biomaterial-based strategy to control the temporal release of BMP4 and enhance ESC mesoderm differentiation to ultimately develop novel directed stem cell regenerative therapies

Poster # 74

Liane Tellier-Degradation of GAG-based Microparticles in Mesenchymal Stem Cell Spheroids

Email: ltellier3@gatech.edu

Affiliation: Georgia Institute of Technology

Title of Abstract: Degradation of GAG-based Microparticles in Mesenchymal Stem Cell Spheroids

Presenting Author: Liane E. Tellier

Affiliation: Georgia Institute of Technology

Complete Author's List :

Liane E. Tellier, Todd C. McDevitt, Johnna S. Temenoff

All Author Affiliations :

Biomedical Engineering, Georgia Institute of Technology (1); The Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology (2)

Abstract:

Mesenchymal stem cells (MSCs) are a promising multipotent cell type for chondrogenic, osteogenic, and adipogenic differentiation. MSCs grown as cell aggregates (spheroids) are injectable through standard needles and their differentiation can be controlled by localized delivery of growth factors. For growth factor delivery, microparticles (MPs) can be incorporated into MSC spheroids and provide efficient, homogenous delivery to the entire spheroid. Glycosaminoglycans (GAGs), a natural extracellular matrix component, are used in biomaterials to electrostatically bind positively-charged growth factors. However, a limitation to this system is the strong interaction with growth factors, which can prevent release to surrounding cells. Therefore, we engineered enzymatically (MMP-sensitive) and hydrolytically degradable forms of GAG-based MPs. We hypothesized enzyme degradable MPs would degrade more rapidly in MSC spheroids than in saline solution due to MSC secretion of MMPs, whereas hydrolytic MPs would degrade at similar rates in solution and spheroids over 7 days. MPs were formulated with 99% w/w modified 4-arm PEG-acrylate (PEG-4Ac) and 1% w/w thiolated heparin (HepSH). MPs were prepared through water and oil emulsion with PEG-4Ac, HepSH, and cross-linker. MPs were cross-linked with 25 mM of MMP-cleavable peptide (CGGVPMSMRGGGC) or dithiothreitol (DTT) for enzyme MPs and hydrolytic MPs, respectively. MP degradation was monitored in solution by incubating MPs in 1 mL PBS with 1% BSA and .5% NaN3 at 37° C over 7 days. MP degradation was monitored in MSC spheroids (700 cells/spheroid) formed with a 3:1 MP to cell ratio in media (αMEM +10% FBS + 1% antimitotic/antibiotic + 1% L-glutamate). Images of MPs in solution indicate that hydrolytic MPs remained at Day 7. In contrast, enzyme MPs were greatly depleted at Day 7 in solution and none were present at Day 8. Fast Green staining of sectioned spheroids indicate that enzyme MPs and hydrolytic MPs were incorporated at Day 1. At Day 3 and 7, both enzyme MPs and hydrolytic MPs still appeared present within spheroids. Overall, two formulations of GAG-based MPs were fabricated. Our data suggests both were easily incorporated into MSC spheroids and enzymatic MP demonstrated early indications of degradability over 8 days. Based on these results, this system could provide greater release of these growth factors, and ultimately homogenous, sustained differentiation of MSC spheroids.