Poster Session

Sunday, 3/6/2016, 5:00 – 7:00 pm
Presentation of ***odd*** numbered posters

Monday, 3/7/2016, 5:00 – 7:00 pm
Presentation of ***even*** numbered posters

Posters by Category

Cellular and Gene Therapy

#	*Presenter*
1	Salwa Albusaysi
2	Matthew Amdahl
3	Rebecca Ball
4	Kory Blose
5	Evan Delgado
6	Roger Esteban-Vives
7	Gabriella DiBernardo
8	Deborah Galson
9	James Fisher
10	Dave Gau
11	Khalid Hajj
12	Michelle Guaragno
13	Michele Herneisey
14	George Hussey
15	Maritza Jimenez
16	Aimon Iftikhar
17	Mehwish Khaliq
18	Christopher Knapp
19	Matthew Kostek
20	Nicholas Lamson
21	Lu Liu
22	Faina Linkov
23	Yuan Liu
24	Celestina Mazzotta
25	Jacquelyn Russell
26	Thomas Richardson
27	Huseyin Sahin
28	Amrita Sahu
29	Lindsey Saldin
30	Joe Shawky
31	Golnar Shojaati
32	Kenichi Tamama
33	Samer Tohme
34	Matthias Waldner
35	Patrick Wilkinson
36	Tianbin Yang
37	Peng Zhang
38	Yi Zhou
39	Luke Ziegler
40	Chaoming Zhou

Computation and Modeling

#	*Presenter*
41	Megan Cala
42	Fangzhou Cheng
43	Michael Durka
44	Lisa Carey
45	Lin He
46	Piyusha Gade
47	Matthew Markovetz
48	Sanjeev Khanna
49	Florencio Serrano-Castillo
50	Michelle Pressly
51	Chao Sang
52	Qi Mi
53	Andrew Voorhees
54	Rana Zakerzadeh
55	Li Ang Zhang

Medical Devices

#	*Presenter*
56	Elena Bellotti
57	Jacqueline Bliley
58	Patrick Bosch
59	Liza Bruk
60	Bin Cao
61	Patrick Cody
62	Da-Tren Chou
63	Dan Crompton
64	Luis De La Torre
65	Xuan Ding
66	Firuz Feturi
67	James Eles
68	Joerg Gerlach
69	Garrett Jeffries
70	Shawn Kelly
71	Nina Reger
72	Ross Lawrence
73	Elaine Soohoo
74	Shalv Madhani
75	Xin Zheng
76	Alexandra May
77	Salim Olia
78	Avinash Patil
79	Mitali Patil
80	Takashi Kozai
81	Tanchen Ren
82	Cuneyt M.
83	Alkiviadis Tsamis
84	Jingyao Wu
85	Qinghao Zhang
86	Alexander Malkin
87	Brian Martin

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

Posters by Category (continued)

Tissue Engineering

#	*Presenter*
89	Kassandra Allbright
90	Travis Armiger
91	Emily Bayer
92	Ivan Batalov
93	Colin Beckwitt
94	Andrew Brown
95	Michael Buckenmeyer
96	Xiaochu Ding
97	Liwei Dong
98	Vasil Erbas
99	Rebecca Duffy
100	George Fercana
101	Maria Giovanna Francipane
102	Ashlee Greene
103	Harman Ghuman
104	Darren Haskett
105	Yan Huang
106	Isaac James
107	Timothy Jackson
108	Ning-Jiun Jan
109	Ethan Kallick
110	Soo Hyon Lee
111	Chiaki Komatsu
112	Jr-Jiun Liou
113	Samuel LoPresti
114	Daniel Long
115	Katherine Lorentz
116	Chelsea Merkel
117	Christopher Mahoney
118	Asiyeh Golabchi
119	Maxine Miller
120	Mark Murdock
121	Muhammad Nisar
122	Jamie Nowalk
123	Piya-on Numpaisal
124	John Ohodnicki
125	Rachelle Palchesko
126	Akhil Patel
127	Catalina Pineda Molina
128	Alessandro Pirosa
129	Aneesh Ramaswamy
130	Michelle Scarritt
131	He Shen
132	John Sembrat
133	Jihee Sohn
134	Sudhanshu Shekhar
135	Chelsea Stowell
136	Sunita Shinde
137	Yolandi van der Merwe
138	Huong Tran
139	Elizabeth Stahl
140	Zhouguang Wang
141	Aaron Sun
142	Yefei Wang
143	Deepthi Vijayraghavan
144	Michael Washington
145	Yingfei Xue
146	Zhang Wensheng
147	Zhe Zhuang

Corporate and Partners

Cyfuse Biomedical K.K.
McGowan Wound Healing Seminar Series
GLP Consortium
Pediatric Clinical Challenges

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

Posters
Cellular and Gene Therapy

#	*Presenter*				
1	Salwa Albusaysi	15	Maritza Jimenez	29	Lindsey Saldin
2	Matthew Amdahl	16	Aimon Iftikhar	30	Joe Shawky
3	Rebecca Ball	17	Mehwish Khaliq	31	Golnar Shojaati
4	Kory Blose	18	Christopher Knapp	32	Kenichi Tamama
5	Evan Delgado	19	Matthew Kostek	33	Samer Tohme
6	Roger Esteban-Vives	20	Nicholas Lamson	34	Matthias Waldner
7	Gabriella DiBernardo	21	Lu Liu	35	Patrick Wilkinson
8	Deborah Galson	22	Faina Linkov	36	Tianbin Yang
9	James Fisher	23	Yuan Liu	37	Peng Zhang
10	Dave Gau	24	Celestina Mazzotta	38	Yi Zhou
11	Khalid Hajj	25	Jacquelyn Russell	39	Luke Ziegler
12	Michelle Guaragno	26	Thomas Richardson	40	Chaoming Zhou
13	Michele Herneisey	27	Huseyin Sahin		
14	George Hussey	28	Amrita Sahu		

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

The association between BDNF levels and polymorphism with the development of depression during interferon alpha therapy in HCV patients

Francis Lotrich (1), Salwa Albusaysi (2) and Robert Ferrell (3)

(1) Western Psychiatric Institute and Clinics, Department of Psychiatry, University of Pittsburgh Medical Center, (2) University of Pittsburgh, Pharmaceutical Sciences Department, (3) University of Pittsburgh, Department of Human Genetics

Recent studies report that 40% of patients receiving INF-a therapy develop depression. Also, INF-a therapy can influence brain-derived neurotrophic factor (BDNF) release. In addition, the BDNF Val66Met SNP has been associated with reduced BDNF levels. Therefore, the purpose of this study is to determine the relationship between the BDNF Val66Met SNP, serum BDNF levels, and the development of depression.In this study, 149 HCV patients on INF-a therapy were assessed for the development of depression using the Beck Depression Inventory (BDI). Serum BDNF levels were measured using ELISA. Patients were genotyped for the BDNF Val66Met SNP. Mixed model and Kaplan-Meier analysis were performed. INF-a therapy was associated with decreased BDNF levels. Also, lower baseline BDNF was associated with increased BDI scores. In addition, the BDNF Met genotype was associated with lower BDNF levels. These results suggest that patients harboring the BDNF Met genotype may be more likely to develop depression. Moreover, baseline BDNF levels were associated with a greater incidence of developing depression. Collectively, these data suggest that the increased risk to develop depression with INF-a therapy may be mediated by genetic risk factors such as the BDNF Met genotype and that baseline BDNF levels are predictive of future vulnerability to develop depression.

Cytoglobin Reduction by Cytochrome b5 and Ascorbate

Matthew B. Amdahl (1,2), Paola Corti (1), Courtney Sparacino-Watkins (1,3), Jesús Tejero (1), Adam C. Straub (1,4) and Mark T. Gladwin (1,5)

(1) Vascular Medicine Institute, (2) Department of Bioengineering, (3) Department of Pulmonary, Allergy and Critical Care Medicine, (4) Department of Pharmacology and Chemical Biology, (5) Department of Medicine, University of Pittsburgh

Cytoglobin is a recently discovered protein closely related to hemoglobin. Like hemoglobin, cytoglobin possesses a heme group and binds oxygen (as well as many other ligands). In general, hexacoordinate globins autoxidize (a process in which the heme iron changes from the ferrous to the ferric form when bound to oxygen) at rates much faster than these of typical oxygen carrier proteins such as hemoglobin and myoglobin. As most putative functions of these proteins involve the ferrous form (Fe^{2+}) or the ferrous oxy form (Fe^{2+}-O_2), the effectiveness of these proteins may require the presence of a suitable reducing system. Furthermore, our group is examining potential clinical applications of exogenous cytoglobin, which would likewise require that cytoglobin be preserved in the reduced state.

In the case of hemoglobin/myoglobin, the cytochrome b5/cytochrome b5 reductase system converts ferric hemoglobin (methemoglobin) back to ferrous hemoglobin. Ascorbate has also been shown to reduce these globins under certain conditions. Based on cytoglobin's structural similarity to hemoglobin, we hypothesize that both cytochrome b5 and ascorbate can reduce cytoglobin.

To test this hypothesis, we reacted oxidized cytoglobin with both cytochrome b5 and ascorbate under anaerobic conditions, resulting in observable cytoglobin reduction. Globins were oxidized with excess potassium ferricyanide, which was subsequently removed with a filtration column. Following addition of the selected reducing system, the extent and rate of reduction was monitored using absorbance measurements between 450 and 700 nm.

Our data show that both ascorbate and cytochrome b5 rapidly reduce cytoglobin. This suggests that either may function as a physiologic redox partner for cytoglobin, and suggests a potential redox-active function for cytoglobin. These results further indicate the potential for both compounds to preserve exogenous cytoglobin in the reduced state during clinical use.

Lipidoid Nanoparticles for Inflammatory Bowel Disease Therapeutics

Rebecca Ball (1), Kathryn Whitehead (1,2)

(1) Department of Chemical Engineering, Carnegie Mellon University, (2) Department of Biomedical Engineering, Carnegie Mellon University

Inflammatory bowel disease (IBD) is an intestinal malady that is associated with damaging symptoms, frequent relapses, and treatments that are hindered due to the lack of knowledge of the underlying physiological cause of the disease. Recently, a number of proteins have been identified that are upregulated in IBD and may be amenable to ribonucleic acid interference (RNAi) therapy.

Here, we utilize nanoparticles made of lipid-like molecules, which we call lipidoid nanoparticles (LNPs), to deliver short interfering ribonucleic acid (siRNA) for intestinal disease therapeutics. siRNA therapeutics show great promise for the treatment of intestinal diseases such as IBD and gastrointestinal cancer due to siRNA's ability to specifically suppress the expression of a protein of interest. Initial studies demonstrated that siRNA-loaded LNPs mediated potent and dose dependent gene silencing in intestinal epithelial cells with the highest silencing occurring around 24 hours post transfection.

We are particularly interested in the protein myosin light chain kinase (MLCK), which is known to be upregulated with IBD, causing the loosening of the tight junctions between the epithelial cells. As well, an increase in IL-18 production and signaling in intestinal epithelial cells has recently been correlated with the prevention of mucus production and progression of intestinal inflammation.

The LNPs are able to mediate potent and dose-dependent gene and protein silencing of multiple disease relevant proteins in Caco-2 intestinal cell monolayers while showing no significant effect on cell viability. In an in vitro inflammatory model, the siMLCK LNPs are able to modulate the MLCK upregulation. The high siRNA entrapment efficiency and potency of the lipidoid nanoparticles underscore their potential use as inflammatory bowel disease therapeutics.

Adipose-derived Mesenchymal Stem Cells Stimulate Elastin Production by Adult Human Smooth Muscle Cells in a 3D Fibrin Scaffold

Kory J. Blose (1), Justin S. Weinbaum (1,5), and David A. Vorp (1,2,3,4,5)

(1) Department of Bioengineering, University of Pittsburgh, (2) Department of Cardiothoracic Surgery, University of Pittsburgh, (3) Department of Surgery, University of Pittsburgh, (4) Center for Vascular Remodeling and Regeneration, University of Pittsburgh, Pittsburgh, PA, (5) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Adult human vascular smooth muscle cells (SMCs) do not normally produce elastin in vivo, but do so in vitro with external stimuli such as transforming growth factor beta-1 (TGF-β1). As human adipose-derived mesenchymal stem cells (hADMSCs) are known to produce TGF-β1 and other growth factors, we hypothesized that adult SMCs co-cultured with hADMSCs would produce elastin.

Constructs were made by embedding 6x10^4 commercially sourced SMCs in fibrin gels. After two days, 9x10^5 commercially sourced hADMSCs embedded in fibrin gels were added on top of the constructs. Additional experimental constructs were made without hADMSCs but were treated with hADMSC conditioned media. Positive and negative control constructs lacking hADMSC were treated with or without TGF-β1, respectively. After 28 days of culture at incubator conditions, constructs were imaged with an Olympus multiphoton microscope to visualize elastin via its intrinsic autofluorescence, and total elastin content was measured using a ninhydrin assay.

All groups showed elastin production via ninhydrin assay. From a qualitative standpoint, the hADMSC/SMC co-culture experimental group produced a similar elastin network as the TGF-β1 treated positive controls. The conditioned media showed a less developed elastin network than the hADMSC/SMC co-culture group and positive control group. While the negative control did show elastin production in the ninhydrin assay, a developed elastin network was non-existent in the autofluorescent images.

The results of this study show promise for using hADMSCs as a possible elastogenic therapy, stimulating new elastin production by adult SMCs in vivo - ideally in the context of elastolytic diseases such as aneurysms. Interestingly, the underdeveloped elastin network seen in the conditioned media group may indicate that the co-culture of hADMSCs and SMCs allow the cells to communicate and better form an elastic network compared to growth factor treatment alone.

MicroRNA-122 Regulates Polyploidization in the Murine Liver

Evan R. Delgado (1*), Shu-hao Hsu (1*), P. Anthony Otero (1), Kun-yu Teng (2), Huban Kutay (2), Kolin M. Meehan (1), Justin B. Moroney (1), Jappmann K. Monga (1), Nicholas J. Hand (3,4), Joshua R. Friedman (3,5), Kalpana Ghoshal (2) and Andrew W. Duncan (1)

(1) Dept. of Pathology, McGowan Institute for Regenerative Medicine, Univ. of Pittsburgh, (2) Dept. of Pathology, The Ohio State Univ., Columbus, OH, (3) Children's Hospital of Philadelphia Research Institute, Dept. of Pediatrics, Philadelphia, PA, (4) Current Affiliation: Dept. of Genetics, Univ. of Pennsylvania, Philadelphia, PA, (5) Current Affiliation: Janssen Research & Development, Spring House, PA, (*) Equal contributions

A defining feature of the mammalian liver is polyploidy, a numerical change in the entire complement of chromosomes. The first step of polyploidization involves cell division with failed cytokinesis. Although polyploidy is common, affecting ~90% of hepatocytes in mice and 50% in humans, the specialized role played by polyploid cells in liver homeostasis and disease remains poorly understood. The goal of this study was to identify novel signals that regulate polyploidization, and we focused on microRNAs (miRNAs). First, to test whether miRNAs could regulate hepatic polyploidy we examined livers from Dicer1 knockout mice, which are devoid of mature miRNAs. Loss of miRNAs resulted in a 3-fold reduction in binucleate hepatocytes, indicating that miRNAs could indeed regulate polyploidization. Secondly, we surveyed age-dependent expression of miRNAs in wild-type mice and identified a subset of miRNAs, including miR-122, differentially expressed at 2-3 weeks, a period when extensive polyploidization occurs. Thirdly, we examined Mir122 knockout mice and observed profound, life-long depletion of polyploid hepatocytes, proving that miR-122 is required for complete hepatic polyploidization. Next, we identified direct targets of miR-122, Cux1, Iqgap1, Mapre1, Nedd4l and Slc25a34, that regulate cytokinesis. Inhibition of each target induced cytokinesis failure and promoted hepatic binucleation. Finally, we examined expression in a subset of human hepatocellular carcinomas (HCC) with reduced miR-122. Consistent with the mouse data, target expression was inversely proportional to miR-122. Conclusion: Our data suggest a novel regulatory role for miR-122 in liver polyploidization. Moreover, differential expression of miR-122 targets in HCC provides new insights into miR-122-mediated tumorigenesis. These studies will serve as the foundation for future work investigating miR-122 in liver maturation, homeostasis and disease.

Characterization of human fetal dermal-derived fibroblasts for cell banking and exploring their potential use for wound healing therapies

Roger Esteban-Vives (1), Myung Sun Choi (2), Matthew T. Young (1), Alain Corcos (3), Jenny Ziembicki(3), Jörg C. Gerlach (1)

(1) Bioreactor Group, McGowan Institute for Regenerative Medicine, University of Pittsburgh, PA (2) University of Pittsburgh School of Medicine, PA, (3) University of Pittsburgh Medical Center, UPMC Mercy Hospital Trauma Services and Burn Unit, Pittsburgh, PA

Introduction

Fetal dermal-derived fibroblasts are involved in a scar-free tissue repair, which shows low immunological rejection properties and high division rates that make them suitable for cell banking and transplantation. Expanded human fetal-derived dermal fibroblasts (hFDF) were previously used in burn wound healing as a temporary skin substitute. These cells have similarities with mesenchymal stem cell showing markers and the ability to differentiate into osteogenic, chondrogenic, and adipogenic lineages. However, the cell characterization and possible cell differentiation, along with the culture process, is poorly described.

Material and Methods

8-9 week gestational age fetal limb tissues from different donors were obtained from abortion clinic donations (IRB: PR007060159, University of Pittsburgh) and cultured in vitro. Cultured cell populations were sorted and characterized using cell sorting (FACS) for MSC markers CD105+/CD90+/CD73+/CD34-/CD45-/CD79-/CD14-/HLA-DR- and cultured for 6 passages. Gene expression analysis for MSC markers and paracrine effectors (HGF, IL-6) were carried out at passages 3 and 6 using real-time polymerase chain reaction (PCR). Enzyme-linked immunosorbent assay (ELISA) was carried out for paracrine effectors at passage 4. Flow cytometry was used at passage 6 to characterize the final cell population.

Results

hFDF cells exhibit MSC-like characteristics showing a significantly higher doubling time expansion rate (k= 0.4 days during 14 passages) than the commercially available human bone marrow, MSC. However, hFDF cells start to differentiate during the in vitro process. hFDF cell sorting using MSC markers and their subsequent cell expansion were performed to test cell lineage stability and differentiation. Gene expression results indicated that hFDF, at passage 3 and 6, has a lower gene expression of CD105, CD73 and CD90 and higher expression of CD34 and CD45 when compared to BM-MSCs indicating the cells started to differentiate. This loss of MSC marker expression was consistent with the flow cytometry results. However, expanded hFDF showed potential wound healing properties producing a significantly higher amount of wound healing hormone HGF and a lower amount of pro-inflammatory cytokine IL-6 when compared to BM- MSC cells.

Conclusions

Allogeneic fetal-derived dermal fibroblast in combination with spray transplantation could enhance skin regeneration with third-degree burn wounds. Despite hFDF showing interesting wound healing properties, the cell source origin and cell differentiation during in vitro expansion makes for an unpredictable cell fate because it blurs the possible clinical use.

The Effects of Cold Storage and Poloxamer 188 Treatment on Stromal Vascular Fraction Viability and Volume Retention of Fat Grafts

Gabriella DiBernardo (1), Jacqueline Bliley (1), Debra Bourne (1), Emmanuel Havis (2), Ryan Schroth (1), Aaron Dees (3), Damian Grybowsky (1), Sheri Wang (5), Lauren Kokai (1,4), Arta Kelmendi-Doko (1,3), Christopher Mahoney (3), Wesley Sivak (1), Kacey Marra (1,3,4), J. Peter Rubin (1,3,4)

(1) Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, (2) Pierre and Marie Curie University, Paris, France, (3) Department Bioengineering, University of Pittsburgh, Pittsburgh, PA, (4) McGowan Institute for Regenerative Medicine, Pittsburgh, PA, (5) University of Pittsburgh School of Medicine, Pittsburgh, PA

Fat grafting is used to reconstruct soft-tissue defects, including those resulting from breast cancer resection and craniofacial trauma. Unpredictable retention is characteristic of all fat grafting procedures and many variables may lead to it, one of which may be ischemic injury. Cold storage is commonly used and is believed to reduce tissue damage during ischemia in whole organ transplants. Poloxamer-188 (P188) has been used to prevent apoptosis of damaged cells, increase graft survival and histology of grafted fat. This study aims to determine whether tissue cooling combined with P188 increases graft retention and viability of injected fat.

Fat was obtained from surgical patients by liposuction and subsequently, incubated at 4C, room temperature, 4C+P188 and RT+P188 for various time points. The Stromal Vascular Fraction was isolated at each time point to assess cell yield and viability. Lipoaspirate samples were collected up to 24 hours to assess gene expression of apoptotic (Bax/Bcl2 ratio), angiogenic (VEGF, AGPT1) and senescent factors (p21). Treated fat grafts were injected onto flanks of athymic nude mice and explanted 6 weeks postoperatively to assess volume retention and tissue architecture.

Viability of the SVF was decreased in RT and 4C groups at 4.5 hours however, P188 appeared to preserve cell viability with both treatments. Gene expression indicated 4C treated fat had downregulated p21 and PPAR gamma expression at 7.5 and 24 hours compared to RT groups, which could signal apoptosis or senescence of adipocytes within the graft. A subsequent downregulation of angiogenic factors was observed at 24 hours with 4C treatment compared to RT. No significant difference between groups was observed in volume retention at 6 weeks postoperatively. However, 4C treatment tended to have a more inflammatory histological appearance with oil cysts compared to RT or P188. Preliminary histology suggests increased adipocyte viability in RT and P188 treated fat grafts.

These results suggest cooling negatively impacts SVF and adipocyte viability, gene expression, and histological appearance of transplanted grafts. Future work will assess apoptosis and necrosis in lipoaspirate-treated samples to correlate these outcomes with our findings.

EZh2 or HDAC inhibition reverses myeloma-induced Gfi1-mediated epigenetic repression of Runx2 and rescues osteoblast differentiation

Juraj Adamik (1,2), Wei Zhao (3), Peng Zhang (1,2), Quanhong Sun (1,2), Kurt R. Weiss (2,4), G. David Roodman (3,5) and Deborah L. Galson (1,2)

(1) Department of Medicine, Hematology-Oncology, University of Pittsburgh, Pittsburgh Cancer Institute, Pittsburgh, PA, (2) McGowan Institute for Regenerative Medicine, Pittsburgh, PA, (3) Department of Medicine, Hematology-Oncology, Indiana University, Indianapolis, IN, (4) Department of Orthopaedic Surgery, University of Pittsburgh Medical Center, Cancer Stem Cell Laboratory, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, (5) Richard L. Roudenbush Veterans Administration Medical Center, Indianapolis, IN

Multiple myeloma (MM), a malignant plasma cell disorder, is the most frequent cancer to involve bone. Over 80% of MM patients develop osteolytic bone lesions that can result in severe pain and frequent pathological fractures, which are a major source of morbidity and contribute to patient mortality. These lesions rarely heal even after therapeutic remission due to persistent MM-induced suppression of bone marrow stromal cell (BMSC) differentiation into bone-forming osteoblasts (OB). We reported that the MM-induced pro-inflammatory bone marrow microenvironment causes upregulation of the multifunctional transcriptional repressor Gfi1 in BMSC via TNF, which correlated with repression of the key OB transcription factor, Runx2, required for OB differentiation. Therefore, we characterized the molecular mechanisms responsible for Gfi1 repression of Runx2 expression. Chromatin immunoprecipitation analyses revealed that MM cells induce repressive epigenetic histone changes at the Runx2 locus and recruitment of Gfi1 to a specific binding site within the Runx2 gene promoter. Ectopic Gfi1 in a pre-osteoblast cell line, MC4, was capable of binding the Runx2 gene promoter, recruiting histone modifiers, altering the chromatin architecture, and repressing endogenous Runx2 mRNA expression. Gfi1 knockdown in MC4 cells blocked MM-induced recruitment of HDAC1 and EZh2 to the Runx2 gene, acquisition of repressive chromatin architecture, and suppression of OB differentiation. These results indicate that MM-induced repressive chromatin architecture at the Runx2 gene is mediated by Gfi1 binding and recruitment of histone modifiers HDAC1, LSD1, and EZh2. Importantly, inhibition of HDAC1 or EZh2 activity in pre-osteoblast cells after MM exposure in vitro or osteoblast precursors from MM patients reversed the repressive chromatin architecture at the Runx2 gene and rescued osteoblast differentiation. These results suggest that treatment of MM patients with EZh2 or HDAC1 inhibitors may reverse the profound osteoblast suppression in MM patients and allow repair of lytic lesions.

Biomimetic Microparticles can Establish Dominant Tolerance in Vascularized Composite Allotransplantation via Endogenous Regulatory T Cell Enrichment

James D. Fisher (1,2), Wensheng Zhang (5), Riccardo Schweizer (5), Chiaki Komatsu (5), Jignesh Unadkat (5), Jonas Schnider (5), Angus Thomson (1,3), Mario Solari (5), Michael Davis (6), Vijay Gorantla (1,5), Steven R. Little (1,2,3,4)

(1) University of Pittsburgh School of Medicine, University of Pittsburgh Departments of Bioengineering (2), Immunology (3), Chemical Engineering (4), Plastic Surgery (5) and the United States Army Institute for Surgical Research (6)

Background
Vascularized composite allotransplantation (VCA)—encompassing transplantation of hands/limbs and face is an emerging field with potential to restore the appearance and function of damaged tissue. Clinically, the process of rejection is suppressed via systemic immunosuppression, which is associated with a host of deleterious side effects. It is known that our bodies contain a subset of lymphocytes called regulatory T cells (Treg) that play a critical role in establishing and maintaining immunological homeostasis. To this end our group has recently developed controlled release microparticle (MP) systems capable of enriching Tregs at given location via synthetic cell constructs referred to as Expansion MP. Accordingly, we hypothesized that Expansion MP could promote long-term graft survival in a rodent VCA model.

Methods
Expansion MP (consisting of IL-2, TGF-β and rapamycin) was fabricated using standard double emulsion chemistry. Following fabrication, Expansion MP was tested in an allogeneic rat hind limb transplant model. All animals received the same baseline immunosuppression protocol. Animals receiving Expansion MP received two subcutaneous 1 ml doses (10mg/ml) at days 0 and 21 respectively in the transplanted limb. Limbs were graded on a daily basis using a 5-point rejection scale. Our primary indictor of effectiveness was mean graft survival compared to controls receiving just the baseline immunosuppression protocol (n=6).

Results
Our results strongly suggest that Expansion MP appears to
prolong graft survival indefinitely (>400days) whereas controls reliably reject limbs by day 40. Secondary skin grafts demonstrate that Expansion MP treatment appears to confer donor specific tolerance to recipients.

Conclusions
Given these promising results, we believe that Expansion MP has the potential to dramatically impact the field of reconstructive transplantation (with possible applications to solid organ transplantation). Future work will consist of optimizing our treatment strategy as well as scaling up to a large animal model in anticipation of an IND application to the FDA.

SRF-Independent Role of MKL in Angiogenesis

Dave Gau (1), Marion Joy (1), Partha Roy (1)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

This project will look at the novel SRF (serum-response factor)-independent role of MKL (Megakaryoblastic leukemia) in its regulation of angiogenesis, or new blood vessel formation. Aberrant angiogenesis is at the stem of pathologies such as cancer, diabetes, retinopathy, and wound healing failure. Attempts in the past to regulate angiogenesis have met only limited success due to high toxicity from treatments that aim to globally disrupt angiogenesis or selectively enhance specific pro-angiogenic pathways while inhibiting others. Selective targeting and regulation of the invasive phenotype of endothelial tip cells (endothelial cells (EC) which are located at the growing ends of capillaries and direct new vessel formation) may be a more suitable method to controlling angiogenesis. For dynamic remodeling of the actin cytoskeleton to occur, some de novo or new synthesis of cytoskeletal and adhesion molecules are required. MKL family transcriptional co-activators are partially responsible for this synthesis. Traditionally, it is thought that MKL performed its function through activation of SRF in response to actin polymerization to create actin cytoskeletal related proteins. Loss of MKL or SRF has been shown to cause a defect in tip cell phenotype and blocks vascularization in vivo, however, targeting this signaling axis is therapeutically challenging since SRF inhibition can cause widespread damages. Preliminary data indicates that MKL can influence expression of certain important regulators of actin dynamics and cell migration such as members of the profilin (Pfn1) family of actin-binding proteins while SRF does not affect such expression. This could be potentially through STAT (signal transducer and activator of transcription) family proteins. Successful completion of this study will provide a mechanism behind the SRF-independent role of MKL in regulation of angiogenesis. In addition, this study will determine if Pfn1 (the major isoform of the Pfn family) can be a putative downstream target in the MKL signaling axis for regulating angiogenesis and provide a conceptual framework for developing therapeutics for diseases related to aberrant angiogenesis.

In Vitro mRNA Delivery using lipid-like Nanoparticles for Gene Replacement Therapy

Khalid Hajj (1), Kathryn Whitehead (1,2)

(1) Department of Chemical Engineering, (2) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA

Messenger RNA (mRNA) is an exciting class of therapeutic molecule for use in gene replacement therapy, cancer immunotherapy and also in the emerging field of genomic engineering. Any disease that is characterized by abnormal protein expression, including cancers and genetic disorders such as hemophilia, could potentially benefit from mRNA drugs encoding for the missing functional protein. Protein replacement therapy by mRNA offers several advantages over DNA, which requires nuclear entry and carries a risk of genomic integration. Unfortunately, mRNA delivery remains a challenge compared to more established nucleic acid drugs such as short interfering RNA (siRNA) because of its large size, instability in solution, and unique immunostimulatory considerations. Thus, mRNA requires an effective delivery vehicle in order to be considered a viable therapeutic. Here we demonstrate efficacious in vitro mRNA delivery of firefly luciferase using lipid-like nanoparticles into a variety of cell lines, including HeLa, Raw 264.7 Macrophages and Caco-2 cells, as well as a difficult-to-transfect suspension cell line. Lipid nanoparticle efficacy compares favorably to commercially available transfection reagents, but to varied degrees according to cell line. We show that although these materials are able to deliver other types of nucleic acids, the nanoparticle formulation and composition must be tailored to the mRNA system in order to observe efficacious delivery. Furthermore, we provide an analysis of the variables that categorize an effective mRNA delivery vehicle such as particle size, zeta potential and surface pKa. Our results underscore the potential of lipid nanoparticles in the delivery of mRNA for protein replacement therapy.

Biomimetic Drug Delivery of a Chemokine to Recruit Endogenous Regulatory T cells(Tregs) for the Treatment of a Model of Dry Eye Disease

Michelle Guaragno (1,4), Andrew J. Glowacki (2,4), Morgan V. Fedorchak (2,4,5), Abhinav P. Acharya (2), Julia Polat (5), and Steven R. Little (1,2,3,4,5,6)

(1) Department of Bioengineering, (2) Department of Chemical and Petroleum Engineering, (3) Department of Immunology, (4) The McGowan Institute of Regenerative Medicine, (5) Department of Ophthalmology, (6) Department of Pharmaceutical Sciences, University of Pittsburgh

Dry eye disease (DED) is an inflammatory disorder that affects millions of patients worldwide. Studies have shown that DED appears to be characterized by inflammation on the ocular surface and lacrimal functional unit, which can impair tear production and in severe cases lead to vision loss. Moreover, an imbalance of effector T-cells and anti-inflammatory cells known as regulatory T-cells (Tregs) cause the ocular inflammation. If there were a technique to increase the number of Tregs locally and selectively in the lacrimal gland, the underlying inflammatory imbalance could be circumvented. Interestingly, studies have demonstrated that the chemokine CCL22 released from a controlled release system is able to recruit functional Tregs to the injection site in vivo. We hypothesize that by using a biodegradable controlled release system, we will be able establish a gradient of CCL22 in the lacrimal gland in order to recruit Tregs and restore the immune balance. We demonstrate in an experimental murine model of DED that CCL22 microparticles improves aqueous tear production, maintains the health of the ocular surface, and could potentially be a viable therapy for DED.

Active targeting of inflammatory macrophages with folate decorated nanoemulsions containing bimodal fluoropolymers

Michele Herneisey (1), Simai Liu (2), Jelena M. Janjic (1,3,4)

(1) Graduate School of Pharmaceutical Sciences, Mylan School of Pharmacy, Duquesne Univ., Pittsburgh, PA, (2) Bayer School of Natural & Environmental Sciences, Duquesne Univ., Pittsburgh, PA, (3) Chronic Pain Research Consortium, Duquesne Univ., Pittsburgh, PA, (4) McGowan Institute for Regenerative Medicine, Pittsburgh, PA

Macrophages are key immune cells driving inflammatory processes and are directly involved in the development of chronic inflammatory diseases.[1] Therefore, active macrophage targeting approaches have the potential to improve chronic inflammation treatments. Folate receptor beta is specifically expressed by activated macrophages, so nanosystems that incorporate folate enable targeted delivery of therapeutic, anti-inflammatory compounds to activated macrophages.[2] Our group has previously developed a nanoemulsion platform containing resveratrol, a natural anti-inflammatory compound, and two imaging modalities (a near-infrared fluorescent moiety and a 19F magnetic resonance imaging (MRI) tracer) for the imaging supported delivery of resveratrol to the macrophage.[3] Here, we have improved this resveratrol nanoemulsion platform in two ways. First, we have incorporated folate onto the surface of the nanoemulsion by anchoring a lipid-polyethylene glycol (PEG)-folate conjugate to the oil phase of the nanoemulsion. Second, we have directly conjugated the fluorescent moiety (fluorescein, FITC) to the 19F MRI tracer (perfluoropolyether, PFPE) through modification of previously reported approaches,[4,5] thus developing a dual-mode imaging reagent. Briefly, PFPE ester was conjugated to FITC-cadaverin through amide coupling, thus producing fluorescent blended PFPE amides (FBPAs). This direct conjugation is advantageous, as it prevents the differential distribution of the fluorescent moiety and the MRI tracer in tissues.[4] The developed nanoemulsions were tested for colloidal stability under cell culture relevant conditions. Nanoemulsions were also evaluated for toxicity in a model macrophage cell line. These nanoemulsions show promise for the bimodal imaging supported delivery of anti-inflammatory compounds through active targeting to the macrophage for the treatment of chronic inflammation.

References

1. C. Nathan and A. Ding, Cell, 2010, 140, 871-882.
2. N. K. Jain et al, Expert Opin Drug Deliv, 2013, 10, 353-367.
3. M. Herneisey et al, Food Chemistry, 2016, submitted.
4. J. M. Janjic et al, Journal of the American Chemical Society, 2008, 130, 2832-2841.
5. M. J. Patrick, J. M. Janjic et al, Journal of the American Chemical Society, 2013, 135, 18445-18457.

Development of Biologic Scaffolds from Human Glioma Tumors as an Organotypic Model to Study Disease Pathogenesis

George S. Hussey (1,3), Yoojin Lee (1,2), Divya Raghu (1), Zain Mehdi (1), Nduka M. Amankulor (4), Johnathan A. Engh (4) and Stephen F. Badylak (1,2,3)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Bioengineering, (3) Department of Surgery and (4) Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA

Advancements in tissue engineering and regenerative medicine have led to the development of extracellular matrix (ECM)-based scaffolds that closely reflect the biochemical and structural environment of native tissue ECM. Although much attention has been given to the therapeutic applications of scaffold-based systems in regenerative medicine, their utility as in vitro physiological models to study disease pathogenesis is also of interest. Applying this strategy toward cancer research, (decellularized) matrix from human tumors provides an ideal in vitro microenvironment that closely mimics the ECM composition and biochemical property of native tumors.

The objective of this study was to develop decellularization protocols for surgically resected human glioma tumor tissue. Biochemical analysis identified ECM proteins differentially expressed in decellularized glioma ECM compared to healthy brain ECM. Results show a distinct molecular readout of glioma ECM components reflective of tumor heterogeneity. Furthermore, increased deposition of collagen and sulfated glycosaminoclycans occurred within tumor ECM, both hallmarks of neoplastic progression in many forms of cancer. The bioactive components retained in glioma ECM scaffolds are neurotrophic as evident by the increased formation of neurite extensions in differentiating neural stem cells, and increased migration when compared to the non-tumorigenic brain ECM, observations which may have important implications for neural stem cell based therapies currently proposed to treat glioblastoma.

The research described herein is the first reported ECM hydrogel specifically derived from human glioma tissue. This study provides evidence that decellularized glioma extracellular matrices are promising substrates for improved modeling of the tumor microenvironment. The implications of this work extend beyond an in-depth understanding of the glioma microenvironment, and offer insights into the identification of novel candidate tissue biomarkers and potential molecular targets for therapeutic intervention. Furthermore, the methods described can be applied to other tumor types and demonstrate the utility of tissue engineering concepts to advance tumor biology research.

Platelet nucleation on arrested neutrophils drives vaso-occlusion in Sickle Cell Disease

Maritza Jimenez (1,3), Enrico M. Novelli (1,4), Gregory J. Kato (1,4), Prithu Sundd (1,2,3)

(1) Pittsburgh Heart, Lung and Blood Vascular Medicine Institute, University of Pittsburgh, (2) Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, (3) Department of Bioengineering, University of Pittsburgh, (4) Division of Hematology and Oncology, University of Pittsburgh, Pittsburgh, PA

Sickle Cell Disease (SCD) is an autosomal recessive genetic disorder that leads to sickling and hemolysis of RBCs under hypoxic conditions. As a result of chronic hemolysis, SCD is associated with a hyper-inflammatory, which accounts for enhanced adhesion of leukocytes, platelets, and RBCs leading to vaso-occlusion. Vaso-occlusive pain crisis is the primary reason for emergency medical care in SCD patients. Although neutrophils have been shown to play a role in the on-set of vaso-occlusion by interacting with sickle RBCs in cremaster venules of transgenic SCD mice; the cellular, molecular and biophysical mechanisms that promote vaso-occlusion in SCD patients are not completely understood.

Freshly collected heparinized blood from SCD (SS) patients and control subjects was perfused through polydimethylsiloxane based microfluidic channels with a glass bottom coated with a cocktail of recombinant human P-selectin, ICAM-1 and IL-8 at a physiological shear stress. Fluorescent Abs were added to the blood for in-situ staining of neutrophils and platelets. Cellular interactions were recorded using quantitative microfluidic fluorescence microscopy1.

Neutrophils were observed to roll, arrest and then capture freely flowing platelets leading to the formation of vaso-occlusive aggregates. RBCs were observed getting trapped within the platelet-neutrophil aggregates. The number of platelet-neutrophil interactions, lifetime of these interactions and were several folds higher in SS than control subject blood. The enhanced platelet-neutrophil aggregations in SS blood was attenuated to the level observed in control blood by blocking P-selectin on platelets and Mac-1 on neutrophils with function blocking Abs.

Our data demonstrates that the vaso-occlusive pathophysiology in SCD involves sequential steps of neutrophil arrest, nucleation of platelets on arrested neutrophils, formation of platelet-neutrophil aggregates and trapping of RBCs. Vaso-occlusion can be ameliorated in SS blood by simultaneous inhibition of platelet P-selectin and neutrophil Mac-1. Understanding the molecular mechanism of vaso-occlusion will enable the development of therapies that can prevent VOC in SS patients.

Host Macrophage Response to Heavy and Light Weight Polypropylene Mesh

Aimon Iftikhar (1,3), Katrina Knight (1,4), Charelle Carter (2,4), Pamela Moalli (1,2,3,4), Bryan Brown (1,2,3)

(1) Department of Bioengineering, University of Pittsburgh, (2) Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, (4) Magee Womens Research Institute

Pelvic organ prolapse affects nearly a million women each year in the United States. Over a quarter of these women are diagnosed and undergo a reconstructive procedure, increasingly including the use of polypropylene mesh, to restore mechanical support to the pelvic floor. However, complications including chronic pain, bacterial infection, mesh erosion, or exposure have been observed at rates as high as 10-20%. Recently, the US Food and Drug Administration reclassified surgical mesh for transvaginal repair of pelvic organ prolapse from a class II, moderate risk device to a class III, high-risk device. It is now more crucial than ever to understand the mechanisms that lead to the negative complications.

Mesh properties, such as stiffness, porosity, and weight have been shown to correlate with the degree of mesh integration with vaginal tissue. Previous research in rhesus macaques implanted with polypropylene mesh differing in stiffness, porosity and weight showed differences in vaginal deterioration following mesh implantation. These differences were correlated with a foreign body response, consisting primarily of activated, proinflammatory M1 macrophages. However, lighter weight, higher porosity mesh implants were associated with an attenuated proinflammatory response. Macrophages typically are characterized on a spectrum ranging from a pro-inflammatory M1 phenotype to an M2 anti-inflammatory phenotype. Previous studies have determined that the early macrophage polarization profile following biomaterial implantation is a strong indicator of overall tissue integration downstream. However, it is not feasible to observe these early responses in rhesus macaques.

Therefore, we are developing a rabbit model to implant heavy and light weight mesh into two different sites, including the vagina and the abdomen. The main objective of this work was to compare the macrophage phenotype at early (14 days) vs. late (90 days) stages in the host following mesh implantation. The mesh-tissue complex was removed from each rabbit and processed for hematoxylin & eosin staining as well as immunolabeling of macrophages with a pan-macrophage marker, Ram11. Although mesh implantation at the vaginal site displays an increased host response at 14 days, the contrary is true in the case of abdominal implantation at 90 days. An ongoing investigation continues to determine M1/M2 specific markers for the rabbit model.

The role of Stat3 in biliary epithelial cell-driven liver regeneration

Mehwish Khaliq (1), TaeYoung Choi (1), Donghun Shin (1,2)

(1) Department of Developmental Biology, University of Pittsburgh, Pittsburgh PA and (2) McGowan Institute of Regenerative Medicine

The only definitive treatment for end-stage liver disease is liver transplantation. Unfortunately, the demand for liver transplants exceeds the availability of donor livers, underpinning the need for harnessing the liver's innate regenerative capacity. Following acute liver injury, regeneration manifests as either: (1) hepatocytes regenerating to restore the lost mass or (2) if hepatocyte proliferation is compromised, biliary cells (BECs) proliferating and contributing to the regenerating hepatocytes. To induce biliary-driven liver regeneration, our lab generated the zebrafish transgenic line, Tg(fabp10a:CFP-NTR), in which hepatocytes can be specifically and genetically ablated. Consequently, BECs de-differentiate into hepatoblast-like cells (HB-LCs), proliferate and then re-differentiate into newly generated hepatocytes, eventually proliferating to restore the liver mass. During this regenerative process, inflammatory pathways are considered important regulators of the HB-LC response as increased cytokine production is linked with HB-LC proliferation. One of the downstream mediators of inflammatory cytokine signaling is the evolutionarily conserved transcription factor, signal transducer and activator of transcription 3 (Stat3). The binding of certain cytokines, such as IL-6, to its respective receptor activates JAKs, which subsequently phosphorylate and activate Stat3; once active, Stat3 dimerize, translocate to the nucleus and activate downstream target genes. Using the zebrafish liver regeneration model, the purpose of this study is two-fold: (1) to elucidate the role of Stat3 and (2) its upstream immunologic activators in biliary-driven liver regeneration. In the regenerating liver, in addition to stat3 expression, we discovered the upregulation of interleukin, il11a, its receptor, il11ra, and co-receptor, gp130. When Stat3 activation was blocked with a chemical inhibitor, JSI-124, HB-LC induction occurred normally. However, hepatocyte maturation was delayed and the number of mature BECs was significantly reduced in inhibitor-treated regenerating livers, manifesting in a reduction of liver size. Interestingly, JSI-124 treatment caused no significant difference in cell death. In addition, the defective intrahepatic biliary network in JSI-124-treated livers resulted in the loss of proper liver function. For future studies, we will determine the roles of stat3, il11a, and ill1ra in biliary-driven liver regeneration by examining this process in their mutants.

Lipidoid Nanoparticles Delivering siRNA for Treatment of Mantle Cell Lymphoma

Christopher M. Knapp (1), Jia He (2), Kathryn A Whitehead (1,2)

(1) Department of Chemical Engineering, (2) Department of Biomedical Engineering, Carnegie Mellon University, 5000 Forbes Ave., Pittsburgh, PA 15213

Mantle cell lymphoma (MCL) is a subtype of non-Hodgkin lymphoma with a very poor prognosis due to inevitable relapse and resistance after treatment. In MCL, common genetic mutations of genes that regulate the cell cycle and cell survival can lead to their overexpression making this cancer an ideal candidate for gene therapies, such as RNA interference. Proteins with abnormal expression in MCL include D-type cyclins and members of the Bcl-2 family, proteins that regulate cell cycle progression and apoptosis, respectively. Additionally, chemotherapy resistant cancers are commonly overly dependent on these proteins.

To take advantage of these genetic abnormalities to enhance MCL therapy, a potent, degradable lipidoid nanoparticle was formulated to deliver siRNA to MCL cells. siRNA is processed by the RNA interference machinery within the cell leading to gene-specific gene silencing. Silencing two anti-apoptotic Bcl-2 family members, Mcl-1 and Bcl-2, simultaneously led to about 40% of cells undergoing apoptosis after 72 hours of treatment compared to 30% when either gene was silenced individually. Silencing CCND1 in addition to Mcl-1 and Bcl-2 increased the fraction of cells undergoing apoptosis to about 70% of all cells after 72 hours of treatment. Simultaneous targeting of D-type cyclins and anti-apoptotic Bcl-2 family members led to a larger fraction of cells undergoing apoptosis than siRNAs targeting single Bcl-2 family members or D-type cyclins. These results indicate that nanoparticles delivering siRNAs targeting different proteins regulating cell survival and the cell cycle may lead to enhanced initiation of apoptosis in MCL cells compared to nanoparticles only targeting one pathway or protein. In a preliminary study, nanoparticles delivering siRNA targeting CCND1, Mcl -1, and Bcl-2 were able to slow tumor growth in a xenograft model compared to control nanoparticles. These results suggest that lipidoid nanoparticles delivering siRNAs targeting multiple genes have potential as a new treatment option for MCL.

Structural protein changes in skeletal muscle in response to physical strain

Matthew Kostek (1,2), Erika Ross (1), Rock Vomer (1)

(1) Duquesne University, Department of Physical Therapy, (2) McGowan Institute for Regenerative Medicine

Background: Skeletal muscle can respond to physical strain by increasing the amount of structural proteins. How the strain is applied is likely to affect the response of the muscle cells. There is likely a lower and upper limit to this strain/muscle response relationship. Because this relationship may be affected by muscle disease, specifically diseases of muscle structure, it would be helpful to characterize this response in a muscle disease to delineate the biologic underpinnings. This understanding could eventually lead to the development and application of strain-related therapies.

Purpose: The goal of the present study is to determine the effect of different levels of muscle strain on the response of muscle structural proteins.

Design: Randomized strain protocol, 42 male mdx mice and 7 control (healthy) mice, approximately 5 weeks of age we subjected to various levels of muscle strain and muscle structural proteins were examined.

Methods: The mice were randomized into 2 experimental groups and 1 control group for each time point. Control mice received no treatment and are used for comparison. The two mdx experimental groups received either low intensity or high intensity muscle stimulation via the sciatic nerve, three times each week for 3 or 6 weeks.

Results: At study conclusion muscle muscles were extracted and examined for structural protein changes. The major structural protein utrophin showed no changes between strain protocols or at any study time point. Currently other structural proteins are being examined in our laboratory. The lack of response of utrophin is surprising but will be better understood in light of other structural protein changes.

pH-Predicted Behavior of Piperazine Derivatives as Transepithelial Permeation Enhancers

Nicholas G. Lamson (1), Gabrielle Cusimano (1), Kanika Suri (1), Anna Zhang (1,2) and Kathryn A. Whitehead (1,2)

(1) Department of Chemical Engineering and (2) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA

The oral delivery of macromolecular drugs, including proteins and nucleic acids, is one of the greatest unmet needs in biomedicine. Although engineering solutions have been used to overcome enzymatic degradation and the low pH in the stomach, poor absorption across the intestinal epithelium into the bloodstream continues to pose the most significant challenge to clinical translation. One common approach to increase the transepithelial flux of macromolecules is the use of chemical permeation enhancers. Unfortunately, the vast majority of effective enhancers have been thwarted by toxicity, and the molecular parameters that contribute to this behavior are poorly understood. Previous work has shown that select piperazine-derived molecules favorably affect transepithelial and intracellular delivery outcomes, suggesting that piperazine-derived molecules interface uniquely with cellular barriers. To gain better understanding of piperazine-mediated permeation enhancement, this work examined piperazine and thirteen of its simple, hydrocarbon-substituted derivatives using Caco-2 monolayers to model the intestinal epithelium. After evaluating each piperazine for permeation enhancement efficacy and cytotoxicity at three concentrations, it became clear that piperazine derivatives consistently enhance permeability, with each derivative resulting in non-cytotoxic permeation enhancement at one or more concentrations. In attempting to identify structure-function relationships for the piperazine derivatives, it was found that neither treatment concentration, structural characteristics, nor molecular pKa were reliable indicators of permeation potential. Interestingly, the pH of the enhancer solution was identified as a controlling parameter, even when accounting for effects from pH change alone. Specifically, piperazine treatments with pH between 9.2 and 9.6 guaranteed non-cytotoxic efficacy. Furthermore, all effective treatments resulted in pH values between 8.7 and 9.6, a behavior that was not shared by additional small, noncyclic amines studied. These data have important implications in the design of oral biologic delivery systems that employ permeation enhancers, and underscore the need to account for local treatment pH at the intestinal epithelium.

Optimization of macrophage targeted theranostic nanoemulsions for extended anti-inflammatory action

Lu Liu (1), Takaaki Komtsu (1,2), Jelena Janjic (1,3)

(1) Graduate School of Pharmaceutical Sciences, Mylan School of Pharmacy, Duquesne University, Pittsburgh, PA, (2) Laboratory of Chemical Pharmacology, Daiichi University of Pharmacy, Fukuoka, Japan, (3) McGowan Institute for Regenerative Medicine, Pittsburgh, PA

Theranostic nanomedicine is an emerging field that aims to personalize patient treatment by combining imaging and drug delivery into one nanosystem. Earlier, we reported a macrophage targeted theranostic nanomedicine strategy for the imaging and treatment of inflammation. It is known that infiltrating macrophages have an important role in most chronic inflammatory diseases by the release of prostaglandin E2 (PGE2) through the cyclooxygenase-2 (COX-2) pathway. The nanoemulsions deliver celecoxib (COX-2 inhibitor) directly to activated macrophages. Our nanomedicine design is based on macrophage-targeted perfluorocarbon (PFC) nanoemulsions with dual imaging properties: near infrared (NIR) and 19F magnetic resonance imaging (MRI). In this study, we focused on improving the current formulation for increased loading and extended release. We hypothesized that these changes would lead to a reduction of injected dose and would achieve prolonged anti-inflammatory effects in vivo. Further, we expanded the pharmacological in vitro assessment to include dosing and time course studies using PGE2 ELISA and COX-2 expression evaluations by in cell western. New redesigned nanoemulsions were prepared by microfluidization. We assessed the nanoemulsions' colloidal stability by measuring droplet size, polydispersity index (PDI), and zeta potential with dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern). Nanoemulsions exposed to different biological media at an elevated temperature did not show significant changes in particle size distribution for 72h. These data demonstrated retention of colloid properties (size, PDI) and stability with the increased loading of the drug. The redesigned control drug-free nanoemulsions did not show toxicity at high doses (up to 80μL/mL of media) in cells. LPS induced a dose-dependent increase in expression of COX-2 in macrophages. Using PGE2 ELISA assay a half-log dose curve was constructed (0-40μM). In the time-course study, we varied LPS exposure time (0-48h) and varied post-nanoemulsion treatment culture period. The goal of these tests was to assess the time the nanoemulsions remain active against COX-2 in macrophages. Taken together our current results demonstrate that dual mode imaging capable nanoemulsions can be modified to improve their anti-inflammatory action in cells. Nanoemulsions, as presented, allow us to study prolonged effects of anti-inflammatory drugs on these cells without the need for repeated dosing, as the drug continues to be available inside the cells for extended periods of time. Animal studies and extensive phenotype analysis of macrophages exposed to large doses of the nanoemulsions are both underway.

Adipose-derived stem cells and endometrial cancer: current work and future directions

Faina Linkov (1), Lauren Kokai (1), Robert P. Edwards (1), Kacey Marra (1), J. Peter Rubin (1)

(1) University of Pittsburgh

BACKGROUND:
Obesity has been identified as a key risk factor for the development of endometrial cancer (EC), the most common gynecologic malignancy in the US. Our central hypothesis is that adipose tissue from EC patients, as well as patients with other obesity associated malignancies, secretes higher levels of cancer-promoting factors (including inflammatory factors, metabolic factors, and hormones) than healthy adipose tissue.

METHODS:
In our ongoing studies, we generated conditioned media from adipose-derived stem cells (ASCs), an important regenerative cell population within adipose tissue. ASCs were isolated from adipose tissue from over 20 EC patients undergoing hysterectomies and five cancer free control patients undergoing elective abdominoplasties. We performed experiments where we cultured Ishikawa cells in ASC-conditioned media (ASC-CM). Study outcomes include cancer cell proliferation rates and biomarker secretion.

RESULTS:
We developed and published a theoretical model by which ASC influence cancer progression. Our pilot results on limited sample set indicate that ASC-conditioned media significantly increased Ishikawa cell proliferation rate when compared to control Ishikawa culture conditions ($p = 0.002$). Additionally, we found that Ishikawa cells secreted almost 10 % more vascular endothelial growth factor (VEGF) when cultured in EC ASC-CM as compared to Ishikawa cells cultured in healthy (cancer free control) ASC-CM. Other studies involving other biologic markers are on the way.

CONCLUSIONS:
Our findings support the hypothesis that adipose tissue is an important source of secreted factors, which increase the rate of EC cell growth. This study provided preliminary evidence that ASCs may be an important parameter to evaluate in relation to EC development.

Small Molecule Inhibition of the E3 Ligase Subunit Fbxo7 Prevents Mitochondrial Damage

Yuan Liu (1,2), Travis Lear (1), Bill Chen (1) and Rama Mallampalli (1,3)

(1) Department of Medicine, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA and (3) Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, PA

F-box protein, the subunit of the SCF (Skp-Cul1-F-box protein) type ubiquitin E3 ligase, targets substrates for polyubiquitylation degradation, including mitochondrial proteins. Pink1 is a mitochondrial serine/threonine-protein kinase, protecting against mitochondrial dysfunction during cellular stress. We discovered that Pink1 was a short-lived protein (t ½ ~ 30 min), and by employing an unbiased screen identified that F box protein Fbxo7 targets Pink1 for polyubiquitylation and proteasomal degradation. Overexpression of Fbxo7 accelerated Pink1 degradation whereas knockdown of Fbxo7 largely stabilized immunoreactive Pink1 steady-state levels. Ectopically expressed Fbxo7 plasmid caused mitochondrial damage in a dose-dependent manner, while knockdown of Fbxo7 protected mitochondria from injury induced by the membrane potential depolarizer CCCP. A computational simulation based screening approach presented a series of small molecule compounds that potentially could t interrupt the interaction between Fbxo7 and Pink1. We tested one small molecule, termed BC1464, that when exposed to epithelial cells accumulated Pink1 protein levels in both dose and time dependent manners. Further, BC1464 pretreatment protected cells from mitochondrial damage induced by CCCP and tBHP. Thus, Fbxo7 impairs mitochondria through its ability to mediate the ubiquitylation and proteasomal degradation of Pink1, and BC1464 protects mitochondria by inhibiting Fbxo7 and Pink1 interaction, in turn increasing the cellular Pink1 concentrations. The results underscore unique opportunities to employ structure-based design to generate a genus of small molecules that preserve cellular bioenergetics by using selective Pink1 E3 ligase subunit antagonists.

Soluble Klotho (sKl) may play a potential reparative role on healing process: an in vitro study on C2C12 cells

Mazzotta C (1,3), Sahu A (1,2), Cheiki A (2), Winter L (1,4), Vo NV (5), Stolz D (6), Van Houten B (7), Barchowsky A (2), Ambrosio F (1,8)

(1) Dept. of Physical Medicine and Rehabilitation, Univ. of Pittsburgh, (2) Dept. of Environmental and Occupational Health, Univ. of Pittsburgh, (3) Dept. of Experimental and Clinical Medicine, Division of Rheumatology, Univ. of Florence, Florence, Italy, (4) Dept. of Bioengineering, Univ. of Pittsburgh, (5) Dept. of Orthopaedic Surgery, Univ. of Pittsburgh, (6) Dept. of Cellular Biology, Univ. of Pittsburgh, (7) Dept. of Pharmacology & Chemical Biology, Univ. of Pittsburgh, (8) McGowan Institute for Regenerative Medicine, Univ. of Pittsburgh

Introduction: The Klotho protein (Kl) is composed of a large extracellular domain, a trans-membrane domain, and a short intracellular domain. After ectodomain shedding, the extracellular domain is released into the extracellular space and is detectable in blood, urine, and cerebrospinal fluid. Thus, Kl exists as membrane (mKl) and secreted (sKl) forms. Moreover, alternative mRNA splicing of exon 3 generates another transcript of sKl, and this truncated protein represents the major sKl of the Klotho gene. The role of sKl on muscle cells remains to be established, but Klotho mutant mice manifest systemic aging phenotypes, suggesting that klotho plays a role of aging suppressor.

Objectives: The aims of this in vitro study were 1.) to evaluate if sKl improves the in vitro wound healing process on C2C12 cells by regulating proliferation and 2.) to determine if Kl is highly expressed at the site of injury, as we have previously observed in vivo.

Material and methods: An in vitro wound healing assay was performed on C2C12 cells cultured in either standard conditions, with 5ng/Ml of sKl recombinant protein or with 5nmol/Ml of siRNA for Klotho. Images at the injury site were captured after 0, 24 and 48 hours. Immunostaining was performed across the same experimental conditions to evaluate the Ki67/Klotho expression level.

Results: The addition of sKl accelerated wound closure in C2C12s, when compared to normal conditions. On the contrary, C2C12s treated with siRNA to Klotho displayed a dramatically impaired wound healing response. Immunofluorescence demonstrated that Ki67 expression level was significantly higher in C2C12 supplemented with sKl after injury, when compared to C2C12 cells after injury without sKl or siRNA. Klotho expression was markedly increased at the wound edges.

Conclusions: These findings demonstrate that sKl ameliorates the healing process by C2C12s, possibly by stimulating proliferation. Further studies are necessary to determined the underlying mechanisms by which sKl may be useful in the development of interventions to improve muscle regeneration in an aged population.

Lack of Beta-catenin in Hepatocytes Impairs Proliferation and Leads to Increased Morbidity in Response to a Choline-Deficient Ethionine-Supplemented Diet

Jacquelyn Russell (1), Hirohisa Okabe (1), Sucha Singh (1), Minakshi Poddar (1), Satdarshan P. Monga (1)

(1) Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA

Despite the liver's capacity for regeneration, hepatic disease is the 12th leading cause of death in the United States. Treatments for chronic liver disease are limited due to poorly understood mechanisms of liver regeneration. Although activation of liver stem cells (LSCs) is thought to contribute to liver regeneration when hepatocyte proliferation is impaired, the role and origin of these cells remains poorly characterized and controversial. The choline-deficient ethionine-supplemented (CDE) diet model of liver injury is known to induce proliferation of LSCs. However, recent evidence has supported the repair to be primarily driven by hepatocyte self-duplication in this model. As a member of the WNT signaling pathway, Beta-catenin plays an important role in liver regeneration by promoting hepatocyte proliferation. However, the role of Beta-catenin in liver regeneration after injury from the CDE diet has not been previously characterized. In the current study, we investigate the role of Beta-catenin in mediating hepatic repair in the CDE diet model. We show that lack of Beta-catenin-driven WNT signaling in mouse livers leads to a defect in hepatocyte proliferation and increased morbidity in response to the CDE diet. Mice with liver-specific deletion of Beta-catenin (Beta-catenin KO) and control wild-type littermates (WT) on the CDE diet showed comparable initial levels of liver injury and inflammation. However, after 14 days on the CDE diet, WT mice demonstrated significant hepatocyte proliferation and reduced liver injury markers. In contrast, Beta-catenin KO mice displayed a lack of hepatocyte proliferation, sustained liver injury, and increased fibrosis. Mice with disrupted WNT signaling but intact Beta-catenin via liver-specific deletion of WNT co-receptors LRP5 and LRP6 (LRP5-6 DKO), also displayed sustained liver injury after 14 days on the CDE diet. Interestingly, we observed continued proliferation of LSC in the form of ductular reaction in WT, Beta-catenin KO, and LRP5-6 DKO mice, suggesting liver repair occurs independent from LSC activation. Therefore our results indicate an indispensable role of WNT-Beta-catenin signaling in liver regeneration through hepatocyte proliferation and support a model of regeneration driven by hepatocyte self-duplication.

Capsule Stiffness Regulates the Efficiency of Pancreatic Differentiation of Human Embryonic Stem Cells

Thomas Richardson (1), Joe Candielle (2), Sierra Barner (1), Prashant N. Kumta (1,2,3,4,5), Ipsita Banerjee (1,2,4)

(1) Department of Chemical and Petroleum Engineering, (2) Department of Bioengineering, (3) McGowan Institute for Regenerative Medicine, (4) Mechanical Engineering and Materials Science, (5) Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA

Encapsulation of donor islets using a hydrogel material is a well-studied strategy for islet transplantation, which protects donor islets from the host immune response. Replacement of donor islets by human embryonic stem cell (hESC) derived islets will also require a means of immune-isolating hESCs by encapsulation. However, a critical consideration of hESC differentiation is the effect of surrounding biophysical environment, in this case capsule biophysical properties, on differentiation. Thus, our objective is to evaluate the effect of alginate capsule properties on growth, viability, and differentiation of encapsulated hESCs throughout pancreatic induction. It was observed that even in the presence of soluble chemical cues for pancreatic induction, substrate properties of bulk alginate capsules can significantly modulate pancreatic differentiation, hence necessitating careful tuning of capsule properties. Capsules in the range of 4-7 kPa supported cell growth and viability, whereas capsules of higher stiffness suppressed cell growth. While an increase in capsule stiffness enhanced differentiation at the intermediate definitive endoderm (DE) stage, increased stiffness strongly suppressed pancreatic progenitor (PP) induction. Signaling pathway analysis indicated an increase in pSMAD/pAKT levels with substrate stiffness likely the cause of enhancement of DE differentiation. In contrast, sonic hedgehog inhibition was more efficient under softer gel conditions, which is necessary for successful PP differentiation. Research is currently underway for encapsulating hESCs in a high throughput alginate array platform. This will allow for a more thorough investigation of the effect of substrates stiffness on differentiation, as well as delineating the signaling pathways which govern pancreatic differentiation. Results of these studies will be presented and discussed.

Safety and Efficacy of Topical Immunotherapy in Vascularized Composite Allotransplantation

Huseyin Sahin (1), Firuz Feturi (2), Vasil Erbas (1), Liwei Dong (1), Matthias Waldner (1), Lin Hu (1), Mario Solari (1), Raman Venkatramanan (2), Vijay Gorantla (1)

(1) Department of Plastic and Reconstructive Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, (2) Department of Pharmaceutical Science, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction:
Vascularized composite allograft (VCA) gives promising results on treatment of devastating tissue defects. Systemic tacrolimus plays critical role in prevention and treatment of VCA rejection but it causes diabetes mellitus and nephrotoxicity. Topical immunotherapy provides local immunosuppression to prevent/treat VCA rejection and reduce the need for systemic immunotherapy with its complications. Here we evaluated the beneficial effects of topical immunotherapy when administered as adjunctive to the systemic immunotherapy in VCA.

Methodology:
Following orthotopic hind-limb transplantation, rats in group 1 and 2 (n=4/group) were treated with tacrolimus, injected intraperitoneally, in a daily dose of 1 mg/kg of body weight (BW) for 7 days. At day 8, doses were maintained at 1 mg/kg of BW for group 1 and dropped into 0.1mg/kg of BW for group 2. Rats in group 3 (control, syngeneic grafts) were treated with no tacrolimus. Rats in group 4 were treated with topical tacrolimus, in a daily dose of 0.5mg/kg of BW, plus systemic tacrolimus in a daily dose of 0.1mg/kg of BW. Plus evaluating the allograft survival, we evaluated the change in BW, glucose level, and creatinine clearance at days 1, 7, 14, and 30 during the treatment period.

Results and Conclusion:
All animals in group 1 had 100% allograft survival, and 80 % of animals in group 2 showed grade III rejection, on post-operative day 30. 80% of animals in group 4 reached to day 100. Animals in group 1 showed about 2-fold decrease in the BW than animals in other groups. A tendency towards increased glycaemia levels was observed in animals receiving tacrolimus (group 1 and 2) comparing to control respectively (120.9±14 and 111.8±13 vs. 99.75±5 mg/dl). A significant decrease in the creatinine clearance (CCr) value was observed in group 2 compared to group 1 and 3 (1.3 ml/min vs. 2.4 and 2.8 ml/min). Average Tacrolimus trough levels were significantly lower in group 1 compared to other groups. We may conclude that topical immunotherapy maintains allograft survival and may reduce the morbidity associated with systemic immunotherapy in VCA.

Age-related declines in Klotho impairs muscle stem cell mitochondrial function

A. Sahu (1,4), L. Winter (2), B. Van Houten (3), A. Barchowsky (4), N. Vo (5), D. Stolz (6), F. Ambrosio (1,7)

(1) Department of Physical Medicine and Rehabilitation, (2) Department of Bioengineering, (3) Department of Pharmacology and Chemical Biology, (4) Department of Environmental and Occupational Health, (5) Department of Pathology, (6) Department of Cell Biology, (7) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Young skeletal muscle displays a remarkable regenerative capacity following an acute injury. One of the major concerns with aging, however, is the dramatic decrease in the regenerative tissue response. This decrease appears to be attributed, at least in part, to an unsupportive micro-environment, and the exposure of aged muscle to a young circulating micro-environment dramatically restores muscle-healing capacity. The identification of circulating bio-markers that may underlie these effects is an important step in better understanding these age-related declines. One such bio-marker is Klotho, an anti-aging protein that has been associated with maintenance of cellular resistance to oxidative stress. Whereas disruption of Klotho promotes an accelerated aging phenotype, over-expression confers increased longevity. Here, we propose that Klotho is a regulator of muscle regeneration and muscle stem cell bioenergetics.

In this study, we investigated the expression of Klotho in actively regenerating young and old skeletal muscle and muscle stem cells (MuSC). Confocal microscopy of both, muscle sections and MuSC indicated significantly lower Klotho expression in old samples as compared to their young counterparts. Loss-of-function analyses were performed by knocking down Klotho in MuSC using a silencing RNA (siRNA) to Klotho. Seahorse analyses demonstrated that older cells have a lower bioenergetics profile as compared to their younger counterparts. When Klotho is inhibited in young cells through siRNA, the bioenergetics profile is diminished to the level of old cells. We also find that Klotho modulates satellite cell senescence and mitochondrial structure, as determined by immunocytochemistry. These experiments suggest that Klotho plays a novel role in regulating stem cell bioenergetics and mitochondria with aging.

Understanding Esophageal Adenocarcinoma Progression Using Inflammatory and Neoplastic Extracellular Matrix Hydrogels

Lindsey Saldin (1,2), Luai Huleihel (2), David Cardenas (2), Maria Quidgley-Martin (3), Madeline Cramer (2), Tim Keane (1,2), George Hussey (2), Ricardo Londono (2,3), Lori Kelly (4), Chris Chung (1,2), Julie Kosovec (4), Emily Lloyd (4), Ashten Omstead (4), Daisuke Matsui (4), Amit Shetty (4), Ali Zaidi (4), Blair Jobe (4), Stephen Badylak (1,2,3,5)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, (3) School of Medicine, University of Pittsburgh, Pittsburgh, PA, (4) Esophageal and Thoracic Research Laboratories, West Penn Hospital, Allegheny Health Network, Pittsburgh, PA, (5) Department of Surgery, University of Pittsburgh, Pittsburgh, PA

Significant effort has been devoted to the development of technologies that can profile the genome of cancer cells and identify intracellular cancer biomarkers such as miRNAs - a stark contrast to the static progress made for tools to profile the aberrant extracellular matrix (ECM) component of the tumor microenvironment. Collagen hydrogels and MatriGel are the “gold standard” 3D culture scaffolds in cancer biology, used as “ECM mimics” for the past 50 and 35 years, respectively. The stimulus of the present study was to develop a new cancer biology technology, disease-specific ECM hydrogels.

A novel approach was used to develop normal, inflammatory, and neoplastic ECM hydrogels from decellularized normal, metaplastic, and neoplastic adenocarcinoma (EAC) esophageal tissue and to identify mechanisms by which these ECM hydrogels influence cell behavior. We identified and isolated exosomes from ECM and showed their ability to rapidly and markedly affect cell phenotype. We will characterize exosomes derived from normal, metaplastic, and neoplastic ECM. Important and unanswered questions are: 1) What is the profile of extracellular miRNA, contained within the ECM via exosomes, to drive the progression of EAC? 2) How does diseased ECM activate an important cell type in an inflammatory driven cancer, the macrophages, via dynamic reciprocity?

Normal, metaplastic, and EAC tissue from a rat model of EAC was decellularized using the same protocol, assessed for absence of nuclei, and formed into hydrogels as previously described. Next-generation sequencing was used to identify the miRNA profiles contained within normal, inflammatory, and neoplastic ECM exosomes, and compared to EAC tissue miRNA profiles. Finally, macrophages are a plastic innate cell and one of the first responders to inflammation or injury. The activation state (M1/M2) of human naïve macrophages exposed to normal, metaplastic, and neoplastic ECM hydrogels in vitro was determined by qPCR. A better understanding of diseased ECM exosomal miRNA profiles and disease-specific activation of macrophages will guide regenerative strategies for patients of this increasingly devastating form of cancer.

Structural control of tissue mechanical properties: Tissue architecture, cell size and F-actin cortex thickness

Joseph Shawky (1) and Lance Davidson (1,2,3)

(1) Department of Bioengineering, (2) Department of Developmental Biology and (3) Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

To understand the connection between genetics, the environment, and birth defects we are particularly interested in physical and genetic factors that regulate tissue mechanics in the developing embryo. Tissues within embryos of the aquatic frog Xenopus laevis increase in stiffness six-fold during early development; however, the origin of this increase is not well understood. This stiffening happens at a time where the tissue is reductively cleaving to form smaller cells and drastically changing shape to form complex architectures.

We compare the mechanics of embryonic tissues to the predictions of the cellular solids model which has been used to understand the mechanical properties of closed-cell foam materials. The CSM relates bulk stiffness of a foam to the density, or unit-size of individual cells, their microstructural organization, and their material properties. Tissues with relatively large cells were created by arresting the cell cycle of reductively dividing cells using hydroxyurea and aphidicolin (HUA). Tissues treated with HUA exhibited a 25% decrease in the measured elastic modulus, similar to the 23% decrease predicted by the CSM given our measured cell size change. Additionally, since one of the factors contributing to bulk stiffness in foams is thickness of the “cell-wall,” studies to measure F-actin cortex thickness within cells at different developmental stages are underway.

To investigate the contribution of tissue architecture to bulk tissue stiffness, we isolated and dissociated dorsal embryonic tissues and reaggregated them into their native macrostructural shape. Fibronectin staining revealed a complete loss of bulk tissue architecture however local cell domains surrounded by fibronectin still remained. There was no significant difference in modulus between reaggregated tissues and native dorsal tissues (2-way ANOVA p-val = 0.29) suggesting that the source of stiffness in these embryonic tissues is inherent to individual cell stiffness, rather than the architecture in which the cells are arranged. Understanding how cell size, F-actin cortex, and tissue architecture affect tissue stiffness is imperative to uncovering the programs in development responsible for stiffening the embryo.

Controlling the Regenerative Potential of Corneal Stromal Stem Cells

Golnar Shojaati (1,2), Martha L. Funderburgh (1), Mary Mann (1), James L. Funderburgh (1)

(1) Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, (2) Department of Ophthalmology, University of Zurich, Zurich, Switzerland

Purpose: Mesenchymal stem cells from human corneal stroma (CSSC) induce regeneration of transparent stromal tissue during wound repair in mice and are in process to be used in clinical trials for therapy of existing stromal scars. The mechanism by which corneal fibrosis is prevented, however, is not fully understood. In the absence of adhesive substratum, CSSC associate into spheroids. In an effort to understand factors that control CSSC regenerative potential, this study compared properties of sphere-derived CSSC (Sp-CSSC) with substrate-attached CSSC (At-CSSC).

Methods: Limbal stromal tissue of donor corneal rims was dissected, collagenase digested, and CSSC expanded at clonal density as described (PMID: 25504883). Spheres from passage 3-4 CSSC were formed in polyhema-coated dishes or in polypropylene tubes in DME/F12, B27, FGF2, and EGF at 10^5 cells/ml for 3 days, then dissociated with Tryple to yield Sp-CSSC. Gene expression was examined by qRT-PCR. Suppression of scarring was examined in a mouse model of corneal wound healing with 2x10^4 CSSC in a fibrin gel applied at the time of wounding. Neutrophil infiltration was assessed by ELISA for myeloperoxidase at 48 hr after wounding. Statistical significance was determined with t-test analysis of replicates using $p<0.05$ as a criterion.

Results: Sp-CSSC had a marked upregulation of genes associated with immunosuppressive activity including TSG-6, IL10, and COX2 compared to At-CSSC. TGFβ3, a cytokine associated with scarless wound healing, was upregulated > 100-fold as were CXCR4 and CXCL12, proteins involved in stem cell homing. In corneal wounds, Sp-CSSC completely suppressed neutrophil infiltration. At 14 days, expression of fibrotic markers (Fap, Tnc, Acta2, Tgfb1, Col3a1, Sparc) was significantly reduced in Sp-CSSC-treated wounds, and similar to that of unwounded tissue. Suppression of fibrosis by Sp-CSSC was not statistically different from that of At-CSSC using a dosage of 2x10^4 cells.

Conclusions: Sphere formation selects a CSSC population expressing high levels of genes associated with regenerative potential. In vivo, Sp-CSSC suppressed inflammation and prevented stromal fibrosis. These results suggest that sphere formation may help standardize the regenerative potential of cell lines from different donors, and may allow use of lower cell dosages in therapeutic applications.

Soft elasticity-associated signaling and bone morphogenic protein 2 are key regulators of mesenchymal stem cell spheroidal aggregates

Kenichi Tamama (1,2), Zoe Cesarz (1)

(1) Department of Pathology, SOM and (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Cell therapy with adult mesenchymal stem cells (MSCs) is a promising approach in regenerative medicine and autoimmune diseases. There are various approaches to improve the efficacy of MSC-based therapeutics, and MSC preparation as spheroidal aggregates, or MSC spheroids, is a novel preparatory and delivery method. Spheroid formation induces a dramatic change in the gene expression profile of MSCs. Self-activation of interleukin-1 (IL1) signaling was shown to be upstream of both pro- and anti-inflammatory genes in MSC spheroids, but the molecular pathways that initiate IL1 signaling remain unknown. As BMP2 up-regulation precedes that of IL1B expression during spheroid formation, we hypothesized that BMP2 signaling triggers IL1 signaling in MSC spheroids. Contrary to expectations, BMP2 signaling decreased expression of IL1B and downstream genes in a SMAD6-dependent manner. Conversely, IL1B signaling enhanced BMP2 expression. Another major difference between 2D monolayer culture and 3D spheroid culture is the Young's elasticity modulus, or stiffness, of the materials surrounding the cells, as there is a million-fold difference between a plastic surface for standard 2D culture (GPa) and 3D spheroidal aggregates (0.1 kPa). We tested another hypothesis that soft elasticity-associated mechano-signaling initiates the gene expression change during spheroid formation. Results showed that both BMP2 expression and inflammatory signaling are up-regulated in an elasticity-associated signaling dependent manner in MSCs. Lastly, BMP2 signaling enhanced cell survival and cell spreading of MSC spheroids. In summary, our study suggests that BMP2 signaling is critical for MSC spheroids.

Protective Effects of Drag Reducing Polymers on Liver Ischemia-Reperfusion Injury

Samer Tohme (1), Hai Huang (1), Allan Tsung (1), Richard L. Simmons (1), Marina V. Kameneva (1,2,3)

(1) Department of Surgery, (2) McGowan Institute for Regenerative Medicine, (3) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

Ischemia-reperfusion (I/R) injury is a process whereby an initial hypoxic insult and subsequent return of blood flow lead to the propagation of innate immune responses and organ injury. Drag reducing polymers (DRPs) are blood-soluble macromolecules that can increase tissue perfusion and decreasing peripheral vascular resistance. Moreover, these polymers reduced so called near-vessel-wall cell-free layer which is developed in microvessels by moving RBCs toward the vessel center, and allows for near-wall accumulation of platelets and leukocytes. DRPs prevent traffic of RBC toward the centerline and reduce near-wall concentration of leukocytes, suggesting significantly reduced inflammatory reactions. This study tested whether DRP can alter the innate response and ameliorate liver I/R injury. To study the potential role of DRP in this setting, experiments were performed utilizing a segmental ischemia model of mice livers for 60 minutes followed by reperfusion for 6 hours. Different concentrations and volumes of DRP were given intraperitoneally right at the onset of reperfusion prior to closing the abdomen. Treatment with DRP significantly protected the livers after I/R as evidenced by a significant 2-3-fold decrease in hepatocellular injury (as measured by ALT levels and extent of necrosis on H&E stained liver sections, $p<0.01$ in both). Additionally, we found that DRPs were protective through inhibition of neutrophil recruitment into the liver and subsequently decreased formation of neutrophil extracellular traps in the liver parenchyma. Furthermore, DRPs significantly reduced the formation of micro thrombi within the liver sinusoids. We then considered the inflammatory events associated with liver I/R that cause tissue damage to the liver. It was found that DRPs significantly attenuated the complexity of the inflammatory cytokine storm initiated by liver I/R as correlated with reduced hepatocellular damage. Therefore, this study demonstrated a novel strategy to counter liver I/R by using DRPs which are able to decrease neutrophil recruitment, microthrombi formation and attenuate the acute inflammatory responses.

Adipose derived stem cells and bone marrow derived stem cells for immunomodulation in the presence of immunosuppressive drugs

Matthias Waldner (1,2), Wensheng Zhang (1), Jacqueline Bliley (1), Isaac James (1), Aurora Almadori (3), Emmanuelle Havis (1), Riccardo Schweizer (2), Jan Plock (2), Kacey Marra (1), Mario Solari (1), Vijay Gorantla (1), Peter Rubin (1)

(1) Department of Plastic Surgery, University of Pittsburgh, PA, (2) Department of Plastic Surgery, University Hospital Zurich, Switzerland, (3) Department of Plastic Reconstructive and Aesthetic Surgery, Second University of Naples, Italy

Background: Mesenchymal stem cells derived from bone marrow (BMSC) and adipose tissue (ASC) have clinically useful immunomodulatory effects and low immunogenicity. Their ability to suppress innate and adaptive immune cells makes them ideal candidates for immunomodulative cytotherapy to reduce alloreactivity. The aims of this study were to assess and compare the immunomodulatory capacities of BMSCs and ASCs and their susceptibility to immunosuppressive drugs. This is the first study to compare paired ASCs and BMSCs isolated from the same human donors.

Methods: Tissue samples of omental fat, s.c. fat and bone marrow aspirate from 9 human organ donors were retrieved and MSCs isolated. The effect of immunosuppressive drugs (Tacrolimus and Rapamycin) on survival and immunomodulative capacity of the MSCs were examined. In mixed lymphocyte reactions (MLR), the capacity of ASCs and BMSCs to suppress immune response was assessed and compared within individual donors. Various HLA mismatched stimulation settings were analyzed in co-culture with different concentrations of immunomodulating MSCs and in the presence of immunosuppressive drugs.

Results: The immunomodulating effects of all MSC types were dose dependent. The presence of Rapamycin and Tacrolimus influenced the immunomodulating properties of MSCs. Proliferation of responder cells was suppressed by both ASCs and BMSCs and the combination of both cell types deriving from the same donor, resulted in a highly sufficient immunomodulation. ASCs provided an inhibition of stimulated PBMCs of more than 80% (Graph 1).

Conclusion: Human ASCs and BMSCs both showed effective immunomodulation across different HLA barriers. The combination of both cell types is an exciting possibility to modulate the immune response. Immunosuppressants affect the immunomodulating potency of MSC and must be further investigated.

Characterizing the role of diploid and polyploid hepatocytes in liver regeneration and disease using E2f7/E2f8 liver-specific knockout mice

Patrick D. Wilkinson (1,2), P. Anthony Otero (1,2), Evan R. Delgado (1,2), Francis Alencastro (1,2), Matthew P. Weirich (1,2) and Andrew W. Duncan (1,2)

(1) Department of Pathology, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Liver regeneration research is critical because the most effective treatment for end-stage liver disease is whole organ replacement. Polyploidy, a numerical change in the complement of chromosomes, affects 50-90% of hepatocytes in adults; the functional role of polyploidy is largely unknown. Liver-specific deletion of E2f7 and E2f8 (E2f7/E2f8-lko) leads to a profound loss of polyploidy and, according to published reports, does not affect liver development or function. E2f7/E2f8-lko mice, thus, represent a “polyploidy knockout” model by which liver polyploidy can be studied. We hypothesized that diploid and polyploid hepatocytes are functionally equivalent. We first compared proliferation/regeneration of E2f7/E2f8-lko (i.e., profoundly diploid) and control (i.e., profoundly polyploid) hepatocytes. We co-transplanted hepatocytes into mice with liver failure and measured donor-specific chimerism following repopulation. Additionally, we examined proliferation of E2f7/E2f8-lko and control hepatocytes within the first 30 days of birth, and measured liver regeneration kinetics in E2f7/E2f8-lko and control mice after 2/3 hepatectomy. We also examined proliferation of E2f7/E2f8-lko and control hepatocytes in culture. Surprisingly, profoundly diploid livers from E2f7/E2f8-lko mice had a significant proliferative advantage. Finally, we examined the relationship between hepatic polyploidy, E2F7/E2F8 and non-alcoholic fatty liver disease (NAFLD), since it was suggested that mammalian polyploid hepatocytes contribute to and E2F8 contributes to NAFLD in zebrafish. In summary, the data show that livers enriched for diploid hepatocytes have a significant regenerative advantage compared to exclusively polyploid livers. Although E2f7/E2f8-lko mice represent a robust “polyploidy knockout” model, future work is required distinguish between the functional consequences caused by E2F7/E2F8 deletion (and associated gene expression changes) and effects caused by loss of polyploid hepatocytes.

Mechanistic studies on the ability of the probiotic organism Lactobacillus plantarum to mitigate scar

Tianbing Yang (1), Phillip H Gallo (1), Fang Liu (1), Latha Satish (1,2), Sandeep Kathju (1,2)

(1) Department of Plastic Surgery, (2) McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Background: Bacteriotherapy is the concept of using probiotic organisms to displace pathogenic ones. Use of probiotic therapy to enhance wound healing is an attractive novel intervention due to its potential to inhibit pathogen colonization and regulate the immune response. In previous studies in a rabbit model, the local application of the probiotic bacterium Lactobacillus plantarum limited burn wound infection and alleviated scar even in the absence of infection. In the present study, we explored the mechanism by which the probiotic bacteria may be eliciting this anti-scarring effect.

Materials and Methods: Hs68 cells, which are normal human diploid fibroblasts, were used to study the effects of fractioned cell-free L. plantarum conditioned medium using the fibroblast-populated collagen lattice (FPCL) assay. Real time RT-PCR, Western blot and casein zymogram assays were performed to determine the expression and secretion levels of matrix metalloproteinase-1 (MMP-1), also known as fibroblast or interstitial collagenase.

Results: Interestingly, we found that the L. plantarum supernatant fraction of <3000 molecular weight stimulated fibroblasts to digest the collagen lattice matrix rather than contracting it. This effect was abolished when the supernatant fraction was subjected to heat at 90C for 2hrs. Treatment with Proteinase K (37C, overnight) did not significantly affect the subsequent digestion of the collagen lattices. We found that the mRNA and the secretory protein levels of MMP-1 were increased, suggesting that a factor secreted by L. plantarum may have stimulated the production of MMP-1 in fibroblasts.

Conclusions: Our results indicate that L. plantarum may contribute to scar reduction in adult fibrotic wound healing by elaborating a factor that alters MMP production and enhances the breakdown of extracellular matrix proteins, especially collagen. Our observations also suggest that said factor is of comparatively low molecular weight (<3KD), is heat sensitive but not proteinaceous. Future studies are underway to determine if L. plantarum can similarly stimulate the secretion of other MMPs, especially MMP-2 and MMP-9, as these are known to play a significant role during normal scirrhous wound healing.

Loss of Gfi1 impairs osteoclast migration and bone resorption via cytoskeletal rearrangement

Peng Zhang (1,3), Quanhong Sun (1,3), Juraj Adamik (1,3), Rong Chong (2,3), Konstantinos Verdelis (2,3), Deborah L. Galson (1,3)

(1) Division of Hematology/Oncology, Department of Medicine, School of Medicine, UPCI, (2) Department of Oral Biology, School of Dental Medicine, Center for Craniofacial Regeneration, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Gfi1 plays a pivotal role in nervous development, HSC maintenance, cell fate commitment, reprograming, oncogenesis, and epigenetic gene silencing. Our previous studies have shown that Gfi1 is a novel transcriptional repressor of the critical osteoblast differentiation factor Runx2 in bone marrow stromal cells and has an important role in MM-induced osteoblast suppression. Here we identify that Gfi1 has a novel and critical function in osteoclastogenesis. Gfi1 is upregulated during osteoclast differentiation. Gfi1 knockout 8w-old mice have a decrease in bone resorption assessed by serum CTX in vivo. Cell-based assays indicated that Gfi1-null monocytes formed fewer, smaller osteoclasts on a plastic plate, and have fewer resorption traces on bone slices. There was a progressive rescue of Gfi1-deficient osteoclast formation with increasing initial plating density suggesting Gfi1 deficiency impairs osteoclast migration. Moreover, tracking the process of osteoclast formation using living cell imaging confirms that Gfi1 regulates osteoclast migration. Mechanistically, lack of Gfi1 regulates osteoclast migration by modifying actin rearrangement; including actin polarization, polymerization, and F-actin ring formation. Our study identifies a previously unknown role for Gfi1 in osteoclastogenesis by regulating actin dynamics that control osteoclast migration and bone resorption. The lack of Gfi1-deficient OCL function in concert with our findings that decreasing Gfi1 in BMSC reduces multiple myeloma suppression of bone formation suggests that Gfi1 is a promising target for treatment of multiple myeloma bone disease since that would decrease OCL function and increase bone formation.

Human Adipose-derived Stem Cells Integrate into Normal Mouse Trabecular Meshwork

Yi Zhou (1,3), Hongmin Yun (1), Enzhi Yang (1), Xiaobo Xia (3), Joel S. Schuman (1,2), Yiqin Du (1,2)

(1) Department of Ophthalmology, University of Pittsburgh Medical Center, Pittsburgh, PA, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, (3) Department of Ophthalmology, Xiangya Hospital, Central South University, Changsha, Hunan, China

Purpose: We have previously induced human adipose-derive stem cells (ADSCs) into trabecular meshwork cells (TMs) as a regenerative therapeutic candidate for glaucoma. This study aims to compare the effects of differentiated ADSCs and undifferentiated ADSCs after intracameral micro-injection into non-glaucomatous mouse eyes.

Methods: Human ADSCs were obtained from the Adipose Stem Center (Drs. Kacey Marra and J. Peter Rubin) at University of Pittsburgh. ADSCs were induced to differentiate into TM cells by culturing on extracellular matrix produced by TM cells in TM-conditioned media. After 14 days, both differentiated and undifferentiated ADSCs were collected and stained with membrane dye Vybrant DiO. Cells were then diluted at the density of 10,000 cells/μl in DMEM/F12, and 2μl of cells were injected at the speed of 0.22 μl/min into C57BL/6 mouse anterior chamber using a 5-μl capillary tube micropipette connected to a syringe pump. Sham injection and normal mice served as controls. Intraocular pressure (IOP) was measured at day 0, 3, 6, 10, 14, week 3, 4, 5, 6 using an iCare tonometer. Anterior chamber structure images were taken to observe inflammation outcome. Outflow facility was monitored using an ex vivo perfusion system to test the IOP-regulating function. Confocal microscopy on whole-mounts and cryosections was performed to detect the location of injected cells.

Results: At various time points post injection, there was no significant difference in IOP and outflow facility among each group. Anterior chamber structure in each group remained intact without visible vascularization or adhesion. Both differentiated and undifferentiated ADSCs homed to the TM region. The homed cells were viable and integrated into the cell layers of the original TM structure.

Conclusions: Both differentiated ADSCs with TM phenotype and undifferentiated ADSCs homed to the TM tissue and did not increase IOP after intracameral injection. Long-term effects on restoring trabecular meshwork function in glaucomatous animal models are needed for further investigation.

Encapsulation of Donor Hemoglobin in Autogenic RBCs for Potential Treatment of Sickle Cell Disease

Luke Ziegler (1,2), Katrina Zougari (1,2), Salim Olia (1,2), Jonathan Waters (1,2,3), Marina Kameneva (1,2,4)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Anesthesiology, (4) Department of Surgery, University of Pittsburgh, Pittsburgh, PA

Study: Sickle Cell Disease (SCD) causes abnormal shape and rigidity of red blood cells (RBCs) due to pathological hemoglobin (HbS) which results in vaso-occlusion, tissue hypoxia, and many other severe and painful complications. Current long-term treatments for SCD are limited: pharmacotherapy with hydroxyurea leads to both leukopenia and thrombocytopenia while repeated blood transfusions results in alloimmunization in over 50% of patients. We propose to replace HbS in the SCD patient's RBCs with healthy donor Hb, and return these modified RBCs to the patient. With this therapy, it is hoped that vaso-occlusion and alloimmunization will be minimized or eliminated. Methods: Experiments are currently performed using donor RBCs (Valley Biomedical Products and Services Inc.). To maximize endogenous Hb removal, RBCs are processed using a novel two-cycle lysing and resealing process. Lysing is achieved by an osmotic pressure gradient, and resealing is achieved by a combination of an osmotic pressure gradient and temperature changes. The majority of native Hb is removed from the RBCs in the first lysing and resealing cycle. These membranes are then re-lysed and refilled with either a pure Hb solution of varying concentrations (experimental cells) or PBS (control cells). Pure, concentrated Hb solution is created by destruction of RBCs with organic solvent followed by purification and filtering of the supernatant. The degree of Hb encapsulation is evaluated by measurement of total hemoglobin concentration in the modified RBCs (OSM 3 Hemoximeter). Results: Encapsulated experimental RBCs reached ~7 g/dl total intracellular Hb concentration which is about 25% of the clinically relevant Hb amount, which compares to the essentially immeasurable (~0.2 g/dl) original intracellular Hb leftover present in the control RBCs. Based on the obtained results, encapsulation of much higher concentrations of Hb is hypothesized and currently is under examination in on-going studies. Initial results indicate that rheological and morphological properties of these modified cells are comparable to natural cells, as investigated by use of Linkam Shearing Stage device.

Anti-Fibrotic Effects of Pirfenidone in Dupuytren's Contracture-Derived Fibroblasts

Chaoming Zhou (1), Fang Liu (1), Mark E Baratz (2), Sandeep Kathju (1,3), Latha Satish(1,3)

(1) Department of Plastic Surgery,(2) Department of Orthopedic Surgery, (3) McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Background: Dupuytren's disease (DD) is a complex fibro-proliferative disorder of the hand that is often progressive and eventually can cause contractures of the affected fingers. Transforming growth factor (TGF-β1) has been implicated as a key stimulator of myofibroblast activity and fascial contraction in DD. Pirfenidone (PFD), is an active small molecule with potential to inhibit TGF-β1mediated action in other fibrotic disorders. This study investigates the efficacy of PFD in vitro in inhibiting TGF-β1 mediated cellular functions leading to Dupuytren's fibrosis.

Materials and Methods: Fibroblasts harvested from Dupuytren's disease (DD) and carpal tunnel (CT)-derived fibroblasts were treated with or without TGF-β1 (10 ng/ml) and/or PFD (200, 400, 800 ng/ml) and were subjected to cell migration, cell proliferation and cell contraction assays. Western blots and real time RT-PCR assays were performed to determine the levels of alpha-smooth muscle actin (α-SMA), type I collagen and type III collagen respectively.

Results: Our results indicate that PFD effectively inhibits TGF-β1-induced cell migration/ proliferation and cell contractile properties of both CT-and DD-derived fibroblasts. Statistically significant inhibition of TGF-β1-induced alpha-SMA mRNA levels was achieved at the higher concentration of PFD (800 ng/ml). Interestingly, TGF-β1 induction of type I and type III collagens was inhibited by PFD in DD- derived fibroblasts but not in CT-derived fibroblasts. We also found that PFD down-regulated TGF-β1-induced phosphorylation of SMAD2/3, a key factor in the TGF-β1 signaling pathway.

Discussion: These in-vitro results indicates that PFD has potential anti-fibrotic effects on Dupuytren's fibroblasts. Further in-vivo studies with PFD may lead to therapeutic application in preventing the progression or recurrence of Dupuytren's disease.

Posters
Computation and Modeling

#	*Presenter*
41	Megan Cala
42	Fangzhou Cheng
43	Michael Durka
44	Lisa Carey
45	Lin He
46	Piyusha Gade
47	Matthew Markovetz
48	Sanjeev Khanna
49	Florencio Serrano-Castillo
50	Michelle Pressly
51	Chao Sang
52	Qi Mi
53	Andrew Voorhees
54	Rana Zakerzadeh
55	Li Ang Zhang

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

Coupling Lattice Boltzmann and Discrete Element Methods to Model Platelet Behavior During a Thromboelastogram

Megan P. Cala (1), Robert S. Parker (1,2,3,4) and Joseph J. McCarthy (1)

(1) Department of Chemical and Petroleum Engineering, Swanson School of Engineering, (2) Department of Bioengineering, Swanson School of Engineering, (3) Department of Critical Care Medicine, School of Medicine, (4) McGowan Institute for Regenerative Medicine, University of Pittsburgh Medical Center

A thromboelastogram (TEG) is a hemostatic assay that evaluates the whole-blood clotting process from aggregation to fibrinolysis and guides the administration of blood product therapies. In short, an initially stationary pin is suspended in an oscillating cup containing a sample of whole blood. Changes in torque between the pin and the cup resulting from thrombosis are traced through a computer-processed signal of the pin rotations. Parameter values from TEG tracings are compared to reference ranges to guide treatment of blood abnormalities, but the connection between TEG morphology and the in vivo mechanism of thrombosis still remains more art than science.

A combination of a computational fluid dynamics technique, lattice Boltzmann method (LBM), and a particle-based mechanistic model, discrete element method (DEM), of the TEG has the potential to bridge the gap between the in vivo micro-scale mechanistic contribution of individual components of thrombosis and the macro-observables of the TEG. Coupling LBM and DEM increases the resolution of the fluid flow at the solid-fluid boundary. As a preliminary simplification, blood will be simulated within the TEG cup as fluid plasma and solid platelets. LBM handles the fluid-fluid and fluid-wall interactions by calculating the hydrodynamic forces exerted by plasma subject to the oscillatory wall velocity of the TEG. DEM addresses the platelet-platelet bonding that occurs during thrombosis, as well as the force calculations from plasma-platelet and platelet-wall interactions. Additional coagulative and anti-coagulative factors will be incorporated to expand the model including, but not limited to, thrombin, fibrin, and plasmin, as a topic of future study. The increased accuracy of the force and torque calculations from the combined model will solidify the relationship between thrombosis and TEG dynamics, potentially elucidating additional parameters of the TEG that are more sensitive to blood product therapies and enabling a more precise interpretation of TEG morphologies to guide treatment decisions.

Study of Layer Dependent Recruitment of Collagen Fibers during Loading of Urinary bladder Tissue

Jack Hornsby (1), Fangzhou Cheng (2), Lori A. Birder (3), F. Aura Kullmann (3), Donna Daly (4), Paul Watton (5), Mark Thompson (1), Anne M. Robertson (2)

(1) Department of Engineering Science, University of Oxford, (2) Department of Mechanical Engineering &Materials Science, University of Pittsburgh, (3) Department of Pharmacology & Cell Biology, University of Pittsburgh, (4) Department of Biomedical Science, University of Sheffield, (5) Insigneo Institute of in silico Medicine, University of Sheffield

Bladder disorders such as lower urinary tract symptom (LUTS) occur frequently in older people. An estimated 15-30% of people over the age of 60 are affected. Symptoms including overflow continence, changed in frequency, urgency, and urge incontinence can cause significant morbidity and dramatically lower quality of life. The two main bladder functions, filling and voiding, are largely governed by bladder wall structure. Previous work has shown a gradual loss of compliance during aging which can significantly impair bladder filling function. From a mechanical point of view, the bladder filling process is dominated by the passive structural components such as elastin and collagen fibers. To explore the roles these components play in bladder filling, we chose 24 month old aged rat bladder and employed a planar biaxial testing device compatible with multiphoton microscopy (BA-MPM) to visualize the collagen fibers in lamina propria during mechanical loading.

Results from MPM imaging suggest two distinct layers of fibers in the lamina propria with qualitatively different fiber architecture. The layer closer to the urothelium is comprised of fine fibers with a large distribution of orientations that was maintained a distributed orientation under increasing equi-bixial load. The second layer consisted of larger diameter fibers that were wavy in the absence of stretch and straightened and reoriented with increasing stretch. We found the strain at which fiber recruitment in the outer layer started, is consistent with the transition strain in the loading curve. In the future, this same approach will be applied across the entire bladder wall, including the detrusor and adventitia layers, enabling the development of a microstructurally motivated mechanobiological model of the bladder wall.

CFD Modeling of Cerebral Aneurysms: Hemodynamic Sensitivity to Choice of Waveform in the Elderly Population

Michael J. Durka (1), Isaac H. Wong (1), David F. Kallmes (2), Dario Pasalic (2), Juan R. Cebral (3), Pablo J. Blanco (4), Manoj Jagani (2), Anne M. Robertson (1)

(1) Department of Mechanical Engineering & Materials Science, University of Pittsburgh, Pittsburgh, PA, USA, (2) Mayo Clinic, Rochester, MN, USA, (3) Department of Bioengineering, George Mason University, Fairfax, VA, USA, (4) Laboratório Nacional de Computação Científica, Petrópolis, Brazil

Intra-aneurysm hemodynamics has been hypothesized to strongly influence cerebral aneurysm pathology. Computational fluid dynamics models have been heavily utilized to estimate hemodynamic quantities. However, the required time-varying boundary data for well-posed models is often not available on a per-patient basis. Holdsworth et al. have revealed the remarkable similarity in waveforms among young healthy subjects from which an archetypal waveform was derived. However, the vascular dynamics of these subjects may not accurately reflect those of elderly patients with cardiovascular diseases: a group much more likely to possess cerebral aneurysms. Our aim therefore was to first assess the variation among waveforms in this population, and then to quantify the differences in the hemodynamic variations produced when different waveforms from this group are used as boundary data in a computational model of a basilar bifurcation aneurysm. A custom written MATLAB script was used to extract waveform characteristics from the Doppler data. Waveforms were automatically placed into one of four categories based on the length and magnitude of systole relative to diastole; parameters hypothesized to strongly effect the aneurysmal hemodynamics. Three waveforms for each group were selected for CFD study for a total of 12 cases. The variation in three metrics of wall shear stress magnitude (WSSM) was studied: time and space averaged WSSM (TSAWSSM), maximum time averaged WSSM (MTAWSSM), and maximum WSSM (MWSSM). With all waveforms normalized to produce an identical flowrate, the TSAWSSM range among all cases was 26% of the average TSAWSSM; the MWSSM range was 145% of the average. The MWSSM contours, however, demonstrated remarkably similar qualitative features despite having different magnitude scales. These results suggest that WSSM at any given location is sensitive to patient specific flow, while relative, qualitative WSSM features (e.g. high versus low areas of WSSM) are largely insensitive to the choice of time-varying boundary data.

A Bayesian Model to Predict Risk of Stroke after CF-LVAD Implantation

Lisa Carey (1,2), Manreet Kanwar (3), Robert Kormos (4), Srivinas Murali (3), James Antaki (2)

(1) Department of Bioengineering, University of Pittsburgh, (2) Department of Biomedical Engineering, Carnegie Mellon University, (3) Cardiovascular Institute, Allegheny General Hospital, (4) Department of Cardiology, University of Pittsburgh Medical Center, Pittsburgh, PA

The risk of stroke continues to be a major adverse event post-left ventricular assist device (LVAD) implantation, limiting the utility of LVADs in the potential, less-sick patient population. Many factors are involved in contributing to the risk of stroke, making it difficult to assess. Our study employs a Bayesian machine-learning approach, which allows for studying complex variable correlations. We utilized the INTERMACS dataset to predict risk of both ischemic and hemorrhagic stroke after continuous flow LVAD (CF-LVAD) implant and understand the pre-implant risk drivers.

Methods: The dataset includes 3,574 patients from INTERMACS who had a primary LVAD from April 2014 - March 2015. Variables were selected for inclusion in the models based on a chi-sq analysis. The final dataset consisted of 60 pre-implant variables and event of stroke, including hemorrhagic and ischemic, post-implant was predicted for 90 days and 1 year after implant. The model was developed using a Tree Augmented Naïve Bayesian analysis and was assessed with a 5-fold cross validation and test validation with a separate data set.

Results: The stroke prediction models identified key hemodynamics, demographics, medications, and lab results as contributing factors that drove risk of stroke at both time points. Validation with the 5-fold cross validation showed great predictive accuracy at 93 and 88%, for 90 days and 1 year.

Conclusion: By using a Bayesian approach, we were able to derive accurate, clinically relevant models that reveal predictive pre-implant factors for stroke. These models may be utilized to identify optimal candidates for LVAD implantation with lower risk of stroke.

Evaluation of Viability, Structural Integrity and Functional Return after Whole Eye Transplantation

Lin He (1), Maxine R. Miller (1), Yang Li (1), Chiaki Komatsu (1), Hongkun Wang (1), Liwei Dong (1), Bo Wang (3), Yolandi van der Merwe (4), Edward H. Davidson (1), Mario G. Solari (1), Shuzhong Guo (2), Jeffrey L. Goldberg (5), Larry Benowitz (6), Valeria Fu (3), Gadi Wollstein (3), Joel S. Schuman (3), Kevin C. Chan (3), Vijay S. Gorantla (1), Kia M. Washington (1,7)

(1) Dept. of Plastic Surgery, UPMC, Pittsburgh, PA (2) Dept. of Plastic Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, China, (3) UPMC Eye Center, Eye and Ear Institute, Ophthalmology and Visual Science Research Center, University of Pittsburgh SOM, Pittsburgh, PA, (4) Dept. of Bioengineering, Swanson School of Engineering, University of Pittsburgh, PA (5) Dept. of Ophthalmology, Shiley Eye Center, University of California San Diego, La Jolla, CA (6) Dept. of Neurosurgery, Harvard University, Boston, MA (7) VA Pittsburgh Healthcare System, Pittsburgh, PA

Purpose: Whole eye transplantation offers the opportunity to provide retinal ganglion cells and an entire optical system to recipients with vision loss. The purpose of this study is to evaluate the viability, structural integrity and function of our whole eye transplant model.

Methods: Syngeneic transplants were performed in 5 Lewis rats. MRI Protocols: Rats were intraperitoneally injected with 0.3mmol/kg Gd-DTPA (Magnevist). Four animals were scanned at 3 weeks and 1 animal was scanned at 10 weeks after transplantation using a 9.4-T Varian/Agilent scanner. The aqueous humor dynamics using gadolinium (Gd)-enhanced MRI, optic nerve structural integrity with diffusion tensor MRI (DTI). ERG Protocol: Rats were housed in a dark box overnight. Diagnosys and reference electrodes were placed and light stimuli were delivered and responses recorded.

Results: At 3 weeks after transplantation, the right anterior chamber (AC) had a similar time to peak but a significantly lower peak intensity and lower initial increase rate than left. At 10 weeks, the right AC had comparable peak intensity to left. Limited Gd enhancement was observed in the vitreous with no significant difference. T2-weighted images showed the donor optic nerve had comparable morphology with the uninjured intraorbital optic nerve 3 weeks after transplantation, whereas in the prechiasmatic optic nerves, DTI quantitation of the right injured optic nerve showed significantly lower fractional anisotropy and axial diffusivity by 54±6.1% and 24.9±5.7%, respectively, and a significant increase in radial diffusivity by 83±29.5% compared to the left uninjured optic nerve ($p<0.05$). ERG revealed the lack of an electrical response in the transplanted eye to light stimuli.

Conclusion: Our whole eye transplant model revealed the presence of aqueous humor dynamics and preserved integrity of blood-ocular barriers after transplantation. Future DTI and ERG studies will examine approaches for regaining neuronal structure and function of our whole eye transplant model.

Degradation Characterization of Acellular Porous Tissue Engineered Vascular Grafts

Piyusha Gade (1), Keewon Lee (1), Blaise Pfaff (6), Yadong Wang (1,2,3,4,5), Anne M. Robertson (1,2,5)

(1) Department of Bioengineering, (2) Department of Mechanical Engineering and Materials Science, (3) Department of Chemical Engineering, (4) Department of Surgery, (5) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsbburgh, PA, (6) Department of Chemical Engineering, University of Pennsylvania, Philadelphia, PA

With over 300,000 small caliber replacement surgeries performed annually in the United States, cell-free tissue engineered vascular grafts (TEVGs) represents an emerging technology that provides an alternate solution to the problems faced by autografts and cell-seeded TEVGs (Kannan et. al, 2005). Growth and remodeling (G&R) computational tools (Miller et. al, 2014) are used to guide rational design of these TEVGs since much of TEVG development is otherwise based on a trial and error investigation.

TEVG to neoartery transformation hinges on the interplay between PGS degradation and cell proliferation with extracellular matrix (ECM) deposition. Since it has been postulated that one of the main mechanisms of ECM deposition is through the stress driven mechanobological response of synthetic cells, accurate modeling of load transfer from the graft to cells is crucial. However, currently there is little experimental data to guide even the qualitative features of the degradation models needed for such analysis. This work aims to characterize the degradation mechanism of the porous poly-glycerol sebacate (PGS) core of a bi-layered composite graft which has shown mature elastin and nerve regeneration in small animals (Allen et. al, 2014). This data will be used to improve the predictive capability of the G&R computational tool and aid in TEVG design.

The results show nearly linear rate of mass loss with almost 85% loss at day 21 for enzymatic degradation. This is higher than the 70% mass loss reported at day 35 for solid PGS (Wang et. al, 2003) and departs from the commonly assumed sigmoidal form in models. An exponential loss in modulus (with about 40% loss at day 4) is also observed which suggests rapid transfer in load to incoming cells and to the outer PCL sheath. This investigation provides the much needed data to drive predictive modeling using G&R theories.

Identification and Characterization of a Defect in Liquid-Solute Transport and Junctional Integrity in Airway Epithelia with Cystic Fibrosis

Matthew R Markovetz (1), Timothy E Corcoran (1,2,6), Stefanie Brown (2), Carol A Bertrand (7), Michael M Myerburg (2), Joseph M Pilewski (2), Robert S Parker (1,3,4,5)

(1) Dept. of Chemical and Petroleum Engineering, Swanson School of Engineering, (2) Pulmonary, Allergy, and Critical Care Medicine, (3) Cell Biology, (4) McGowan Institute of Regenerative Medicine, (5) Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) Laboratories, Dept. of Critical Care Medicine, (6) Univ. of Pittsburgh Cancer Institute, (7) Dept. of Bioengineering, Swanson School of Engineering, Univ. of Pittsburgh, Pittsburgh, PA

Respiratory health and function in the lung rely on proper performance of the airway liquid regulatory mechanisms present in human airway epithelia. In patients with Cystic Fibrosis (CF), absence or dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein leads to airway surface liquid (ASL) hyperabsorption. This hyperabsorption increases mucus viscosity, reduces mucociliary clearance, and subsequently promotes the chronic infections and inflammation and airway damage that are the hallmarks of CF lung disease. There is, however, a lack of consensus about how these transport processes interact and contribute to CF lung disease. Identifying these key contributors to airway disease is essential for identifying new therapies and optimizing optimal CF treatment plans.

We have developed a cell-scale compartmental ordinary differential equation (ODE) model in order to characterize liquid and solute transport at the airway epithelium. This model accounts for the transport of liquid water, ions, and solutes, including the radiopharmaceutical probe Tc99m-DTPA, a surrogate marker for ASL absorption in vivo, between apical, cellular, and basolateral compartments. We have previously trained and validated the parameters of our model and its ability to capture the dynamics of liquid and DTPA absorption by measuring the response in CF and non-CF cell primary human bronchial epithelial cell cultures to apical volume addition. Here we describe model predictions of increased liquid and DTPA transport by CF epithelia via paracellular pathways. This effect was verified experimentally by applying transepithelial osmotic gradients to CF and non-CF epithelia and measuring DTPA transport. These data suggest a possible defect in the integrity of the tight junction in CF. Targeting this defect may allow for the development of more effective therapies directed at maintaining proper airway hydration for patients with CF.

Cell type functional connectivity during eye movement planning

Sanjeev Khanna (1,2,3), Adam Snyder (1,3), Matt Smith (1,2,3,4)

(1) Dept. of Ophthalmology, (2) Dept. of Bioengineering, (3) Ctr. for Neural Basis of Cognition, (4) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Pairs of nearby neurons exhibit correlated spiking activity. There is a strong link between the correlation among neurons and the amount of information that can be represented in a neuronal population. We investigated correlation, by measuring functional connectivity, between neurons in the frontal eye fields (FEF), a part of prefontal cortex that has both visual and motor functions. FEF neurons respond to visual stimuli (visual neurons), eye movements (motor neurons), or both (visuomotor neurons). Determining the connectivity between these cell classes will lead to a better understanding of the circuits that bridge the sensory and motor divide. We investigated the functional connectivity of FEF neurons, predicting certain relationships between connectivity and neuronal properties would be consistent with those found in visual cortex, reflecting a conserved architecture across neocortex. We also predicted functional connectivity to be low between visual and motor neurons, reflecting a compartmentalization of visual and motor circuits in FEF. We used a laminar probe to record from groups of FEF neurons in alert rhesus macaque monkeys performing memory guided eye movements, and measured neuronal correlation on both short and long time scales. We found that the structure of neuronal correlation in FEF mirrored that seen previously in visual cortex. Additionally, correlated spiking activity was strongest in pairs of neurons with similar response properties, and depended on the planned eye movement direction. Future work will be aimed at understanding how population activity in FEF is modulated during active visual perception.

Towards the Development and Validation of an Electrophysiological Model of Primary Human Bronchial Epithelial Cells

Florencio Serrano-Castillo (1), Timothy Corcoran (1,2,3), Carol Bertrand (4), Michael Myerburg (2), Joseph Pilewski (2), Robert Parker (1,4,5,6,7)

(1) Department of Chemical and Petroleum Engineering, (2) Division of Pulmonary, Allergy, and Critical Care Medicine, (3) Department of Bioengineering, (4) Department of Pediatrics, (5) McGowan Institute for Regenerative Medicine, (6) Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) Laboratories, (7) University of Pittsburgh Cancer Institute

The airway epithelia is responsible for a wide range of functions, including water transport, electrolyte balance, and pH regulation. These functions are performed by a multitude of ion channels, transporters, and other proteins that comprise a very tightly regulated network. Dysregulations in this network can have significant repercussions, as is the case in Cystic Fibrosis (CF). CF is caused by a mutation in the CFTR gene, which codes for a Chloride ion channel localized on the apical side of the epithelium. The dysfunction or absence of this channel causes an electrolyte imbalance that leads to hyperabsorption of water, mucus dehydration and failed clearance, chronic infections, and respiratory failure. This makes understanding the complex interactions of the airway epithelium a great priority.

The present work focuses on the development, and validation, of a dynamic model of electrolytes and water transport across the airway epithelia. The model is informed by traditional electrophysiological measurements made on primary human bronchial epithelial (HBE) cell cultures. All measurements were performed in an Ussing chamber; this technique is routinely used in the characterization of intact epithelia, primarily due to its simplicity and versatility. Modeling airway epithelium based on measurements obtained from this technique also facilitates the implementation of the model as a tool to help us better inform future experimental inquiries. In this manner, the model can provide a new level of resolution to routine experiments by letting us dissect the individual contributions of different players in the epithelial network and yielding insight into the subtle differences in the way that HBE cultures respond to different challenges and therapeutics.

Modeling Platelet Dynamics in Critically Ill Patients

Michelle A. Pressly (1), Robert S. Parker (1,2,3,4), Gilles Clermont (1,2,4,5,6)

(1) Dept. of Chemical and Petroleum Engineering, Swanson School of Engineering, (2) Dept. of Critical Care Medicine, School of Medicine, (3) Dept. of Bioengineering, Swanson School of Engineering, (4) McGowan Institute of Regenerative Medicine, University of Pittsburgh Medical Center, (5) Dept. of Industrial Engineering, Swanson School of Engineering, (6) Dept. of Mathematics, Dietrich School of Arts and Sciences

Platelets play a significant role in cardiovascular health and disease, particularly in critical care. Patients may be at significant risk for bleeding or clotting events leading to organ damage. To mitigate this risk, transfusions or heparin are often administered. For example, heparin-induced thrombocytopenia (low platelet count), experienced by 1-5% of patients, is a condition that can lead to life-threatening clotting events. Disseminated intravascular coagulation (DIC), also manifested by thrombocytopenia, leads to uncontrolled bleeding. The resulting clinical challenge lies in early detection of thrombocytopenic phenotypes and other clotting abnormalities more commonly seen in the critically ill.

The thromboelastogram (TEG) is a viscoelastic assay of clot formation dynamics obtained by taking a blood sample and applying forces that simulate sluggish blood flow and testing the strength of the formed clot. Additionally, it serves as a common clinical measurement in critical care. Using TEG tracings and a compartmental model structure, we can capture platelet response given initial concentrations of clotting factors, platelets, and fibrinogen. The model will use dynamic mass balances and treat the TEG sample as a batch reactor where reactions between the clotting factors, fibrinogen, and platelets, lead to clot formation on the TEG wire, and ultimately a TEG trace. The kinetics of the reactions will be modeled as either bilinear or Hill-type reactions. The model will be constructed in Pyomo and parameters will be estimated from TEG tracing data using IPOPT. Analysis of the resulting model, including its parameterization and initial conditions, may identify different disease endotypes of platelet dynamics in critically ill patients. Furthermore, incorporating this type of model into the TEG tracing analysis provided to the clinician may provide a deeper mechanistic understanding of expected treatment responses in critical care patients.

A study of the mechanical properties and fiber structure in rabbit and human aneurysms

Chao Sang (1), Xinjie Duan (1), David Kallmes (2), Ram Kadirvel (2), Yonghong Ding (2), Daying Dai (2), Khaled Aziz (3), Juan Cebral (4) and Anne Robertson (1)

(1) Department of Mechanical Engineering & Materials Science, University of Pittsburgh, Pittsburgh, PA, (2) Department of Radiology, Mayo Clinic, Rochester, MN, (3) Department of Neurosurgery, Allegheny General Hospital, Pittsburgh, PA, (4) Department of Bioengineering, George Mason University, Fairfax, VA

An intracranial aneurysm (IA) is a saccular enlargement in the wall of a cerebral artery. As the aneurysm grows there is a greater risk of rupture – this can lead to severe hemorrhage, and other complications, including sudden death. Due to the risks associated with treatment of an IA, it is important to be able to identify which aneurysms are at risk for rupture and treat those while avoiding treatment in others. The most reliable indicator of risk is the size of IA at present. However, the majority of ruptured aneurysms are small, due to the large number of small aneurysms. Hence, size is insufficient for risk stratification.

Enhanced understanding of the evolving wall composition and structure in time, will contribute both to improved risk stratification as well as provide knowledge critical for developing new treatments. Resected tissue from human aneurysms is extremely valuable, but only represents one time point in the pathology. Animal models for IAs provide a means of evaluating the wall progression in time. However it is vital to prove the relevance of these models. In earlier work, we have shown the relevance to human IAs of the geometry and flow for this model. Here, we address the similarity in uniaxial mechanical properties between the aneurysm wall of the rabbit model and eight unruptured human IAs in our earlier work. We include results for the changing collagen architecture during loading, assessed using multi-photon microscope during mechanical loading.

Optimal Stratification for Patients with Traumatic Injury

Qi Mi (1,3), Rami Namas (2), Dongmei Liu (2), Jason Sperry (2), Yoram Vodovotz (2,3) and Timothy Billiar (2)

(1) Departments of Sports Medicine and Nutrition, (2) Department of Surgery and (3) Center for Inflammation and Regenerative Modeling, McGowan Institute for Regenerative Medicine, University of Pittsburgh

BACKGROUND:Traumatic injury is one of most prevalent health problems in the US, accounting for 41 million emergency department visits and incurring $585 billion annually. Prolonged intensive care unit (ICU) stay, multiple organ dysfunction syndrome (MODS), and nosocomial infection (NI) are the major adverse post-trauma outcomes. The development of a strategy to optimize patient stratification may help to identify patients prone to develop MODS and NI as well as allocation of resource intensive care.

METHODS: A cohort of 376 blunt trauma survivors with available MODS scores from day 2 - 5 post-admission were studied. Data mining analyses were performed to explore the relationships between various MODS calculations and adverse outcomes. Area under the curve (AUC) for the percentage of developing adverse outcomes over different MODS values was calculated to select the optimal MODS calculation. The best cut-off values was subsequently determined by Decision List analysis which could segregate the largest sample size and highest likelihood of developing adverse outcomes.

RESULTS: MODS score based on the average values from day 2-5 shows the maximum AUC for outcomes such as NI, ICU length of stay (LOS), total hospital LOS, and disposition. Decision List analysis suggested a MODS cut-off value of 3 by which 2 groups were identified, 304 patients (81%) with MODS ≤ 3 and 72 patients (19%) with MODS > 3. This analysis revealed that patients with MOD >3 had higher incidence of NI (61% vs 24%, p< 0.0), longer ICU LOS (mean 16.3 vs 5.9 days, p<0.0) and less chance to discharge to home (23.6% vs 48.4%, p<0.0). For patients with MODS ≤ 3, patients with NI had higher MODS score compared to no-NI patients (mean 1.3 vs 0.7 p<0.0).

CONCLUSIONS: Data mining analysis may provide a quantitative tool to identify trauma patients prone to develop adverse outcomes.

Predicting neural tissue insult in the lamina cribrosa due to intraocular pressure using a microstructure based finite element model

Andrew P. Voorhees (1), Ning-Jiun Jan (1,2), John G. Flanagan (3), Jeremy M. Sivak (4), Ian A. Sigal (1,2)

(1) Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA, (2) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, (3) Department of Optometry and Vision Science, University of California Berkeley, Berkeley, CA, (4) Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, ON, Canada

Purpose: To study how the microarchitecture of the lamina cribrosa (LC) affects the mechanical insult to retinal ganglion cell (RGC) axons as they exit the eye. We developed two models, a simplified homogeneous model (HM) and a specimen-specific, microstructure-based model (uM). The uM included the mechanics of neural tissue and captured the non-linearity, anisotropy, and inhomogeneity of the LC beams and sclera with detail that previous models have not considered.

Methods: A 30 um thick section of a sheep optic nerve head was imaged using polarized light microscopy from which collagen fiber density and orientation were obtained. A highly detailed finite element model (average element side length under 6um) of a small temporal region of the LC and sclera was created. For uM, material properties were assigned according to the observed architecture, whereas HM had homogenized sclera and LC regions. We simulated a 2% scleral canal expansion, corresponding to a 5 mmHg increase in intraocular pressure (IOP).

Results: In HM, maximum stretch was 3% and occurred at the peripheral LC. Stresses in the LC of HM were highly uniform. The distribution of tensile stretch in the neural tissue pores of uM had a larger median and variance as compared to the collagen beams of uM and the homogenous LC of HM. Mean stretch in some pores was more than 3 times that in neighboring pores and sometimes exceeded 30%. Stresses in uM were concentrated in the collagen beams.

Conclusions: Our uM demonstrates that small changes in IOP can cause large deformations in RGC axons. Our model predicts large pore-to-pore variance in stretch, agreeing with recent experimental findings. This phenomenon is not explained by previous models. Microstructure-aware modeling improves our understanding of how neural tissues suffer mechanical insult due to elevated IOP and provides insight into the development of glaucoma.

Computational Fluid-Poroelastic Structure Interaction in Arteries

Rana Zakerzadeh (1), Paolo Zunino (2)

(1) Computational Modeling and Simulation PhD program, (2) Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA

Considering arterial wall as an elastic structure is a common assumption in Fluid-Structure Interaction simulations. However; it neglects realistic arterial wall model. In reality, arterial wall like other soft tissues is viscoelastic and it shows poroelastic behavior as well. The present study attempts to investigate the effect of both poroelasticity and tissue viscoelasticity on fluid-structure interaction in arteries and analyze the role of extracellular fluid flow in the apparent viscoelastic behavior of the arterial wall.

We discuss a computational framework for modeling multiphysics systems of coupled flow and mechanics problems via finite element method. Blood is modeled as an incompressible, viscous, Newtonian fluid using the Navier-Stokes equations and the arterial wall consists of a thick material which is modeled as the Biot system. Physically meaningful interface conditions are imposed on the discrete level via mortar finite elements or Nitsche's coupling. We discuss stability of the loosely coupled non-iterative time-split formulations and the use of the loosely coupled scheme as a preconditioner for the monolithic scheme. Energy estimates are derived for each constitutive model of the arterial wall from the weak formulation of the fluid/solid coupled problem and are applied to assess the distribution and dissipation of the energy delivered to the artery during one heart cycle. We further investigate the interaction of an incompressible fluid with a poroelastic structure featuring possibly large deformations. The numerical results investigate the effects of poroelastic parameters on the pressure wave propagation in arteries, filtration of incompressible fluids through the porous media, and the structure displacement.

Identifying Disease Endotypes and Time of Onset from Interleukin-6 Trajectories in Septic Patients

Li Ang Zhang (1), Robert S. Parker (1,3,4), Ipsita Banerjee (1,3,5), Gilles Clermont (1,2,3,4)

(1) Department of Chemical and Petroleum Engineering, (2) Department of Critical Care Medicine, (3) CRISMA Center, (4) McGowan Institute for Regenerative Medicine and (5) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

Sepsis is a syndrome characterized by a severe and widespread inflammatory response to pathogen in the body. The high inter- and intra-patient variability of this syndrome as well as the myriad of confounding factors has made sepsis difficult to treat. Despite this, serum cytokine interleukin-6 (IL-6) levels in sepsis always seem to follow a distinctive shape and are similar across patients. We sought to cluster patients with similar IL-6 trajectories in order to identify different endotypes of sepsis and subsequently to identify time of sepsis onset.

164 IL-6 trajectories from the ProCESS Sepsis trial were analyzed. Measurements were taken at 0, 6, 24, and 72 hours post trial enrollment. Patients with IL-6 trajectories below the lower limit of detection and/or missing measurements were excluded. The treatment arms of the trial were not taken into consideration.

Each IL-6 trajectory was modeled as a step response to a second order differential equation. Equations were generated to represent under-damped and over-damped systems to capture behaviors such as oscillations and overshoot. Once model parameters had been fit, they were clustered using a Gaussian mixture model. The centers of each cluster generated a characteristic IL-6 response to sepsis (a master curve) for that sub-population. Additionally, this master curve informed the absolute “disease time” of a patient relative to their onset of sepsis.

Statistical testing of clinical biomarkers in disease time within each cluster revealed significant differences in cardiovascular and kidney function biomarkers. Furthermore, several clusters exhibited high mortality, suggesting the existence of higher-risk sepsis endotypes. The knowledge of disease time as well as endotype risk can be used to guide treatments in order to shift sepsis patients towards better outcomes.

Posters
Medical Devices

#	*Presenter*
56	Elena Bellotti
57	Jacqueline Bliley
58	Patrick Bosch
59	Liza Bruk
60	Bin Cao
61	Patrick Cody
62	Da-Tren Chou
63	Dan Crompton
64	Luis De La Torre
65	Xuan Ding
66	Firuz Feturi
67	James Eles
68	Joerg Gerlach
69	Garrett Jeffries
70	Shawn Kelly
71	Nina Reger
72	Ross Lawrence
73	Elaine Soohoo
74	Shalv Madhani
75	Xin Zheng
76	Alexandra May
77	Salim Olia
78	Avinash Patil
79	Mitali Patil
80	Takashi Kozai
81	Tanchen Ren
82	Cuneyt M.
83	Alkiviadis Tsamis
84	Jingyao Wu
85	Qinghao Zhang
86	Alexander Malkin
87	Brian Martin

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

Real time in vivo MRI for the monitoring of long term delivery of glaucoma treatment

Elena Bellotti (1), Morgan V. Fedorchak (1,2,3,4), Ian P. Conner (2), Kevin C. Chan (2,3,4,5), Tim Knab (1), Anthony Cugini (5), Joel S. Schuman (6), Steven R. Little (1,2,4,5,7)

(1) Dept. of Chemical Engineering, Swanson School of Engineering, Univ. of Pittsburgh, Pittsburgh, PA, (2) UPMC Eye Center, Ophthalmology and Visual Science Research, Dept. of Ophthalmology, Univ. of Pittsburgh SOM, Pittsburgh, PA, (3) Louis J. Fox Center for Vision Restoration of UPMC and Univ. of Pittsburgh, Pittsburgh, PA, (4) The McGowan Institute for Regenerative Medicine, (5) Dept. of Bioengineering, Swanson School of Engineering, Univ. of Pittsburgh, Pittsburgh, PA, (6) Dept. of Ophthalmology, New York Univ. (7) Dept. of Immunology, Univ. of Pittsburgh, Pittsburgh, PA

Glaucoma is the leading cause of blindness in industrialized countries. One of the biggest challenges associated with the development of new drug formulation for treatment of this pathology is the measurement of the biodistribution of the released drug directly in vivo, which is a requirement of FDA. An accurate method of measuring ocular drug distribution would allow for determination of the real amount of drug administrated to the target tissue, reducing potential systemic side effects. However, today the primary limitation in the investigation of spatiotemporal drug distribution in vivo is the large number of sacrificed animals needed to determine the fate of the released active agent.

Our goal is to develop and optimize a new and non-invasive method for monitoring the release of a hypotensive drug and its distribution into the target tissue in live animals over a treatment period of one month. For this purpose, we propose to use MRI to investigate in real time in vivo release of Gd-DOTA, a commonly used contrast agent, as a surrogate of the anti glaucoma drug brimonidine tartrate (BT), given its similar size and ocular half life. Gd-DOTA was first encapsulated into gel/PLGA-based microparticle eye drops originally designed to produce a specific BT release profile. In vitro release studies with these formulations confirm that Gd-DOTA release behavior is similar to BT-release. Subsequently, preliminary in vivo studies were performed based on a post mortem model for scanning rabbits after administration of Gd-DOTA-loaded microparticles. Ongoing studies include modifying Gd-DOTA release to increase the duration of the delivery up to one month and to include zero order release kinetics through geometry/size and microstructure of the controlled release microspheres. These formulations will then be utilized for in vivo MRI studies. Eye drops containing contrast agent will be administrated by subconjunctival injection (to facilitate retention) and optimization of the acquisition methods and on the number of time points to use will be performed. Finally, preliminary scans to determine the most appropriate quantification method will be carried out. The key aspect of this study will be real-time in vivo scans of live animals to investigate the spatial and temporal distribution of the Gd-DOTA released from gel/microparticle eye drops.

Evaluation of Clinical Alternatives to Nerve Autograft in Long Gap Median Nerve Defects

Jacqueline Bliley (1), Matthias Waldner (1,2), Gabriella Dibernardo (1), Ryan Schroth (1), Chris Mahoney (1), Damian Grybowski (1), Debra Bourne (1), Isaac James (1), Tyler Simpson (5), Casey Tompkins-Rhoades (1), Anna Taylor (1), Aaron Dees (1), Donald Crammond (3), Kacey G. Marra (1,5,6)

(1) Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, (2) Department of Plastic Surgery and Hand Surgery, University Hospital Zürich, Switzerland, (3) Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA, (5) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, (6) McGowan Institute for Regenerative Medicine

Background: Standard treatment for peripheral nerve injuries involves excising a donor nerve and transplanting it into the defect (i.e. an autograft). Donor site morbidity, including numbness and the formation of neuromas, is a disadvantage of autografts. Limited donor nerve availability also complicates situations where multiple injuries are sustained. Current clinically available nerve guides do not actively support regeneration through growth factor delivery. We are investigating a poly(caprolactone) (PCL) nerve guide combined with a local neurotrophic factor delivery system as well as a clinically available decellularized nerve allograft in non-human primates (NHPs) to support regeneration over long gaps (>3 cm).

Methods: 5-cm median nerve defects were repaired with autograft, decellularized nerve allograft, a poly (caprolactone) (PCL) conduit, or a PCL conduit with glial cell line-derived neurotrophic factor (GDNF) microspheres. NHPs were trained to retrieve treats from a Klüver board. Successful retrieval percentage was recorded. Intraoperative explant nerve conduction velocity (NCV), muscle evoked potentials (MEPs), sensory nerve action potentials were obtained. Nerve explants were also evaluated for Schwann cell and nerve fiber density.

Results and Conclusions: Prior to the operation, NHPs utilized a thumb and forefinger pinch 70-80% of the time. A trend towards increased functional recovery was observed in the GDNF-treated and decellularized nerve groups compared to our negative control PCL/empty microsphere group. Nerve conduction and MEPs were evident at one year in both PCL/GDNF and decellularized nerve groups suggesting that both treatments are able to regenerate nerve across the 5-cm gap and support muscle reinnervation. However, increased nerve conduction and MEP amplitude were seen in the autograft group (40% of baseline for NCV and 50% of baseline for MEP). No significant differences were observed in Schwann cell density between autograft, PCL/GDNF, or decellularized nerve; however, significantly more nerve fibers were evident in the autograft and decellularized nerve groups compared to PCL/GDNF.

Athletic shoe-wear for adolescents with Sever disease

Patrick Bosch (1)

(1) Department of Orthopaedic Surgery, Children's Hospital of Pittsburgh of UPMC

Adolescent children between the ages of 8 and 14 years will often have irritations of growth plates (physes) with activities. Calcaneal apophysitis, Sever's disease, is one such irritation in the heel. It is particularly common in children wearing cleats for soccer, baseball or football. There are no shoes designed to accommodate the sensitivity of a growing physis. A design of cleats to compromise between athletic performance and comfort for this age group could reach a significant demographic. These children are participating in higher numbers and obviously outgrow and update shoes more than adult athletes. A cushioned calcaneal apophysis and suspensory system to relieve areas at risk in the growing foot could be developed.

Autonomous Topical Drug Delivery to the Middle Ear

Liza Bruk (1,2), Cuneyt Alper (3) and Morgan Fedorchak (2,4,5,6,7)

(1) Department of Bioengineering, (2) Ophthalmic Biomaterials Laboratory, (3) Department of Otolaryngology, (4) Department of Ophthalmology, (5) Department of Chemical Engineering, (6) Clinical and Translational Science Institute and (7) Louis J Fox Center for Vision Restoration

One-third of children experience six or more cases of acute otitis media (AOM) – middle ear infection – before the age of seven, accounting for over 20 million pediatric physician visits annually in the United States alone. Existing treatments of systemic antibiotics and ear drops have low efficiency due to low patient compliance, difficulty of administering drug to the middle ear, and the potential of harmful side effects. This study focuses on developing and testing a safe and simple method of transporting drug across the ear drum. Only a single drop is needed for treatment, treatment is localized to the middle ear, and applied drug concentration is minimized, thus mitigating side effects. A topical controlled release system may significantly improve patient care in treatment of AOM.

Previous studies have shown hydrogel-based drug delivery to the ear is possible with intratympanic injection or with the help of chemical permeation enhancers. However, these approaches come with risk of side effects. This group has already shown success in topical treatment of ocular diseases using a controlled release system of drug-loaded microspheres applied to the target area via a hydrogel drop depot.

Fabrication of microspheres and hydrogel are modified from previous studies focused on treating ocular diseases with a similar system. Microspheres are fabricated via a standard double emulsion procedure using a polymer that is commonly used in FDA approved biomedical applications - poly(lactic-co-glycolic) acid. Drug is loaded into the microspheres during the fabrication process. A reverse thermoresponsive hydrogel is fabricated from N-isopropylacrylamide via a procedure previously developed by the group.

Microspheres are characterized by scanning electron microscopy and volume impedance measurement. Release profiles are determined by measuring drug release using both spectrophotometry and high-performance liquid chromatography. Ex vivo studies determine permeation of drug across the tympanic membrane and in vivo studies determine pharmacokinetics and toxicity.

A platform copolymer coating strategy for biomedical devices

Bin Cao (1), Xinyan Tracy Cui (1,2,3)

(1) Department of Bioengineering, (2) Center for the Neural Basis of Cognition, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Surface chemistry plays a key role in mediating interactions between biological environment and biomedical devices. For most biosensors and diagnostic devices, to improve their performance and longevity, it is very important to have a surface coating that can promote specific interactions for the device while reducing non-specific interactions with proteins and cells in the complex media. We have developed a platform copolymer coating strategy with both a hydrophilic zwitterionic domain (either polycarboxybetaine (PCB) or polysulfobetaine (PSB)) to reduce non-specific biofouling on the surface of the device, and an bioactive epoxide (GMA) domain for further conjugation with biomolecules to promote specific bindings. The copolymers were prepared by a solution atom-transfer radical-polymerization (ATRP) from an initiator with a biomimetic catechol end group for surface adhesion. The ratio between zwitterionic domain and epoxide domain can be easily tuned by adjusting initial feeding ratio of each component. Surface coating was realized by a dip-coating method. This coating strategy has been successfully applied to many applications, including various implantable neural electrodes and biosensors.

Poster number 60 *Category: Medical Devices*

Neural cell adhesion molecule, L1, coated neural probes for improved chronic recordings

Patrick Cody (1), Takashi D. Y. Kozai (1), Carl Lagenaur (2), X. Tracy Cui (1)

(1) Department of Bioengineering, (2) Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA

Neural probes are used in brain-machine interface based prosthetics to restore movement to paralyzed individuals. Intracortical neural electrodes provide the greatest spatiotemporal resolution compared to other recording approaches to enable optimal decoding of neural activity for prosthetic control. Inflammatory brain responses including glial scarring and neural degeneration degrade and limit the longevity of recordings thereby severely hindering the clinical potential of neural probes. Our lab has demonstrated that the neural cell adhesion protein, L1, may be covalently conjugated to silicon neural probe surfaces to reduce inflammatory glial activation, improve neuron survival and enhance neurite outgrowth throughout a chronic period in a rat model as verified with quantitative histological measures. In this work, we employ a repeatable and established visual stimulation paradigm (developed in house) to compare chronic electrophysiological recordings from L1 coated and uncoated parylene-C insulated 4x4 Utah arrays implanted in the rat primary monocular visual cortex. In addition to a battery of histological analyses, laser capture micro-dissection to assay RNA expression changes in the immediate micro vicinity of the probe is developed to better elucidate the mechanism of L1's benefits. Recording performance from L1 coated implants will be quantified with single-unit yield (percent of electrode sites recording single-units), single-unit signal-to-noise amplitude ratios, and multi-unit signal-to-noise firing rate ratios. Understanding the effect and mechanism by which L1 improves neural probe performance will inform approaches to better realize the full clinical potential of neural prosthetics for treating paralysis.

In vivo degradation, bone healing, and biocompatibility of novel Mg alloy pins in a rat femoral osteotomy model

Da-Tren Chou (1,2,4,*), Daeho Hong (1,2,4,*), Sinan Oksuz (5), Riccardo Schweizer (6), Abhijit Roy (1,2,4), Boeun Lee (1), Vijay Gorantla (2,3), Prashant N. Kumta (1,2,4) (* These authors contributed equally to the work)

(1) Dept. of Bioengineering,(2) McGowan Institute for Regenerative Medicine, (3) Dept. of Plastic Surgery, Univ. of Pittsburgh, Pittsburgh, PA, (4) NSF-ERC for Revolutionizing Metallic Biomaterials, (5) Gulhane Military Medical Academy, Dept. of Plastic and Reconstructive Surgery, Istanbul, Turkey, (6) Dept. of Plastic Surgery and Hand Surgery, Univ. Hospital Zurich, Switzerland

Biodegradable magnesium (Mg) alloys are a promising new class of biomaterials which exhibit higher mechanical properties than degradable polymers while degrading over time to circumvent complications associated with permanent metals and the accompanying need to be removed in secondary surgeries. These advantages of Mg alloys make them especially attractive for orthopedic applications wherein degradable devices are necessary to be harnessed to fix fractured bone, providing the desired stability while they heal. This pilot study aims to investigate the safety and efficacy of novel Mg-Zn-Sr-Zr and Mg-Y-Zn-Ca-Zr alloy pins to fix a full osteotomy created in rat femurs. Sharpened pins of the Mg alloys and the control material Ti-6Al-4V were inserted into the intramedullary cavity after an osteotomy was created in the midsection of the femurs. To assess the systemic toxicity, blood cell counts and serum biochemical tests were performed after 2 and 14 weeks implantation. Livers and kidneys were also harvested to observe histomorphological changes and the accumulation of the various alloying elements using ICP-OES. Hard and soft tissue adjacent to the fracture site was also examined using Goldner's Trichrome and ALP staining. Degradation behavior of the Mg alloys was determined using μCT to measure their degradation rate and the morphology of surrounding bone. Blood testing exhibited no significant difference changes due to the implantation of the Mg alloys compared to the control groups. No changes in the morphology of liver and kidney tissue as well as no accumulation of degradation products were observed. Corrosion occurred gradually, with degradation rate slowing after 2 weeks with points of high stress observed near the fracture site resulting in stress corrosion cracking. Nevertheless, normal bone healing was observed in femurs fixed with Mg alloy and Ti-6Al-4V pins confirmed by the presence of osteoids, osteoblast activity, and new bone formation. These results thus demonstrate the feasibility of degradable Mg alloys as a viable candidate for orthopedic and craniofacial fracture fixation applications. Results of these studies will be presented and discussed.

Modulation of rigid RBC traffic to capillaries using soluble drag reducing polymer additives to blood: potential treatment for SCD

D. Crompton (1,2), S. Gudla (1,2), M.A. Jimenez (1,4), P. Sundd (1,4,5) and M.V. Kameneva (1,2,3)

(1) Department of Bioengineering, University of Pittsburgh, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, (3) Department of Surgery, University of Pittsburgh, (4) Vascular Medicine Institute, University of Pittsburgh, (5) Pulmonary Allergy and Critical Care Medicine, University of Pittsburgh

Sickle cell disease (SCD) is an inherited hemolytic disorder that alters hemoglobin within red blood cells (RBCs), causing them to become 'sickle' shaped and rigid. Sickled RBCs (S-RBCs) occlude microvessels leading to vaso-occlusive pain crisis (VOC). Although VOC is the primary reason for emergency medical care by SCD patients, the current treatment is limited to chronic hydroxyurea (HU) and blood transfusion, which is often associated with serious complications such as alloimmunization, and HU related toxicity. We propose to investigate a novel rheological approach which would enable the modulation of RBC traffic through small microvessels. Our approach would prevent VOC by bypassing S-RBCs past the capillary network, thus reducing the number of S-RBCs entering capillaries.

In microvessels with diameters below ~300 micron, deformable RBCs tend to move toward the vessel center creating a cell-free layer near the vessel wall, leaving less- or non-deformable cells such as S-RBCs closer to the vessel wall. This results in an uneven RBC distribution and an overall increase in number of S-RBCs entering capillaries. It was previously demonstrated that nanomolar concentrations of soluble high molecular weight (> ~106 Da) molecules (so-called drag reducing polymers or DRPs) provide equal distribution of normal RBCs across the vessel lumen and can thus reduce the relative number of S-RBCs near the vessel wall. Our study investigates the DRP effect on flow of RBC suspensions at 20-30% hematocrit in bifurcating microchannels. Preliminary studies demonstrated the existence of the cell-free layer resulting in a hematocrit decrease in samples collected from branch outlets. Further studies with RBC suspensions containing normal and heat treated rigid RBCs (R-RBCs) will be performed. The proportion of R-RBCs which have exited through each outlet will be determined by analysis of cell deformability. A decrease in R-RBCs and increase of normal RBCs in branch outlets suggest that DRPs can be therapeutically effective in shunting S-RBCs past the capillary system and reducing VOC in SCD.

Wireless stimulator of muscular fibers for patients with imperforate anus

Luis de la Torre (1), José Neve (2)

(1) Children's Hospital of Pittsburgh of UPMC, (2) Universidad Iberoamericana Puebla, México

Thousands of children are born with imperforate anus every year in the US. Surgical treatment for these malformations requires a surgical technique called "Posterior Sagittal Ano-Rectoplasty" (PSARP). In this technique, it is critical to dissect through the exact middle of the perirectal muscular complex and the anal sphincters. To guide this dissection, surgeons use a device to stimulate the muscular fibers, and based on the observed directionality of the contractions, the surgeon determines where the midline of the muscles are. This approach leaves two major problems: 1) the assessment of the contractile response to the stimulation is only visual and therefore quite subjective, 2) every patient has a different amount of sphincter muscle development, and for those with smaller amounts of muscle, in whom maintaining the midline is the most critical, the assessment of the response is the most difficult, and frequently inaccurate, often resulting in undesirable outcomes such as fecal incontinence.

What is needed is a device that is capable of stimulating these muscular structures and then measuring the response quantitatively. In this way, the surgeon will be able to maintain 50% of the muscular structures on either side of the rectum and anus. This precision will improve the functional outcome and reduce the number of patients with fecal incontinence.

In The Iberoamericana University of Puebla Mexico, we developed an initial prototype as a wireless electro-stimulator that could quantitatively measure the degree of muscle stimulation using color codes.

Improved Estimation of Thermal Strain Imaging Using Pulse Inversion Harmonic Imaging

Xuan Ding (1,2,4), Man Nguyen (2), Isaac James (5), Kacey Marra (1,5,6), J. Peter Rubin (1,5,6), Steven A. Leers (3) and Kang Kim (1,2,3,6)

(1) Department of Bioengineering, University of Pittsburgh School of Engineering, (2) Center for Ultrasound Molecular Imaging and Therapeutics, Department of Medicine & Heart and Vascular Institute, University of Pittsburgh School of Medicine and University of Pittsburgh Medical Center (UPMC), (4) Medical Scientist Training Program, University of Pittsburgh School of Medicine, (5) Department of Plastic Surgery, University of Pittsburgh School of Medicine, (6) McGowan Institute for Regenerative Medicine, University of Pittsburgh and UPMC

Introduction

Ultrasound thermal strain imaging (TSI) and non-invasive thermometry are similar in principle. Studies have shown that TSI is able to identify lipids in ex vivo human plaques and in an in vivo rabbit model. In future in vivo human studies, clutter will degrade image quality and concerns about thermal safety will prevent further temperature increases. Here, we show that even in the presence of clutter and for the same temperature rise, pulse inversion harmonic (PIH) imaging is able to improve the strain signal to noise ratio (SNR) and contrast to noise ratio (CNR) for TSI.

Methods

TSI was performed on a Vantage (Verasonics Inc.) system with an L7-4 linear array. Fundamental images at 3.3 MHz and 6.6 MHz were acquired. A PIH image was formed by summing received data from two 3.3 MHz 180o phase shifted transmits. For heating, a multi-foci beamforming was used to obtain a broad heating beam thereby allowing imaging and heating to be implemented using a single transducer. A homogeneous gelatin phantom was imaged with TSI using heating duty cycles (DC) from 0.1-10%. The temperature rise was measured with a thermocouple (MT-23/5, Thermoworks). Whole human abdominal fat and a carotid endarterectomy sample were obtained with IRB approval and embedded in gelatin. TSI was performed using a 5% DC with and without clutter which was generated using a copper mesh. After TSI, the carotid tissue was stained with Oil red O (ORO). Temporal shifts were tracked with a 1.5λ kernel using Loupas' estimator and a 0.44 X 0.89 mm (axial X lateral) median filter was applied. The strain was estimated using linear regression over a 4 mm or 2.5 mm (carotid) window. We define lambda as the measured temperature rise. is the thermal strain. The strain SNR and CNR were calculated.

Results/Discussion

In the homogeneous phantoms lambda converges to nearly -0.1 %/oC for all imaging methods, PIH-TSI has smaller spatial variation. The strain SNR for PIH-TSI is 36% greater than the SNR for fundamental TSI. Fig. 1c shows that even with clutter, PIH-TSI provides a strain estimate consistent with the shape of the heating beam. Without clutter, the CNR in the fat was 0.80 (3.3 MHz), 0.98 (6.6 MHz), 1.05 (PIH). With clutter, the CNR was 0.62 (3.3 MHz), 0.87 (6.6 MHz), and 1.09 (PIH). PIH TSI improves lipid contrast in the ex vivo carotid.

Locally Implantable Biodegradable Polymeric Tacrolimus Disc provides Allograft Targeted Immunosuppression to Sustain Long Term Vascularized composite allograft Survival

F. G. Feturi (1), J. V. Unadkat (1), V. S. Gorantla (1), M. G. Solari (1), V. R. Ramanan (1), A. M. Spiess (1)

(1) Department of Pharmaceutical Sciences, Department of Plastic and Reconstructive Surgery University of Pittsburgh, Pittsburgh, PA

Widespread application of VCA has been limited due to systemic side effects associated with chronic use of oral immunosuppressive drugs. Polymeric materials are potential drug delivery systems due to their ability to effectively deliver the drug to the target sites. Feasibility and efficacy of use of these materials have not been explored in VCA. We developed polymeric system that can provide controlled and sustained release of tacrolimus (TAC) to the allograft, and evaluated its efficacy in sustaining allograft survival.

TAC loaded discs were prepared by solvent casting. Drug release kinetics was evaluated in vitro in PBS and in vivo by SC implantation of the disc in rats. Following hind-limb transplantation, rats (n=3/group) received one disc in transplanted leg (group 1) or in the contralateral un-transplanted leg (group 2). TAC levels in blood and tissues were measured using LCMS. In vitro, TAC was released in a sustained manner with a total dose released of 36.5 % in one month. In vivo, Single TAC disc (10mg, 9 % w/w) provided sustained release over 2 months achieving therapeutic blood levels (5-10 ng/ml), then undetectable blood levels (<2ng/ml). High local tissue levels (e.g. DLNs and nerves) of TAC were achieved at the disc inserted leg ($P<0.05$).

In vitro/in vivo studies demonstrated the feasibility of the system for local and sustained drug delivery and thereby for potential applications in VCA. To date, our preliminary data shows that the system is effective in sustaining allograft survival via local immunosuppression for at least 15 days, without complications.

In vivo tracking of the meningeal response to chronically implanted neural electrode arrays

James Eles (1,2), Takashi Kozai (1,2), Alberto Vazquez (1,2,3), Tracy Cui (1,2,4)

(1) Department of Bioengineering, University of Pittsburgh, (2) Center for Neural Basis of Cognition, University of Pittsburgh and Carnegie Mellon University, (3) Deparment of Radiology, UPMC, (4) McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh PA

Brain-machine interfaces (BMI) hold the promise to restore lost function to over 3 million paralysis and limb-loss victims in the US. One of the highest barriers to clinical translation is the lifespan of the multi-electrode arrays (MEA) behind BMI control, which is limited by a foreign body response. The response has two elements: a neurodegenerative glial scar that forms around the MEA in the brain, and a collagenous and cellular sheath that forms around the MEA in the meninges. The latter is responsible for over half of device failures, but is relatively unexplored in the MEA biocompatibility literature. The following is the first longitudinal, in vivo study of the meningeal response to chronically implanted MEAs. We implanted planar, silicon MEAs into the cortex of CX3CR1-GFP reporter mice, and observed leukocyte dynamics for one month through a glass cranial window. We report a period of meningeal cell migration along the MEA shaft within the first week post implant, followed by progressive cellular encapsulation of the MEAs through ensuing weeks. We tested how silicone elastomer craniotomy sealants affect the meningeal response, and determined that they can reduce encapsulation, but incite additional foreign body response as determined by the formation of foreign body giant cells. This work not only provides insights to the fundamental understanding of wound healing responses to transcortical implants, but also informs the design of materials and therapeutics to modulate this wound healing response.

Analysis of Cell-Spray Technologies for Spray-Grafting on Burn Patients

Matthew T. Young (1), Roger Esteban-Vives (1), Eva Schmelzer (1), Jenny Ziembicki (3), Alain Corcos (2), Jörg C. Gerlach (1)

(1) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Bioreactor Group, Pittsburgh, PA, (2) UPMC Mercy Hospital, Trauma Services and Burn Unit, Pittsburgh, PA

Introduction

The current treatments for severe partial and deep partial burns are based on mesh grafting. The risk of poor functional and aesthetic outcomes, especially wound contracture and discoloration, increases with delayed closure. Cases in which the burned area comprises a large portion of the total body surface area often require extended periods for wound closure. This is accompanied by and contributes toward the risk of infection, which can significantly complicate healing, further delaying reepithelialization. Our consortium promotes a therapy for severe second-degree burns consisting of a progenitor isolation routine in an on-site setting for same-day cell grafting in combination with a spray grafting technology for cell deposition, which allows treatment of areas many times larger than can be achieved using mesh grafting, efficiently utilizing healthy tissue when it is the most scarce and accelerating wound healing to facilitate desirable functionality and appearance.

Methods and Results

5 L/min of airflow generates a homogeneous pattern distribution, with a droplet size average of 3.6 mm, covering 50 percent of the 600 cm wound surface. The spray process does not inflict cell injury significantly. For further applications, we will use an airflow of 5 L/min and a total liquid volume of 2 ml per application.

Conclusions

Spraying results in a superior distribution pattern compared to needle squirting. Cell spraying causes insignificant cell damage compared to pipetted cell suspensions. Cell spraying requires a much smaller donor area for area treated versus conventional grafts.

Extracorporeal Carbon Dioxide Removal at Ultra-Low Blood Flows

Garrett Jeffries (1,3), Brian Frankowski (3), William Federspiel (1,2,3,4)

(1) Department of Bioengineering, (2) Department of Chemical Engineering, (3) McGowan Institute for Regenerative Medicine, (4) Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA

Clinical adaptation of extracorporeal carbon dioxide removal (ECCO2R) systems for management of hypercapnic respiratory failure is hindered by the high blood flow rates necessary to provide adequate support. We have previously shown that CO2 removal is potentiated by rotation of an impeller array within a gas exchange bundle - achieving the highest efficiency of any reported artificial lung device. In this study, we sought to establish the feasibility of removing clinically relevant levels of CO2 from blood (≥75 mL/min) at operating conditions matching those of renal hemodialysis (blood flow rate ≤250 mL/min) using an ECCO2R device with integrated rotating impellers.

Our Ultra-Low-Flow ECCO2R Device (ULFED) prototype consisted of 23 impellers (4 mm OD, 10 mm length) fixed to a driveshaft that rotated at speeds up to 34,000 RPM. A stainless steel coil separated rotating impellers from a surrounding bundle of microporous hollow fiber membranes with total gas exchange surface area of 0.21 m2 (750 fibers, 30 cm in length). The impeller/bundle assembly was potted concentrically in a cylindrical acrylic tube for total fluid priming volume of 105 mL. In vitro gas exchange in blood was evaluated in a recirculating flow loop per ISO 7199:2009 standards at normocapnia (inlet blood pCO2 = 45 mmHg).

The maximum in vitro CO2 removal rate measured with the ULFED was 75.3 ± 0.3 mL/min at a blood flow rate of 250 mL/min. Performance of the ULFED matches or exceeds the abilities of all available ECCO2R devices, but does so at half the blood flow rate required of other devices, and using only one-third of the total fiber surface area. Under these operating conditions, the ULFED could be connected to patients using dramatically simplified methods directly adopted from routine renal hemodialysis, with potential to be integrated directly into existing dialysis circuitry.

A High-channel-count Retinal Prosthesis for the Blind

Shawn Kelly (1,2,3), Ashwati Krishnan (3), Tracy Netemeyer (4), Chi-Hua Lin (3), Aziz Yousif (3), Meghan Kaffine (3,4), Arya Hezarkhani (3,4), William Ellersick (5), Marcus Gingerich (6), Douglas Shire (6) and Joseph Rizzo (7)

(1) VA Pittsburgh Healthcare System, Pittsburgh, PA, (2) Institute for Complex Engineered Systems, (3) Department of Electrical and Computer Engineering, (4) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, (5) Analog Circuit Works, Sudbury, MA, (6) VA Cleveland Medical Center, Cleveland, OH, (7) Massachusetts Eye and Ear Infirmary, Boston, MA

An implantable, hermetically-enclosed retinal prosthesis with over 256 channels is being developed to restore functional vision to the blind. Image data from an externally-worn camera are processed by a microcontroller and then transmitted wirelessly, along with power from an external battery, to the implanted device by inductive coupling between coils. The image data are received and error-checked by a custom-designed integrated circuit enclosed in a hermetic package attached to the eye. The IC then drives stimulation current pulses based on the image data and monitors the electrode potentials to ensure safety of the tissue. A microfabricated thin-film electrode array carries the current to the patient's retinal ganglion cells, creating visual percepts and coarse vision.

We present here a number of advancements in the device. First, we discuss improvements to the power and data transmission system, describing the class E transmission power amplifier and its operation delivering frequency-shift-keyed data. We have successfully transmitted 565 Kbps data on a nominal 6.78 MHz carrier. Second, we present a method of creating biphasic neural stimulation pulses that improve safety for the tissue. Traditional balanced biphasic current pulses result in a residual voltage at the electrode-tissue interface. We have developed circuits that sense the residual voltage and implement a feedback loop to adjust anodic pulse timing to eliminate the residual voltage. We discuss the implementation of the feedback loop and present results of the feedback system on the electrode voltage. Third, we present an exploration into the safety of retinal ganglion cell stimulation. Biological safety limits are known for cortical stimulation, but retinal stimulation has not been as rigorously explored. We discuss experimental methods and results from a series of experiments stimulating rat retina and staining for cellular death. Finally, we discuss testing methods for next-generation, pseudo-hermetic implant packaging using impedance methods.

Poster number 70 *Category: Medical Devices*

Nitric Oxide Release from PLGA-PVA Nanoparticles to Reduce Escherichia coli Growth

Nina A. Reger (1), Wilson S. Meng (2), Ellen S. Gawalt (1,3)

(1) Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, PA, (2) Division of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Nitric oxide, at low concentrations, has been used to elicit nitrosative stress in bacterial cultures in order to disperse biofilms. Polymer nanoparticles consisting of poly-lactic co glycolic acid and polyvinyl alcohol were formed and the nanoparticle surface was actively functionalized using thin films to deliver nitric oxide. Nanoparticles were modified with an s-nitrosothiol, specifically s-nitrosocysteamine, as the nitric oxide delivery molecule. S-nitrosocysteamine was attached to the nanoparticle surface using thin film reactions and traditional carbodiimide coupling. Surface reactions were confirmed using Diffuse Reflectance Infrared Fourier Transform and ultraviolet-visible spectroscopy. Nanoparticle size and morphology were determined using dynamic light scattering and scanning electron microscopy, respectively. Subsequent attachment of s-nitrosocysteamine resulted in a release of 37.1 ± 2.8 nmol nitric oxide per milligram of nanoparticles. This low concentration of nitric oxide was capable of inhibiting E. coli planktonic growth by 31.9% and increased the effectiveness of the antibiotic Tetracycline by 88.7%. The functionalized nanoparticles were not cytotoxic to mouse embryo fibroblasts in vitro. Thus, this nitric oxide delivery vehicle could be used in pharmaceutical applications to combat bacterial infections, such as in patients with Cystic Fibrosis.

Effectiveness of Robotic Training after an Acute Muscle Injury in Mice

R. Lawrence (1,5), S. Lai (2,5), A. Sahu (3), S. Shinde (3), M. Boninger (3,4), S. Micera (2), F. Ambrosio (3,4)

(1) Department of Bioengineering, (2) Institute of BioRobotics of Scuola Superiore Sant'Anna, (3)Department of Physical Medicine and Rehabilitation, (4) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, (5) co-first author

Although skeletal muscles have the ability to spontaneously regenerate after injury, the healing process is slower and functionally incomplete as the severity of the injury increases. Several studies have shown that mechanical loading and motor exercise has a positive effect on the efficacy of muscle regeneration, but the optimal treatment protocol still has not been determined. Through the use of isolated muscle training from a robotic therapy machine after injury, the superior training method can be observed over time and non-invasively. We analyzed the effectiveness of robotic training after an injury by subjecting mice to an acute muscle injury followed by various training regiments on a robotic platform modeled after therapy machines for post-stroke muscle recovery. Two days following a cardiotoxin injury to mice biceps, the animals were randomly assigned to three different groups of high, low, and no resistance training modules which were performed 10 trials a day, 4 days a week for 3 weeks. After 3 weeks, biceps muscles were harvested and subsequently stained for laminin in order to analyze the effect of training on the myofiber cross-sectional area and total number of the fibers. Mice subjected to heavy loading demonstrated a noticeably faster force recovery after injury during the first week when compared to mice trained with a low intensity training or no training at all. Analyses of the data revealed no notable difference in muscle function and regeneration at the end of the 3 weeks. All groups returned to baseline force-producing capacity by 3 weeks post injury. Accordingly, histological analysis revealed no significant difference between the mean cross sectional area and number of laminin rings for each group. Future studies should look further into the rate and progression of myofiber regeneration throughout the testing phase in order to better understand the mechanisms by which muscle loading enhances skeletal muscle recovery

Poster number 72 *Category: Medical Devices*

Design and Prototype Development of a Torsional Ventricular Assist Device (tVAD)

Elaine Soohoo (1), Lewis K. Waldman (2), Hsuan Ma (1), Anne Alcasid (1), Dennis R. Trumble (1,3)

(1) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, (2) Insilicomed, Inc., La Jolla, CA, (3) Left Field Cardiac, Inc., Pittsburgh, PA

Purpose
To develop a torsion-based ventricular assist device (tVAD) as an alternative to traditional ventricular assist devices (VADs) currently on the market. The tVAD attaches to and rotates the apex of the heart with the intention of reducing wall stress and increasing the ventricles' ability to empty more completely.

Methods
The tVAD prototype was designed using a commercial CAD software package (Solidworks 2015, Dassault Systèmes Solidworks Corp., Waltham MA). The overall design approach was guided by computational simulations of applied apical torsion of the heart and results from in vivo pig experiments wherein a first generation tVAD rotated a hypokinetic heart a quarter turn during the systolic phase of the cardiac cycle. Parametric computational simulations were performed using ContinuityPro software (Insilicomed, Inc., La Jolla, CA) and used to determine design parameters for a second-generation tVAD prototype. These simulations utilized beating heart models attached to a closed-loop circulatory system. Ventricular size, shape and dimensions were based on anatomic measurements taken from both adult porcine hearts and human heart failure patients, while biomechanic and circulatory model parameters were taken from literature values.

Results
We have recently created a working second-generation tVAD prototype design and surgical delivery scheme suitable for clinical use. Model details will be fine-tuned prior to device construction based on performance parameters derived from further computational simulations.

Conclusion
Based on results from preliminary computational simulations and experiments on live porcine hearts, applied apical torsion shows promise as an alternative method to traditional cardiac assist devices currently used to treat congestive heart failure.

In-Vitro and Acute In-Vivo Study of an Integrated Wearable Artificial Lung

Shalv Madhani (1,2), Brian Frankowski (1), Jonathan D'Cunha (3), Robert Kormos (3), Greg W. Burgreen (4), James Antaki (1,5), William Federspiel (1,2,6,7)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Cardiothoracic Surgery, (4) CAVS, Mississippi State University, (5) Department of Biomedical Engineering, Carnegie Mellon University, (6) Department of Critical Care Medicine, (7) Department of Chemical Engineering

We are developing the Passive Paracorporeal Ambulatory Assist Lung (P-PAAL) device, intended to provide 70% to 100% lung support. The P-PAAL approach focuses on improving efficiency through hollow fiber membrane (HFM) bundle design. Gas exchange efficiency is improved by increasing mean velocity past fibers allowing significant size reduction. The P-PAAL is an integrated and portable device, designed to ambulate patients during therapy. This study investigates the in vitro and acute in vivo performance of the P-PAAL.

The P-PAAL features a 1.75 inch diameter cylindrical HFM bundle of stacked sheets, with a surface area of 0.65 m2. A centrifugal pump is integrated into the device to allow pumping against a 27 Fr Avalon Elite Dual Lumen Cannula. In vitro gas exchange was conducted on the P-PAAL fiber bundle in bovine blood, in accordance with AAMI 7199 standards. Hemolysis was also measured on the fiber bundle in accordance with ASTM F1841 standards. The device was then tested in 40-60 kg adult sheep (n=4) for 6h.
The device pumped 3.5 L/min against the DLC at 1900 RPM. Oxygenation of 180 ml/min at 3.5 L/min of blood flow was obtained for the P-PAAL device in vitro, resulting an efficiency of 277 ml/min/m2. The NIH for the device was 0.054 g/dL at 3.5 L/min in vitro. In vivo gas exchange matched in vitro studies. Blood entered the device between 50% and 70% saturation and left the device at 100% saturation at all flows and plasma free hemoglobin remained below 20 mg/dl. No blood transfusions were given and hemoglobin concentration remained 8-10 mg/dL. Few areas of thrombus were noted in the fiber bundle possibly related to low flows at the start of the study. Based on these encouraging results we believe the P-PAAL is ready for 5 day chronic experiments.

Evaluation of a soft elastomeric electrode for intramuscular stimulation and drug release

Xin Zheng (1), James R. Eles (1,4), Gregory Brunette (1), Vasil E. Erbas (3), Lee Fisher (1,2), Vijay Gorantla (3), Tracy Cui (1,4)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, (2) Department of Physical Medicine & Rehabilitation, University of Pittsburgh, Pittsburgh, PA, (3) Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, (4) Center for Neural Basis of Cognition, University of Pittsburgh & Carnegie Mellon University, Pittsburgh, PA

Current technologies in treating muscle atrophy following peripheral nerve injury employ either electrical stimulation or pharmacological therapies coupled with physical therapy. For chronic intramuscular electrical stimulation in rodent models, a stiff stainless steel wire is used to deliver therapeutic stimulation pulses. The limitations for using stiff intramuscular wires are that there is a mechanical mismatch between the stiff electrode and the soft tissue, inducing muscular scarring around the electrode, and worsening the efficacy of electrical stimulation. Additionally, these stiff electrodes do not offer the opportunity for local drug release. We are in the process of developing a soft elastomeric electrode capable of electrical stimulation as well as drug delivery. This soft wire is fabricated with conductive elastomeric polymers which have a much lower young's modulus compared to conventional metallic intramuscular electrodes, making them ideal for chronic implantation. Here we evaluate the mechanical, electrochemical characteristics as well as acute in vivo capabilities of the soft wires for intramuscular electrical stimulation. For drug release, we investigate the electrically stimulated protein (FITC-Albumin) release profile from a PEDOT-CNT coating. Preliminary results show that protein loading at 1mg/ml yield the most releasable protein over a 6-hour release period, meeting the requirement for therapeutically relevant drug release concentration.

Extracorporeal CO2 Removal by Hemodialysis

Alexandra May (1,2,7), Ayan Sen (3,4,7), Matthew E Cove (3,5), John A Kellum (3), William J Federspiel (1,2,3,6)

(1) Department of Chemical Engineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Critical Care Medicine, University of Pittsburgh Medical Center, (4) Department of Critical Care Medicine, Mayo Clinic Arizona, (5) Department of Critical Care, National University, Singapore, (6) Department of Bioengineering, (7) Authors contributed equally

Partial respiratory support aimed at removing carbon dioxide, extracorporeal carbon dioxide removal (ECCO2R), provides an alternative to mechanical ventilation in ARDS patients. Respiratory hemodialysis is an ECCO2R technique that uses traditional hemodialysis to remove bicarbonate from the blood. Previous work in this area has shown that a challenge to this approach is controlling the blood pH post dialyzer. In this work, we sought to design a zero bicarbonate dialysate, designed using Stewart's approach to acid-base chemistry, which would not cause acidosis and still remove at least 40% of the metabolic CO2. Carbon dioxide removal was measured in a scaled down system using a 0.04 m2 dialyzer. At intermittent hemodialysis (IHD) flow rates, and blood flow rates of 247 mL/min and 420 mL/min the system removed 62.6 ± 4.8 mL/min to 77.7 ± 3 mL/min of CO2, respectively. Over the range of dialysate flow rates tested, the CO2 removal rate increased by 50% and 65%, at 247 mL/min and 420 mL/min blood flow rates respectively. By using a zero bicarbonate, alkaline dialysate metabolic acidosis was prevented, with a change in blood pH less than 0.08 at all of the test conditions. The results demonstrate a promising approach to respiratory hemodialysis using a zero bicarbonate dialysate when operated at IHD conditions.

CFD Optimized Reservoir for In-Vitro Testing of Intraventricular Rotary Blood Pumps

Salim E. Olia (1,2,3), Hannah J. Voorhees (1), Trevor A. Snyder (4), Marina V. Kameneva (1,2,5)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Artificial Heart Program, UPMC, (4) Advanced Cardiac Care, Integris Health System, Oklahoma City, OK, (5) Department of Surgery, University of Pittsburgh, Pittsburgh, PA

Study: Benchtop evaluation of intraventricular rotary blood pumps (RBPs) is a unique challenge owing to features on the pump surfaces which impacts hemocompatibility, but are covered or obstructed by attachment to tubing, compliant blood bags, or clinical hard-shell reservoirs. Maintaining inlet clearance and adequate mixing is crucial at lower flows associated with pediatric and partial support ventricular assist devices (VADs). A new blood reservoir was developed, addressing the growing pool of transmyocardial-type VADs, to enable improved in-vitro hemolysis assessment.

Methods: A two-piece, bottom-mount, cylindrical reservoir was designed in SolidWorks® for SLA-printing using an optically clear, ABS-like, biocompatible resin. The assembly consists of a lug-type lid which mates flush with the inner lumen of the reservoir base with radial seal outside of the chamber. The reservoir inlet barb, tangent to the reservoir base, accepts 3/8” ID tubing while the pump insertion site consists of a 1.0 cm long interference fit. CFD was performed to determine optimal reservoir entrance inlet angle and floor slope.

Results: The tapered interference fit successfully secured and sealed around the transmyocardial portion of a RBP prototype. Three luer fittings enable fluid sampling, temperature measurement, and venting of the reservoir. A total capacity of 300 or 500 mL is achieved through interchangeable lids with varied heights. A 30° upward inlet angle and 10° slope through CFD analysis provided complete mixing within the reservoir while allowing for air emboli to be collected at a vent port in a domed section of the lid. This reservoir has been successfully fabricated and hemolysis experiments for intraventricular RBPs are ongoing.

Anticorrosive Coating for Resorbable Magnesium Medical Devices

Avinash Patil (1), Laura Fulton (1), Elia Beniash (1,2,3)

(1) Department of Bioengineering, (2) Department of Oral Biology, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Magnesium (Mg) and its alloys are promising candidates for use as resorbable materials for biomedical devices which can degrade in situ following healing of the defect, eliminating need for second surgery to remove the device. The major challenge for use of the Mg devices in clinic is the initial corrosion burst which generates gas pockets around the device compromising its performance. The aim of the study was to evaluate the potential of alkylsilane self-assembled multilayer coatings to regulate Mg corrosion and to modify physicochemical properties of the coatings using surface functionalization. The coating was formed by co-polymerization of n-Decyltriethoxysilane (DTEOS) and Tetramethoxysilane (TMOS) followed by dip coating of metal discs. Scanning Electron Microscopy (SEM), ATR-FTIR spectroscopy, contact angle and hydrogen evolution studies were performed to assess the functionality of coatings. We used MC3T3-E1 cells to evaluate cytocompatibility of alkylsilane coated Mg discs. Cell viability was assessed using LIVE/DEAD assay. Fluorescence microscope & SEM were used to visualize cytoskeleton structures and the morphology of cells attached to coated discs. The SEM study revealed a homogeneous micron thick and defect free coating. Organosilane coating increases contact angle by two fold but after amination it decrease the contact angle which is close to pure Mg. ATR-FTIR confirmed the presence of alkyl peaks at 3000 cm-1 region and Si-O absorption band at 1000 cm-1. There was significant ($P<0.0001$) reduction in the corrosion rate of Organosilane coated Mg discs compared to uncoated Mg discs. The LIVE/DEAD assay confirmed that alkylsilane coated layer is cytocompatible and % cell death on aminated alkylsilane discs is lower than alkylsilane discs ($p<0.029$). The fluorescence imaging results showed significantly higher cells density on the aminated alkylsilane coated discs compared to alkylsilane coated discs after 15 days. The SEM study showed numerous cells on the aminated alkylsilane coated discs compared to alkylsilane coated discs. Overall our results demonstrate that alkylsilane coating regulates corrosion of resorbable Mg devices and coating is cytocompatible and has great potential to further functionalize surface chemistry of the Mg implantable devices.

Fabrication and optimization of parameters of vertically aligned platinum wire aptasensor arrays (VAPAA) for impedimetric detection of cardiac biomarkers

Mitali Patil (1), Prashant N. Kumta (1,2,3,4,5)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, (2) Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA, (3) Chemical and Petroleum Engineering, University of Pittsburgh, Pittsburgh, PA, (4) School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, (5) McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Cardiovascular diseases (CVD) are the leading cause of death in developed countries all around the world, including the United States. Lack of standard diagnostic methods and slow turnover for processing blood samples in hospital laboratories indicate the dire need of point-of-care (POC) diagnostic biosensors for the rapid and sensitive detection of cardiac markers in blood. Therefore, this study focuses on the development and optimization of impedimetric vertically aligned platinum wire aptasensor arrays (VAPAA) for the detection of specific cardiac markers, brain-natriuretic peptide (BNP - an indicator of myocyte stress) and Troponin-T (TnT – an indicator of myocyte injury). Various parameters such as platinum surface roughness, platinum wire diameter, and concentrations of the self-assembled monolayer components involved in the fabrication of the aptasensor – specifically, cysteamine, glutaraldehyde, streptavidin, and aptamer – were tested against clinically relevant concentrations of BNP and TnT to assess the optimal parameters for detecting BNP and TnT with the best precision and sensitivity. In addition, electrochemical surface and aptamer regeneration strategies were explored to create a reusable aptasensor. The fabricated platinum wire aptasensors were successfully able to detect various concentrations of BNP and TnT prepared in phosphate-buffered saline via electrochemical impedance spectroscopy (EIS) and the use of an applied potential in an electrochemical setup proved to be a simple and facile method for aptamer regeneration for resuse of the biosensors. In addition, the authors were able to determine the optimal platinum wire dimensions and optimal concentrations of self-assembled monolayer components for achieving enhanced biosensor sensitivity and precision of detection. The feasibility of the developed platinum aptasensor and the optimization of parameters for the ideal biosensor can open the door for future experimentation against other cardiac markers, including detection of cardiac markers in clinical blood samples, miniaturization, and possible translation to a functional handheld medical device for rapid and sensitive cardiovascular disease (CVD) diagnosis. Results of these studies will be presented and discussed.

Carbon Fiber for Electrophysiology, Electrical Stimulation, and Wireless Stimulation of Discrete Population of Nerves and Muscles

William E. McFadden (1), Alberto L. Vazquez (1,2), Xin Sally Zheng (1), Vasil E Erbas (3), Vijay S Gorantla (3,5), X. Tracy Cui (1,4,5), Takashi D. Y. Kozai (1,4,5,6)

(1) Department of Bioengineering, (2) Department of Radiology, (3) Department of Plastic Surgery, (4) Center for the Basis of Neural Cognition, (5) McGowan Institute of Regenerative Medicine, (6) NeuroTech Center, University of Pittsburgh Brain Institute

While microelectrodes for brain research and clinical applications have experienced a technological surge due to the perceived stability of brain implants, peripheral nerve interfaces continue to evolve as iterations of previous cuff electrodes. The objective of this project is to develop carbon fiber, fiber sheath, and fiber cable microelectrodes for nerve fiber and muscle fiber closed-loop recording and electrical stimulation. These fibers and fiber cables have demonstrated the ability to withstand significant mechanical strain such as those experienced in the periphery of a healthy, mobile individual. In addition, fiber cable electrodes have been shown to elicit visible muscle contraction with lower current pulse stimulation amplitudes than control electrodes. This newfound efficacy for stimulating both innervated and deinnervated muscle, along with the size and strength of fiber cable electrodes, could lead to applications in rehabilitation and functional electrical stimulation (FES). For neurostimulation, neuromodulation, and even rechargeable drug delivery polymers, significant challenges exist in achieving a highly discrete stimulation area in dense nerve fibers with multiple efferent/afferent bundles. Untethered carbon fibers have further been converted into wireless stimulation electrodes by scavenging energy scattered into the tissue with an energy transducer. In the cortex, wireless stimulation activates a much more discrete population of neurons compared to electrical stimulation and achieved greater depth penetration and energy efficiency compared to Infrared nerve stimulation. Combined, these technology demonstrate promising next-generation closed-looped PNS neuromodulation systems.

Fetal brain derived extracellular matrix hydrogel promotes positive optic nerve tissue remodeling after acute trauma

Tanchen Ren (1,2), Asma Naqvi (1,2), Anne Faust (1,2), Yolandi van der Merwe (1,2,3), Stephen F. Badylak (1,3), Michael B. Steketee (1,2,4)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Ophthalmology, School of Medicine, (3) Department of Bioengineering, Swanson School of Engineering, (4) Center for Neuroscience University of Pittsburgh, University of Pittsburgh, Pittsburgh, PA

Vision loss due to optic nerve trauma or disease is generally permanent due to the inability of retinal ganglion cells (RGCs), like all central nervous system neurons, to regenerate severed axons. A significant gap exists in treating optic nerve injury since we lack a therapeutic platform to alter the default healing response. Regenerative medicine strategies using extracellular matrix (ECM) technology have been widely successful in promoting functional tissue repair, over scar formation, in numerous tissues and organs. ECM from younger, homologous tissue sources appears to be more efficacious in generating a positive outcome. Here we developed fetal brain derived ECM by a vacuum assisted decellularization (VAD) method. DNA was mostly removed while sGAG was highly preserved. Fetal brain ECM hydrogel showed typical fibrous morphologies with dense and randomly oriented collagen fibrils under scan electron microscopy (SEM). Fetal brain ECM conditioned medium improved axon growth of RGCs better than adult brain derived ECM. In vivo, fetal brain ECM hydrogel effectively increased growth associated protein-43 (GAP-43, growth cone marker) expression in the optic nerve after crush, indicating increased axon regeneration. Additionally, the reduced expression of glial fibrillary acidic protein (GFAP) after ECM treatment indicated reduced astrocyte activation and scarring. These studies not only provided a method for preparing fetal brain ECM, but are also an important step toward optimizing ECM technology for treating CNS injury and understanding the underlying mechanisms regulating the default healing response in the CNS.

Development of a Testing/Treatment device for Eustachian Tube Dysfunction

Cuneyt M. Alper (1,2,3), J. Douglas Swarts (1), Miriam S. Teixeira (1), William J. Doyle (1)

(1) Department of Otolaryngology, University of Pittsburgh School of Medicine (UPSOM), (2) Division of Pediatric Otolaryngology, Children's Hospital of Pittsburgh (CHP) of UPMC, (3) Clinical and Translational Science Institute, UPSOM, Pittsburgh, PA

Eustachian tube (ET) connects middle ear (ME) to the nasopharynx (NP). Primarily, ET function (ETF) refers to the efficiency of ET openings and the ability to equilibrate the pressure difference between the ME and the NP. The inability to do so leads to development of negative ME pressure (MEP) due to absorption of gases into the blood, and may lead to tympanic membrane (TM) retraction, otitis media, ME effusion, retraction pockets, cholesteatoma, or medical and surgical treatment failures.

Currently available treatment methods for ETD include insertion of ventilation tubes, which bypasses the ET and buys time but does not address the ETD. Adenoidectomy and treatment of inflammation may also help. Some devices such as Otovent®, and Ear-Popper® may be useful but effectiveness, especially in children is questionable. More recently, more invasive methods such as laser tuboplasty, cartilage tuboplasty and balloon dilation of ET is becoming popular.

A number of methods have been used to assess the ET function, ability to perform Valsalva maneuver, Politzer's test, ET catheterization, Toynbee test, tympanometry, Holmquist test, 9-step test, sonotubometry, manometry, inflation-deflation test, forced response test and pressure chamber test methods. Most of these tests are available only in selected research centers, and often ETD is diagnosed by history.

There is a need for developing a test method that is simple, easy to use, child friendly, easy to interpret, with high sensitivity. Similarly, there is a need to develop a non-invasive treatment method that is easy to use and child friendly. While the testing and treatment concepts with above specifications are different, there is an overlap, and potential new device development for testing and treatment using the features of some of the existing testing and treatment methods, and concepts including intranasal adjustable delivery of controlled air pressure, and ear sensor that detects the ET opening.

ECM-based Nanomechanical Biosensors For Measuring Cell-generated 3D Mechanical Strains During Tissue Morphogenesis

Alkiviadis Tsamis (1), John M. Szymanski (1), Quentin Jallerat (1) and Adam W. Feinberg (1,2)

(1) Department of Biomedical Engineering and (2) Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA

Cell-generated mechanical forces are important during a wide range of processes including wound healing and morphogenesis. To understand how these forces shape tissue formation, we need to be able to track them during this process in-vivo. However, it has proved challenging to do this without perturbing the system. Here, we developed, calibrated and tested fibronectin (FN)-based nanomechanical biosensors (NMBS) that can deform in 3D and provide fluorescent-based strain readout during tissue morphogenesis ex-vivo. Fluorescently-labeled FN-based 2D square-lattice meshes (10×100μm width×space, ~10nm thick) were microcontact printed on poly(N-isopropylacrylamide) surfaces and then transferred to different materials (polydimethylsiloxane (PDMS), fibrin, collagen-I). Imaging of NMBS during uniaxial tensile test of these materials and MATLAB-based analysis validated NMBS tracking of homogeneous 2D strains. Comparison between NMBS and computationally-derived strains around circular defect and spherical indentation in PDMS validated NMBS tracking of heterogeneous 2D and 3D strains. NMBS were also integrated into embryonic chicken skin to measure 3D strains during tissue morphogenesis ex-vivo. NMBS strains agreed with macroscopic strains for fibrin, collagen-I and PDMS, and matched the known material properties of fibrin (anisotropy, compressibility) and PDMS (isotropy, incompressibility). Also, NMBS strains measured for the heterogeneous 2D and 3D strain fields agreed with computationally-derived strains, demonstrating accurate strain tracking in samples with known material properties. In addition, NMBS measured 3D tensile strain 110% and compressive strain -10% in day-8 embryonic chicken skin after 8 hours of development ex-vivo. We have developed an in-vitro calibrated FN-based NMBS, which can measure 3D microscopic tensile and compressive strains during tissue morphogenesis ex-vivo. In the future, we plan to integrate the NMBS into embryonic heart in order to measure 3D microscopic strains during cardiac morphogenesis in-vivo. The ultimate goal is to import 3D strains into constitutive laws of embryonic tissue material to provide 3D stress fields, which could help us better understand how these forces shape embryonic development.

Application of magnesium stents for the treatment of airway obstruction

Jingyao Wu (1,2), Boeun Lee (1,2), Tianbing Yang (3), Sandeep Kathju (3), Prashant N. Kumta (1,2)

(1) McGowan Institute for Regenerative Medicine, (2) University of Pittsburgh Department of Bioengineering, (3) Department of Plastic Surgery, University of Pittsburgh

The treatment of airway obstruction presents a significant clinical challenge and sometimes it is extremely difficult to handle. Bronchoscopy based interventional pulmonology has raised significant interest by providing immediate relief and reduced morbidity and risk. Airway stenting is a valuable adjunct to the other therapeutic bronchoscopic techniques as stenting provides mechanical support to maintain the patency of airway lumen and therefore, secures long-term effectiveness. However, the permanent nature of non-degradable tracheal stents renders them a last resort treatment option for pediatric patients and benign tracheal stenosis. Over the past decade, magnesium and magnesium alloys have emerged as novel degradable metallic materials which will potentially shift the paradigm of degradable materials and also the current medical device market. Orthopedic implants and stents are major applications that have been targeted so far. In this study, we developed magnesium based absorbable stents and evaluated the biocompatibility and in vivo degradation in rat airway bypass model and rabbit tracheal model. First generation magnesium stents were made from commercialized pure Mg and AZ31 alloys and Mg-Y alloys with an outer diameter of 2.25mm, inner diameter of 1.25mm and length of 5mm. Donor female lewis rats were sacrificed and the tracheas harvested to provide a bypass graft for stent evaluation. The stent was placed intra-luminally in the donor trachea, which was anastomosed to recipient rats in and end to side fashion. Animals were euthanized at 1, 8, 16, and 24 weeks for post evaluation by μCT and standard histologic analysis. Second generation stents were machined from ERC-P6 alloy based on the in vivo results of the first generation stents. The size of the stents was scaled up to fit the trachea size of rabbit. Stents were directly delivered inside the trachea of rabbits without a bypass. The animals were euthanized at 4 weeks for post evaluation by endoscopy and standard histologic analysis. In the study of the third generation stents, balloon expandable stents of AZ31 alloys were manufactured and implanted in rabbit model for 4 weeks. Post evaluation includes in vivo degradation and biocompatibility analysis. Results of these studies will be presented and discussed.

Controlling the Fate of Human Umbilical Vein Endothelial Cells (HUVEC) in vitro Using Calcium-Rich Hydroxyapatite Phosphate Scaffolds

Qinghao Zhang (1), Matthew T. Young (2), Eva Schmelzer (2), Ian Nettleship (1) and Jörg Gerlach (2,3)

(1) Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA, (2) Department of Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, (3) Department of Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Our ongoing research is focused on implementing hydroxyapatite phosphate scaffolds used in perfusion bioreactors to maintain hematopoietic stem cell populations and to control their differentiation towards the various hematopoietic lineages. Hemangioblasts, which are bi-potential angio-hematopoietic stem cells, are known to give rise to both hematopoietic stem cells and endothelial cells. In our previous work we have developed three-dimensional scaffolds that mimic the structure and chemistry of the trabecular bone in human. These calcium phosphate scaffolds mimic the micro-environment of the endosteal niche in which hematopoietic stem cells, osteoblasts, stromal cells, and endothelial cells reside. These calcium phosphate scaffolds have been designed to release calcium into the vicinity of the scaffold's surface. In the perfusion-bioreactor culture, these scaffolds promoted the differentiation of bone marrow-derived hematopoietic stem cells. We hypothesized that under these specific culture conditions other cell types with a potential hemangioblast character can be induced towards hematopoietic lineages as well. Therefore, we investigated whether human umbilical vein endothelial cells (HUVECs) have hematopoietic potential when cultured under these inducible conditions. In fact, when HUVECs were cultured under these inducible conditions, surface marker expression analyses by flow cytometry demonstrated an increased number of cells positive for mature hematopoietic markers such as CD45, Lin, and CD235a. Conclusively, our data indicate that our culture model using calcium-rich hydroxyapatite phosphate scaffolds in perfusion bioreactors provides a general hematopoietic inducible microenvironment.

An Extracorporeal Neutrophil Reprogramming Device for the Treatment of Acute Inflammatory Conditions

Alexander D. Malkin (1,2), William J. Federspiel (1, 2, 3, 4), John A. Kellum (2,4) and Kai Singbartl (5)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Chemical Engineering, (4) Critical Care Medicine, University of Pittsburgh, (5) Department of Anesthesiology, Penn State Hershey

Sepsis is a systemic inflammatory response due to an infection. This condition affects 900,000 Americans per year and its incidence is expected to increase over the next 10-20 years as the population ages. As one of the first responders to sites of inflammation, neutrophils play a critical role in innate immune response and sepsis. High quantities of neutrophils migrating to remote organs such as the lungs has been associated with increased morbidity and mortality by initiating multiple organ dysfunction or immune paralysis in septic patients. The migration and activation of neutrophils is regulated by CXCR-1 and CXCR-2, g-protein coupled receptors that bind to interleukin-8 (CXCL-8).

An extracorporeal device is being developed to attenuate neutrophil chemotactic response in inflammatory conditions such as sepsis. This device achieves cell phenotype reprogramming by exposing neutrophils to immobilized CXCL-8 within an artificial microcirculation. First CXCL-8 was covalently immobilized on the inner lumen of amine functionalized fibers [Baxter Intl, Illinois USA] using a bifunctional polyethylene glycol spacer. After perfusion of human whole blood through modules constructed from modified fibers, neutrophil surface receptor expression was quantified using flow cytometry. In scaled devices neutrophil surface receptor expression of CXCR-1 and CXCR-2 decreased by 44% and 87% respectively after recirculation when compared to baseline blood. CXCR-2 downregulation nearly matches the 93% downregulation induced by soluble free CXCL-8 (1ug/ml). Additionally, when hAlbumin was immobilized in place of CXCL-8, downregulation closely resembled baseline blood, indicating covalently bound IL-8 was responsible for receptor downregulation. Future tests will evaluate chemotaxis of neutrophils treated with the extracorporeal device. Beyond sepsis, a similar device platform may be applied to alternative disease states such as ischemia reperfusion and solid organ rejection, which are regulated by cell-cell interactions.

Velocity of Canine Eruption in Cleft Patients: Comparison of Graft Material, Age, and Amount of Canine Root Formation

Andrews, R (1), Helal, N (1), Martin, B (1), Basri, O (1), Macissac, Z (2), Kumar, A (2), Grunwaldt, L (2)

(1) Division of Pediatric Dentistry, Children's Hospital of Pittsburgh of UPMC, (2) Division of Pediatric Plastic Surgery, Children's Hospital of Pittsburgh of UPMC

Comparison of clinical and radiographic outcomes of permanent tooth eruption for pediatric patients with a cleft alveolus treated with either (1) autograft or (2) mixed allograft and autograft. Currently autograft iliac crest bone remains the most commonly used graft material. Autograft from the iliac crest has a high donor site morbidity which may include meralgia paraesthetica,, hematoma, intestinal herniation, infection, and postoperative fracture. Use of allograft may minimize donor site morbidity associated with autograft harvest. Our outcome measure found no difference in velocity of canine tooth eruption between autograft versus mixed autograft and allograft bone. Further investigation is warranted regarding outcome of allograft-only treatment in alveolar bone graft patients.

Posters
Tissue Engineering

#	*Presenter*
89	Kassandra Allbright
90	Travis Armiger
91	Emily Bayer
92	Ivan Batalov
93	Colin Beckwitt
94	Andrew Brown
95	Michael Buckenmeyer
96	Xiaochu Ding
97	Liwei Dong
98	Vasil Erbas
99	Rebecca Duffy
100	George Fercana
101	Maria Giovanna Francipane
102	Ashlee Greene
103	Harman Ghuman
104	Darren Haskett
105	Yan Huang
106	Isaac James
107	Timothy Jackson
108	Ning-Jiun Jan
109	Ethan Kallick
110	Soo Hyon Lee
111	Chiaki Komatsu
112	Jr-Jiun Liou
113	Samuel LoPresti
114	Daniel Long
115	Katherine Lorentz
116	Chelsea Merkel
117	Christopher Mahoney
118	Asiyeh Golabchi
119	Maxine Miller
120	Mark Murdock
121	Muhammad Nisar
122	Jamie Nowalk
123	Piya-on Numpaisal
124	John Ohodnicki
125	Rachelle Palchesko
126	Akhil Patel
127	Catalina Pineda Molina
128	Alessandro Pirosa
129	Aneesh Ramaswamy
130	Michelle Scarritt
131	He Shen
132	John Sembrat
133	Jihee Sohn
134	Sudhanshu Shekhar
135	Chelsea Stowell
136	Sunita Shinde
137	Yolandi van der Merwe
138	Huong Tran
139	Elizabeth Stahl
140	Zhouguang Wang
141	Aaron Sun
142	Yefei Wang
143	Deepthi Vijayraghavan
144	Michael Washington
145	Yingfei Xue
146	Zhang Wensheng
147	Zhe Zhuang

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

Poloxamer Hydrogel Delivery of Adipose-derived Stem Cells for Repair of Transecting Peripheral Nerve Injury: Positive Effects on Regenerative Gene Expression Programs in Re-innervating Muscle

Kassandra Allbright (1,2,3), Jacqueline Bliley (2), Emmanuelle Havis (4), Gabriella DiBernardo (2), Damian Grybowski (2), Isaac James (2), Mayara Silva (2), Sheri Wang (1,2), Wesley Sivak (2), Kacey Marra (2,5,6)

(1) School of Medicine, University of Pittsburgh, (2) Department of Plastic Surgery, University of Pittsburgh, (3) Physician Scientist Training Program, University of Pittsburgh, (4) Universite Pierre et Marie Curie Paris VI, (5) McGowan Institute of Regenerative Medicine, University of Pittsburgh, (6) Department of Bioengineering, University of Pittsburgh

Peripheral nerve damage commonly results from traumatic injuries and is associated with high long-term morbidity. The current gold standard of care for a severe transecting injury is transplantation of a nerve autograft. Unfortunately, this treatment does not typically result in full functional recovery. One major challenge to functional restoration is the degeneration that occurs in the de-innervated muscle units. During the slow process of nerve regeneration and reinnervation, extensive muscle atrophy occurs – further disrupting return to full nerve/motor unit function. Several groups have demonstrated improved functional and histological outcomes in animal models of nerve injury with repair site transplantation of adipose-derived mesenchymal stem cells (ASCs). Despite this success, a clinically relevant method of cell delivery has not been established. We have investigated a thermoresponsive hydrogel based on Poloxamer 407, an FDA-approved inert pharmaceutical additive, for this purpose. We demonstrated high in vitro viability by proliferation analysis and normal morphology in gel culture by confocal imaging. We then investigated the therapeutic efficacy of the transplantation of ASCs in poloxamer hydrogel in vivo. While previously published work has primarily focused on assessment of regenerating nerve alone, we included assessment of regenerating muscle tissue. A 15mm gap injury was made to sciatic nerves of rats assigned to six treatment and control groups (n=6/group), including ASCs delivered in poloxamer hydrogel and autograft. Muscle and nerve tissue was explanted for gene expression and histological analysis. Gene expression data shows that primary members of the muscle regenerative signaling pathway (including Pax7, MyoD, and Myogenin) are increased in poloxamer and ASC treatment groups, relative to controls. Furthermore, Bax (an apoptosis mediator) was down-regulated in treatment groups, while MHCII and Cox4 (markers of muscle fiber health) were up-regulated. Histological analysis of nerve and muscle samples is ongoing. This improvement in the regenerative and viability markers of the re-innervating nerve/muscle unit suggests that delivery of ASCs within poloxamer hydrogel at the site of injury is a potential regenerative treatment for peripheral nerve injury.

The Role of Spectrin in Mechanics and Compressive Resilience of the Cell Nucleus

Travis Armiger (1), Kris Dahl (1,2)

(1) Department of Chemical Engineering, Carnegie Mellon University, (2) Department of Biomedical Engineering, Carnegie Mellon University

Spectrin proteins have been shown to be present in the nucleus where they interact with nucleoskeletal proteins and with DNA repair proteins. Spectrin proteins were originally seen in enucleated red blood cells where they form an elastic protein network which provides mechanical resilience. Unlike in red blood cells, the role of spectrin proteins in the nucleus remains to be understood. The nucleus, composed of the chromatin within the nuclear interior, and its surrounding two dimensional network of structural proteins which make up the nucleoskeleton, creates a significantly more complex system than that of the red blood cell. The intermediate filament protein network of lamins are the primary component of the nucleoskeleton. However, other proteins, such as spectrin, may also help regulate nuclear mechanics.

Spectrins may provide elasticity to the nucleoskeleton, analogous to their role in red blood cells, and may also impact chromatin mechanics as they are involved in DNA damage repair in the nuclear interior. For these reasons we investigate the role of spectrin proteins in the nucleus using two independent techniques. Live cell particle tracking is used to investigate the role of spectrin proteins (in this case alpha II spectrin specifically) on chromatin fiber fluctuations. This technique utilized fluorescently tagged transcription factors, bound to chromatin, which are tracked over time. Interestingly, no significant difference in chromatin dynamics was seen comparing between alpha II spectrin knock-down and control nuclei. A second technique, cellular compression, was performed to determine spectrin's impact on nuclear resilience. In this case, alpha II spectrin knock-down cell nuclei failed to return to their original area upon removal of a static weight, relative to control cell nuclei. Together these results indicate that spectrin proteins have minimal effect on chromatin rheology, as determined through intranuclear particle tracking, but appear to decrease the ability of nuclei to "spring back" to their original area upon removal of an external force, determined via cellular compression assays. This result may be amplified in cells which undergo cyclic stress, ultimately leading to phenotypic changes.

Sequential Growth Factor Delivery for Vascularized Bone Regeneration

Emily Bayer (1,5), Morgan Fedorchak (2,4,5), Riccardo Gottardi (1,5,6), Steven R. Little (1,2,3,5)

Departments of: (1) Bioengineering, (2) Chemical Engineering, (3) Immunology, (4) Ophthalmology, (5) The McGowan Institute for Regenerative Medicine at the University of Pittsburgh, Pittsburgh, PA, USA, and (6) RiMED Foundation, Palermo, Italy

Over 3 million musculoskeletal procedures are performed each year in the U.S., creating an annual bone grafting business of over $2.5 billion. Additionally, many other fracture types require fixation with titanium hardware and grafting with autologous bone, a gold-standard treatment which is associated with severe drawbacks.

Synthetic biomaterials are widely investigated for use in bone tissue engineering because they theoretically can be engineered to exhibit preferred mechanical properties, degradation rates, and biochemical characteristics. While these strategies can be used to bridge gaps in bone fragments, provide stability, and even deliver regenerative factors such as BMP, their efficacy remains limited. This, in part, is due to the fact that bone repair is an incredibly complex process that requires multiple growth factor cues to guide a series of regenerative steps. On a cellular level, bone repair consists of vascular infiltration, osteoblast migration and, ultimately, structured bone tissue formation, steps which are guided by a carefully orchestrated presentation of growth factors.

To address these complexities, we aim to first identify the optimal schedule of growth factor delivery. Recently, our group has developed systematic in vitro testing methods to explore the role of sequence, timing, and dosage of key bone regeneration growth factors. This system allows us to determine cell response and structural formations in response to growth factor delivery variations. Specifically, we have investigated the roles of sequence and overlap in growth factor delivery to evaluate cellular responses to a “hard switch” of sequential growth factor delivery vs. varying degrees of overlap in presentation. Ultimately, we aim to use this information to design a degradable, polymeric microparticle-based system capable of autonomously delivering a schedule of growth factors, which can be integrated within a scaffold for enhanced bone repair.

The Role of Cell-Cell and Cell-ECM Interactions in Cardiomyocyte Alignment on Biomimetic Micropatterned Surfaces

Ivan Batalov (1), Quentin Jallerat (2), Sean Kim (2,3), Adam W. Feinberg (1,2)

(1) Department of Materials Science & Engineering, (2) Department of Biomedical Engineering, (3) Department of Chemical Engineering, Carnegie Mellon University

The primary goal of 2D cardiac tissue engineering is the formation of aligned cardiac muscle tissues capable of synchronous contractions and generating contractile forces comparable to that of the native heart muscle. A previously reported technique induced cell alignment in cardiac muscle cell (cardiomyocyte) monolayers by micropatterning the extracellular matrix protein fibronectin onto the substrate in 20-micron wide lines of alternating high and low protein density (20x20 pattern). Fibronectin is believed to play a major role in cardiomyocyte alignment in the developing embryonic heart. However, the 20x20 pattern does not resemble the structure of native heart fibronectin. To better mimic the process of native myocardium alignment in vitro, we designed a new pattern that recapitulates the fibronectin structure in the developing heart based on confocal 3D images of the fibronectin in embryonic chick myocardium. We then compared chick cardiomyocyte alignment on this biomimetic pattern to the alignment on the 20x20 pattern. Results showed that although cells align similarly on both patterns at a high cell density, the alignment on the biomimetic pattern decreases at lower cell densities but stays the same on the 20x20 pattern. Further, by calculating maps of local cell attachment rate and alignment on both patterns at different densities, we showed that with increasing cell density the effect of cell-substrate interactions on alignment decreases and the effect of cell-cell interactions increases. Additionally, this result has been supported by inhibiting cell-cell interactions using anti-N-cadherin blocking antibodies, which caused the decrease of cardiomyocyte alignment on the biomimetic pattern within a range of intermediate cell densities. This suggests that cell-cell interactions can play an important role in the formation of aligned embryonic myocardium. In the future we will use human induced pluripotent stem cell-derived cardiomyocytes to engineer more clinically-relevant human heart muscle and analyze human cardiomyocyte response to the fibronectin patterns as a function of cell density.

Statin efficacy is dictated by tumor dormancy and oxygen tension

Colin H Beckwitt (1), Amanda M Clark (1), Alan Wells (1)

(1) Department of Pathology, University of Pittsburgh School of Medicine

Metastasis in breast cancer patients heralds mortality, as disseminated disease becomes chemoresistant. In the metastatic cascade, one or a few cancer cells break off from the primary tumor, enter the blood stream, and colonize a distant site. After establishment, this micrometastasis commonly enters a period of quiescence, termed dormancy, which may last for decades before outgrowing, clinically seen as cancer recurrence. Thus, interventions that prevent metastatic outgrowth are needed to treat this mortal stage of tumor progression.

In order to examine the properties of breast cancer cells in the context of the metastatic niche, our lab employs a microphysiological system (MPS) of the most common site for metastases, the liver. Briefly, a micro-hepatic tissue is established by seeding primary human liver cells in a porous scaffold subject to a physiological flow. RFP-labeled breast cancer cells are seeded onto these microtissues and examined after two weeks of culture by immunofluorescence. Liver function and health are monitored by clinical chemistry assays performed on supernatant samples. We have previously shown that this system robustly reproduces tumor dormancy.

The MPS allows for clinically-relevant testing of therapies. We previously showed atorvastatin exhibits cytotoxic effects on many different cancer cell lines in vitro and that membrane E-cadherin expression, a protein re-expressed during the mesenchymal-to-epithelial reverting transition that marks dormancy, confers resistance to statin therapy. We have more recently found that statin lipophilicity and potency dictate efficacy in cancer cells. As E-cadherin expression is lost following the EMT that accompanies micrometastatic outgrowth, we believe statin therapy may be effective at suppressing the formation of clinically evident metastases. More importantly, we believe oxygen tension will be a crucial mediator of emergence from dormancy, consequently potentiating statin therapy. As 26% of adults currently take a statin for other medical conditions, these studies may suggest the best statin to use in the context of breast cancer dormancy.

Pilot in vivo Evaluation of Magnesium-based Guided Bone Regeneration Devices in a Canine Vertical Ridge Augmentation Model

Andrew Brown (1,2), Samer Zaky (2,3), Konstantinos Verdelis (2,4,5), BJ Costello (2,5,6), Charles Sfeir (1,2,3,5)

(1) Department of Bioengineering, (2) Center for Craniofacial Regeneration, (3) Department of Oral Biology, (4) Department of Restorative Dentistry, (5) McGowan Institute for Regenerative Medicine, (6) Department of Oral and Maxillofacial Surgery

Guided bone regeneration (GBR) is a dental bone grafting approach typically used prior to placement of dental implants or to treat periodontal disease. GBR consists of a bone graft substitute and barrier membrane which create a protected space for bone regeneration to take place. Unfortunately, use of gold-standard bone graft substitutes provide unreliable results, while metallic barrier membranes require removal procedures following bone regeneration. Thus, GBR devices that provide more reliable outcomes while eliminating device removal procedures would be highly desirable. Our group hypothesized that magnesium based GBR devices could provide these characteristics and be capable of regenerating bone in a canine vertical ridge augmentation model.

Three magnesium GBR devices were manufactured: Mg tenting screws, Mg/PLGA barrier membranes and Mg micromeshes. Defects were created in the alveolar bone of dogs by extracting the second and fourth premolars and then using a reciprocating saw to produce a standardized 8mm wide by 8mm high, full buccolingual thickness defect. The Mg GBR groups consisted of Mg tenting screws inserted into the remaining alveolar bone to serve as the graft material with either Mg/PLGA barrier membranes (n=2) or Mg micromeshes (n=2) serving as the barrier membrane. The Mg GBR groups were compared to standard-of-care controls consisting of human allograft bone and Ti micromesh.

Mg GBR treated sites exhibited regeneration of full buccolingual thickness with Mg/PLGA barrier membrane samples exhibiting increased alveolar bone height as measured with microCT. Standard-of-care treated sites exhibited minimal bone regeneration with poorly integrated bone graft particles. Histological assessment revealed no signs of chronic inflammation and areas of active osteoid suggesting continued bone regeneration and remodeling. These results show that Mg GBR devices can be successfully manufactured and implanted and support bone regeneration. Future directions include manufacturing improvements and a full-scale study of the devices.

The Effect of ECM Stiffness on Ovarian Follicle Development

Michael J. Buckenmeyer (1), Yong-Hyun Shin (2), Aleksandar Rajkovic (2,3,4), Bryan N. Brown (1,2)

(1) Department of Bioengineering, McGowan Institute for Regenerative Medicine, (2) Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, (3) Department of Human Genetics, Magee-Womens Research Institute and (4) Department of Pathology, Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA

Introduction:
A diminishing ovarian follicular reserve is normal for women throughout their functional reproductive life; however, the cause for this decline is unknown. Cyclic remodeling of extracellular matrix (ECM) with associated changes in mechanical properties is known to initiate signaling cascades via mechanotransduction that significantly influence ovarian follicle development and if not tightly regulated could potentially dictate the exhaustion of the remnant follicle pool. Our findings indicate that increasing ECM stiffness may prematurely trigger follicle activation causing a decrease in the immature follicle population.

Materials and Methods:
An ovarian microenvironment was simulated with a three-dimensional cell culture system using tissue-specific hydrogels derived from decellularized porcine ovaries. Two concentrations (2 mg/mL and 5 mg/mL) of ovarian hydrogels were used to test differing ECM stiffness on follicle development. Newborn mouse ovaries with mCherry labeled oocytes were microdissected and cultured for 7 days on top of the hydrogels in Waymouth's MB 7521 media. After day 7 culture, the ovaries were imaged using confocal microscopy and mCherry labeling quantified with Volocity software to determine the total number of viable oocytes. The ovaries were fixed and serial sectioned for histological analysis using a periodic-acid Schiff (PAS) stain.

Results/Discussion:
Rheology testing of two ovarian hydrogel concentrations confirmed that the 5 mg/mL peak storage (G') and loss (G'') moduli were approximately double the 2 mg/mL gel concentration. The increase in viscoelasticity resulted in a decrease in the total number of oocytes between the two gel concentrations (2 mg/ml ~1850 oocytes; 5 mg/mL ~1300 oocytes) suggesting that modulating ECM stiffness has a significant impact on follicle viability. As a control, ovaries from day 7 wild-type mice were microdissected for oocyte quantification (~1900 oocytes) and showed similar viability to the 2 mg/mL hydrogel ovary culture. PAS stained sections confirmed oocyte presence and morphology.

Dual physical dynamic bond-based injectable and biodegradable hydrogel for tissue regeneration

Xiaochu Ding (1), Jin Gao (1), Hassan Awada (1) and Yadong Wang (1,2,3,4,5)

(1) Department of Bioengineering and the McGowan Institute for Regenerative Medicine, (2) Department of Surgery, (3) Department of Chemical and Petroleum Engineering, Swanson School of Engineering, (4) Department of Mechanical Engineering and Materials Science and (5) Clinical Translational Science Institute, University of Pittsburgh School of Medicine, University of Pittsburgh, Pittsburgh, PA.

Therapeutic proteins play key roles in tissue regeneration through promoting cell proliferation, migration and differentiation to repair a diseased or damaged tissue. But their short half lives due to proteolysis in extracellular matrix sacrificed the therapeutic efficacy. To overcome such weakness, a new shear thinning hydrogel is designed for sustainable release of proteins with prolonged bioactivity. This injectable hydrogel is formed using dual physical dynamic bonds based on host-guest chemistry and electrostatic interaction to build up the network structure. The material was synthesized by simultaneously coupling mono-carboxylic acid terminated poly(ethylene glycol) and arginine to poly(ethylene aspartate diglyceride) to yield a mPEG-grafted poly(ethylene argininylaspartate diglyceride) (mPEG-g-PEAD). When mixing this polymer with α-cyclodextrin and a natural polyanion (heparin), the supramolecular network was formed in a quick gelation with shear thinning properties. The in vitro cytotoxicity was evaluated using primary baboon arterial smooth muscle cells (BaSMCs) and showed that the cell membrane integrity, viability and metabolism were not compromised by this synthetic polycation at concentration as high as 10 mg/mL, a 1000-fold lower toxicity than commercial PEI. The in vitro biocompatibility of the as-made hydrogel was also evaluated using BaSMCs. Neither the hydrogel nor the hydrogel components altered cell behavior in the assays. Fibroblast growth factor 2 was incorporated into the hydrogel and sustainably released in a nearly stable rate up to 16 days without initial burst release, suggesting potential applications in wound healing and ischemic tissue regeneration, among others.

Assessment of neoartery regeneration in cell-free, fast-degrading vascular grafts using a rat carotid artery interposition model

Liwei Dong (1), Keewon Lee (2), Muhammad Umer Nisar (3), Nadim Farhat (2,3), Chiaki Komatsu (1), Kang Kim (2,3,5) , Mario G. Solari (1), Vijay Gorantla (1), Yadong Wang (2,4,5)

(1) Dept. of Plastic Surgery, Univ. of Pittsburgh and UPMC, Pittsburgh, PA, (2) Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA, (3) Center for Ultrasound Molecular Imaging and Therapeutics, Dept. of Medicine and Heart and Vascular Institute, Univ. of Pittsburgh and UPMC, Pittsburgh, PA, (4) Depts. of Surgery, Chemical Engineering, Mechanical Engineering and Material Science, Pittsburgh, PA, (5) McGowan Institute of Regenerative Medicine, Univ. of Pittsburgh and UPMC, Pittsburgh, PA

The clinical need for vascular grafts is increasing with the high incidence of cardiac and peripheral vascular disease. Autologous vascular grafts are the gold standard, but donor site morbidity and additional surgery limit its clinical utility. To address these limitations, we have developed a cell-free, rapidly biodegradable vascular graft with an open cell porous structure, which has demonstrated accelerated cell infiltration and in-host remodeling when used in a rat abdominal aorta model. In this work, we assessed the neoartery regeneration of our grafts using a rat carotid artery interposition model. Compared to the abdominal aorta, the common carotid artery is smaller in inner diameter and more muscular, mimicking a small elastic artery in the human. We fabricated bi-layered composite vascular grafts: with a fast-degrading elastomer, poly(glycerol sebacate) (PGS) core and an electrospun polycaprolactone (PCL) outer sheath. We implanted these vascular grafts into the rat common carotid artery by end-to-end anastomosis (n = 4 per time point) with 10-0 nylon suture with standard microsurgical techniques that addressed the native artery – vascular graft size differential. We checked graft patency at 0, 5, 10, 30 min with flow Doppler after anastomosis, to confirm absence of thrombosis and with ultrasound scanning at 2, 4, 12-week post-implantation, to monitor other complications including stenosis or post-stenotic dilation. We explanted grafts at 12-week post-implantation and assessed in-host remodeling using histology and immunofluorescence staining. Ultrasound monitoring confirmed the patency of the grafts in the carotid artery without thrombosis and stenosis. Histology and immunofluorescence staining demonstrated endothelial monolayer in lumen and organized smooth muscle cells in medial layer. Our results confirmed successful neoartery regeneration in the cell-free, bi-layered, fast-degrading vascular grafts following rat carotid artery interposition. Our vascular graft can be ideally suited for regeneration in vascular pathologies involving small arteries.

Translational Considerations in Whole Eyeball Transplantation

Vasil Erbas (1), Huseyin Sahin (1), Liwei Dong (1), Maxine Miller (1), Sinan Oksuz (2) Golnar Shojaati (1), Matthias Waldner (1), Lin He (1), Huamin Tang (1), Gadi Wollstein (3), Kevin Chan (4), Ian Sigal (3), Nataliya Kostereva (1), Shailesh Raval (5), Tamer Ibrahim (5), Jeffrey Goldberg (6), Larry Benowitz (7), Joel S. Schuman (3), Fatih Zor (7), Mario Solari (1), Kia M. Washington (1), Vijay Gorantla (1)

(1) Department of Plastic Surgery University of Pittsburgh, Pittsburgh, PA, (2) Gulhane Askeri Tip Akademisi, Ankara, (3) Department of Ophthalmology University of Pittsburgh, (4) McGowan Institute for Regenerative Medicine, (5) Department of Bioengineering University of Pittsburgh, Pittsburgh, PA, (6) Stanford University School of Medicine, Palo Alto, CA, (7) Harvard Medical School, Boston, MA

Purpose: Whole eyeball transplantation (WET) is the holy grail of vision restoration and is conceptually the most challenging of vascularized composite allografts (VCA). The swine eye is analogous to the human and is the ideal model for human WET. Our goal was to define technical considerations (surgical planning/procedures/post operative imaging /evaluations) in a robust, large animal, preclinical, translatable WET model.

Methods: WET techniques were optimized in 17 fresh tissue swine dissections. An eyeball-periorbital VCA subunit with extra ocular muscles, and optic nerve (ON) was raised superolaterally and anastomosed to the recipient external ophthalmic artery (EOA) after exenteration. Perfusion was confirmed with methylene blue and vascular territories [central retinal artery (CRA), ciliary and vortex plexuses] defined by microfil. Orbital contents and ON were imaged with dynamic contrast enhanced (DCE-MRI) and diffusion tensor imaging (DTI) [T1/T2 MRI at 3T/7T/9.4T]. Advanced protocols for histopathology, immunohistochemistry, epoxy embedding, corrosion casting, optical coherence tomography (OCT), tonometry, fundoscopy and ERG were optimized and surgical techniques for ON crush, cut and coaptation established

Results: Like the human, the swine retina is holangiotic and the ON has a lamina cribrosa. However, the CRA is absent and the predominant arterial supply is from the EOA. OCT and MRI allowed real-time, high definition, non-invasive, in situ, micron-scale, cross-sectional visualization of structure/topography of ocular structures.

Conclusion: Our study is the critical first step towards a swine WET model optimized for viability, retinal survival, ON regeneration and reintegration while documenting key immune responses, and enabling key neuro-immuno-therapeutic interventions.

Using Micropatterned Extracellular Matrix Cues to Guide Murine and Human Myotube Formation

Rebecca Duffy (1), Yan Sun (1,3) and Adam W. Feinberg (1,2)

(1) Department of Biomedical Engineering, (2) Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA and (3) School of Biological Science and Medical Engineering, Beihang University, Beijing, China

While the process of myogenesis is well understood in vivo, there is a need for a better understanding of how to guide formation of uniaxially aligned skeletal muscle in vitro. Specifically, we wanted to investigate how extracellular matrix (ECM) composition and geometry drive formation of aligned myotubes. To do this, we compared murine and human myotube formation on laminin (LAM), fibronectin, collagen IV, and collagen I. We examined how ECM geometry guided myotube alignment by comparing myotube formation on 20, 50, 100, and 200 um wide LAM lines with 10, 15, 20, and 30 um line spacings. Results showed that for both species, significantly more myotubes differentiated on LAM compared to fibronectin, collagen IV, and collagen I lines. C2C12 mouse myoblasts were highly aligned on 20, 50 and 100 um wide lines with 20 and 30 um spacings, but the highest myotube densities were observed on 50, 100 and 200 um wide lines with 10 and 15 um spacings. Human myotubes, contrastingly, were well aligned and had similar myotube densities on all line patterns. Our findings showed that LAM increases myotube formation for C2C12 and human primary myoblasts, but there are species specific differences for geometric conditions that increase myotube formation while controlling orientation of myotube alignment. Future work will focus on understanding how micropatterned ECM cues influence functional output as measured by twitch force of mouse and primary human skeletal muscle.

A vascular bioreactor model and perivascular hydrogel to study and treat bicuspid aortic valve associated aortopathy

George Fercana (1), Marie Billaud (1,4), Stephen Badylak (2,3,4), Julie Phillippi (1,3,4), Thomas Gleason (1,3,4)

(1) Department of Cardiothoracic Surgery, (2) Department of Surgery, (3) Department of Bioengineering, (4) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation and is associated with degeneration of the ascending aortic wall, potentially leading to highly fatal aortic tears (dissection). Persistent degeneration continues in BAV patients despite aortic valve replacement to correct valvulopathy, and has been associated with over-active tissue degrading matrix metalloproteinase enzymes (MMPs) in the aortic wall, suggesting an underlying cell-based mechanism for the associated aneurysm in proximal ascending aorta. A potential therapy is mammalian extracellular matrix (ECM)-derived hydrogels, which have been shown to retain transforming growth factor-beta 1, a growth factor known to decrease MMP-2 and -9 expression and activity in arterial smooth muscle cells. To investigate this mechanism and potential therapy, we actively leverage an extensive bank of human ascending aortic tissue and primary smooth muscle cell (SMC) populations (500+ specimens), utilize a custom-built perfusion vascular bioreactor system, and will implement a bioactive hydrogel produced from decellularized porcine aorta. These three components will be combined in order to establish an in vitro disease model of the BAV ascending aorta to also test the hypothesis that aortic ECM-derived hydrogels invoke downregulation of MMPs in primary SMCs isolated from BAV patients. Previously we have demonstrated de novo collagen and elastin production within SMC-seeded scaffolds after 4 weeks of bioreactor culture. Additionally, aortic ECM-derived hydrogels exhibit gelation kinetics similar to those of small intestinal sub-mucosa, maintain a fibrous ultrastructure when interrogated via scanning electron microscopy, and demonstrate a mitogenic effect on endothelial cells. To implement this hydrogel into the bioreactor system, a custom airbrush assembly has been developed to spray aerosolized hydrogel directly onto polymeric vascular scaffolds, forming a perivascular sheathe, with a collagen gradient visualized by picrosirius red staining. This hydrogel will be validated in vitro, with the ultimate goal of clinical translation to halt or prevent persistent aortic degeneration in BAV patients undergoing aortic valve replacement surgery.

Regenerating a kidney in a lymph node

Maria Giovanna Francipane (1, 2) and Eric Lagasse (1)

(1) McGowan Institute for Regenerative Medicine, Department of Pathology, University of Pittsburgh, Pittsburgh, PA and (2) Ri.MED Foundation, Palermo, Italy.

The ultimate treatment for end-stage renal disease (ESRD) is orthotopic transplantation. However, the demand for kidney transplantation far exceeds the number of available donor organs. While more than 100,000 Americans need a kidney, only 17,000 people receive a kidney transplant each year (National Kidney Foundation's estimations). In recent years, several regenerative medicine/tissue engineering approaches have been exploited to alleviate the kidney shortage crisis. Although cell-based therapy shows promise in experimental animal models, it cannot be applied when renal structure has been profoundly altered, as in chronically injured kidney. In this respect, kidney regeneration is needed to rebuild a whole functional kidney de novo, eliminating the need for dialysis. To build a neo-kidney, some investigators have proposed to recapitulate early stages of organogenesis by implanting the primordial kidney, also known as the metanephros.

For tissue transplantation to be effective, a hospitable site must be relatively accessible, provide sufficient space for cell expansion and offer access to vasculature to support long-term engraftment. In our search to find an ideal transplantation site, we found the lymph node to be a good candidate. By directly injecting the lymph node with embryonic kidney tissues, we demonstrated engraftment of the donor cells and subsequent organ function.

While there is room for further improvement with regard to a contiguous collecting duct epithelium with a single path for urine, the degree of graft function observed in our study supports lymph node use to model kidney development or act as a bioreactor to grow replacement kidneys from patients' own cells. Lymph nodes adjacent to kidneys could be transplanted and graft-host ureter connection achieved through surgical and/or engineering techniques.

Local Induction of Regulatory Lymphocytes for the Treatment of Periodontal Disease

Ashlee Greene (1), Andrew J Glowacki (1,6,8), Sayuri Yoshizawa (6,7,8), Zhe Zhuang (6,7,8), Siddharth Jhunjhunwala (2,6), Andreia E Vieira (9), Gustavo P Garlet (9), Charles Sfeir (2,6,7,8), Steven R Little (1,2,3,4,5,6,8)

(1) Departments of Chemical Engineering, (2) Bioengineering, (3) Pharmaceutical Science, (4)Immunology, (5)Ophthalmology, (6) The McGowan Institute for Regenerative Medicine, (7) Department of Oral Biology, (8) The Center for Craniofacial Regeneration, University of Pittsburgh, Pittsburgh, PA, USA, (9) Department of Biological Sciences, School of Dentistry of Bauru, Sao Paulo University (FOB/USP), Bauru, SP, Brazil

Periodontal disease affects over 78 million people in the United States alone and is a critical oral health care issue according to the American Dental Association. Periodontitis is characterized by bacterial plaque buildup, gum inflammation, loss of gingival tissue attachment, and pocket formation around the teeth. Invasive bacteria trigger a heightened immune response that destroys the connective gingival tissue and leads to bone resorption. Current treatment emphasizes removal of the oral bacterial load through procedures such as scaling and root planing often accompanied by antibiotic treatments. Literature suggests that the immune imbalance caused by the inflammatory response could be reversed by the local presence of regulatory lymphocytes (Tregs) that restore immunological homeostasis. Recently, we showed that local recruitment of endogenous regulatory lymphocytes aids in reversing the progression of periondontitis. The local, controlled release of the chemokine CCL22, induced Tregs recruitment which re-established periodontal homeostasis. However, this approach relies on the availability of functional, natural, regulatory T cells, and it is becoming more apparent that many inflammatory diseases may result from dysfunctional Tregs. Therefore, we hypothesize that the local induction and expansion of regulatory lymphocytes may provide a more robust mechanism to increase the number of functional regulatory T cells in the periodontium, and reverse disease conditions. To this end, we have fabricated controlled release microparticles containing the Treg-inducing factors; IL-2 (cytokine that promotes the differentiation of immature T cells into regulatory T cells), TGF-β (growth factor essential for Treg differentiation) and rapamycin (mTorr inhibitor), which have been shown to locally induce regulatory lymphocyte generation. Using a mouse model for periodontal disease, we have shown that the use of Tri-factor controlled release microparticles significantly reduced disease outcomes.

ECM hydrogel injection for the treatment of stroke: Time course comparison of hydrogel retention and phenotypic characterization of invading cells

Harman Ghuman (1,2), Julia Donnelly (5), Andre Massensini (1,3,6), Stephen Badylak (1,2,4), Michel Modo (1,2,3)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Bioengineering, (3) Department of Radiology, (4) Department of Surgery, (5) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; (6) Universidade Federal de Minas Gerais, Department of Physiology and Biophysics, Belo Horizonte, Brazil

Stroke is the leading cause of adult disability and a significant effort is under way to develop therapies to repair the damaged tissue. Biomaterials composed of mammalian extracellular matrix (ECM) promote constructive tissue remodeling with minimal scar formation. At ECM concentrations that have similar rheological properties as brain tissue, the biomaterial exists in fluid phase at room temperature, while forming hydrogels at body temperature. ECM with different concentrations (0, 1, 2, 3, 4, 8 mg/mL) was injected into the lesion cavity after stroke to support endogenous repair mechanisms. Retention and gelation of the ECM, as well as host cell invasion and phenotype was analyzed at 1, 14 and 28 days post-injection using immunohistochemistry. Retention of ECM hydrogel within the cavity occurred at concentrations >3 mg/mL, with extensive diffusion into the host tissue at lower concentrations. A significant host cell invasion into the ECM hydrogel was seen at 1 day post-injection, with an average of over 35,000 cells invading in the 8 mg/mL concentration. As the acute inflammatory response was replaced with an ECM remodeling phase at later time points, there was a significant decrease in the total number of cells invading the biomaterial. Initial invading cells were of a microglia and macrophage phenotype and followed specific trails into the ECM biomaterial along topological features conducive to cell migration. The follow-on cells were neural and oligodendrocyte progenitor cells, which are essential for repopulation of the neural tissue. This characterization demonstrates that an ECM hydrogel can be readily injected and retained within the lesion cavity, while promoting an acute endogenous repair response. Longer time points and behavioral studies are necessary to evaluate the therapeutic potential of this approach.

Poster number 103 *Category: Tissue Engineering*

Phenotypic Changes of Stromal Vascular Fraction Cells for Use in a Tissue Engineered Vascular Graft

Darren Haskett (1,6), Katelyn Bruce (5), Jeffrey Krawiec (2,6), Justin Weinbaum (2,6), Antonio D'Amore (1,2,6), William Wagner (1,2,6), Lauren Kokai 3,6), J. Peter Rubin (3,6), David Vorp (1,2,4,6,7)

(1) Department of Surgery, (2) Department of Bioengineering, (3) Department of Plastic Surgery and (4) Department of Cardiothoracic Surgery University of Pittsburgh, Pittsburgh, PA, (5) Department of Bioengineering Carnegie Mellon University, Pittsburgh, PA, (6) McGowan Institute for Regenerative Medicine and (7) Center for Vascular Remodeling and Regeneration, University of Pittsburgh, Pittsburgh, PA

Significance: Small diameter tissue engineered vascular grafts (TEVGs) containing adipose derived mesenchymal stem cells (ADMSCs) have been shown to promote remodeling and vascular homeostasis in vivo and offer a possible treatment solution for those suffering from cardiovascular disease. However, the time needed for culture represents a large hurdle for such a treatment option, whereas TEVGs containing cells taken directly from the stromal vascular fraction (SVF) following liposuction would not suffer such an impediment. SVF is known to be comprised of multiple phenotypes of cells, and preliminary results have shown that TEVGs seeded, shortly cultured, and implanted with SVF from young healthy individuals will remain patent in rats and will undergo remodeling. However, it is unknown which cell types are binding to the scaffold.

Objective: The current study aims to determine the phenotypes of the scaffold-binding cells from SVF (in comparison to the seeded pool) and how these phenotypes change up to four passages from harvest (which is the current standard for ADMSC TEVGs).

Methods: Poly(ester urethane)urea bilayered scaffolds are seeded with SVF immediately after acquisition and at each subsequent passage up to passage 4, incubated for a short period to allow cells to be incorporated into the scaffold and then fixed. Fixed sections are then analyzed using immune-fluorescent chemistry (IFC) for markers that define difference phenotypes.

Results and Discussion: IFC analysis shows positive staining for CD31 and CD34, markers of endothelial cells and endothelial precursor cells respectively, when SVF is used directly (without passage). Gradually the percent of cells staining for both CD31 and CD34 decreases over passage, until it is absent at passage 4. In contrast, staining for CD90 (an MSC marker) is present in all samples that were tested.

Conclusion: Understanding the differences between SVF and passaged ADMSCs for seeding of TEVGs will allow for swifter fabrication and ultimately better clinical translation.

Poster number 104 *Category: Tissue Engineering*

Functional and structural improvements of the hearts in myocardial infarction model by controlled delivery of a heparin-based coacervate of fibroblast growth factor-1

Yan Huang (1), Zhouguang Wang (1), Yadong Wang (1)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

Abstract: Emerging evidence supports the beneficial effect of fibroblast growth factor-1 (FGF1) on heart diseases, but its application has been hindered by the short half-life of the free protein. To achieve effective growth factor delivery, we designed an injectable coacervate which could protects growth factors and preserves their bioactivities. In this study, acute myocardial infarction (AMI) model was established and the cardioprotective effect of the FGF1 coacervate was investigated. As shown in the echocardiographic results, FGF1 coacervate inhibited ventricular dilation and preserved cardiac contractibility more than free FGF1 and saline group within the 6-week duration of the experiments. Histological examination revealed that FGF1 coacervate reduced inflammation and fibrosis post-MI, significantly increased the proliferation of endothelial and mural cells, and resulted in stable capillaries and arterioles. Besides these effects, FGF1 coacervate improved the proliferation of cardiac stem cells at 6 weeks post-MI. On the contrary, identical dosage of free FGF1 showed no statistical difference from saline group. In conclusion, our results showed that injection of FGF1 coacervate attenuated the injury caused by MI and may be a potential new therapy for myocardial infarction.

Poster number 105 *Category: Tissue Engineering*

Novel polysaccharide compound accelerates wound healing in a porcine MRSA-infected wound model

Isaac James (1), Debra Bourne (1), Mayara Silva (1), Peng Chang (1), Sheri Wang (1), Phillip Gallo (1), Latha Satish (1,2), Kacey Marra (1,2,3), J. Peter Rubin (1,2,3)

(1) Department of Plastic and Reconstructive Surgery, (2) McGowan Institute for Regenerative Medicine, (3) Department of Bioengineering

BACKGROUND: Methicillin-resistant Staphylococcus aureus (MRSA) is the most common etiology of wound infection in the US, causing considerable morbidity and mortality, and costing more than $10 billion annually. Current treatments are limited, and antibiotics are often ineffective. SYN01 is a non-toxic, polycationic polysaccharide which may act as a defensin mimetic. It has been shown to disrupt biofilms and prevent bacterial colonization in vitro. Using a porcine model, we sought to investigate SYN01 as a therapeutic for MRSA-infected wounds.

METHODS: Circular, full-thickness excisional wounds 4cm in diameter (n=20) were created on the backs of 6 month-old female Yorkshire pigs. Either 10^5 or 10^8 CFU of a clinical MRSA isolate (Xen31, ATCC:33591) was rubbed into to each wound using a tongue blade. Wounds were covered individually for 30min to allow bacterial adherence and treated with SYN01 or equivalent volume of sterile saline. Treatments were randomized to account for anteroposterior differences in wound healing. Wounds were covered with Tegaderm & OpSite to prevent cross-contamination between wounds. Treatments were reapplied at 48hrs and animals were sacrificed at 5d. 3mm punch biopsies were taken from each wound, homogenized and serially diluted in saline, and plated to agar for CFU quantification. Wounds were traced at 0d and 5d to track closure.

RESULTS: All wounds developed clinically significant MRSA infection by 48hr. Compared to saline control, SYN01 reduced bacterial load by 1.5-fold ($p=0.12$) and 8-fold ($p<0.005$) in the 10^5 and 10^8 CFU wounds respectively. Wound closure trended 13.0% (+/-6.2%) and 9.6% (+/-1.9%) faster in the 10^5 and 10^8 CFU groups respectively.

CONCLUSIONS: Two topical treatments of SYN01 reduced bacterial load and resulted in faster wound closure in MRSA-infected porcine wounds. SYN01 appears to confer greater benefit in wounds with higher bacterial loads and may provide a promising new therapeutic option for difficult wound infections.

Heart progenitor cells undergo a mechanically-regulated mesenchymal-to-epithelial transition that is required for vertebrate heart formation

Timothy R. Jackson (1), Hye Young Kim (1), Lance A. Davidson (1,2,3)

(1) Department of Bioengineering, (2) Department of Developmental Biology, (3) Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA

Congenital heart defects, often resulting from errors in early heart development, are the leading cause of death in infants in the United States. Recent studies have highlighted the importance of how mechanics drive organogenesis and yet the physical mechanisms that underlie early heart formation remain poorly understood. Although the role of sequential epithelial-to-mesenchymal transitions have been extensively studied in cardiogenesis, researchers have ignored the role of the presumptive mesenchymal-to-epithelial transition (MET) that occurs during HPC movement from bilateral fields in the anterior lateral plate mesoderm to the ventral midline. We hypothesize that spatiotemporally specific mechanical signaling from the HPC microenvironment regulates HPC MET, which initiates cell behavior changes necessary for proper heart formation. Using the model organism Xenopus laevis, we manipulate bulk tissue stiffness with small molecule inhibitors and quantify MET progression through epithelial marker localization to the apical surface of HPCs using immunofluorescence. We also use targeted injections to perturb tensile forces generated by the underlying endoderm and evaluate its effects on MET. Reduction of tissue stiffness inhibits MET and results in a two-fold reduction in epithelial marker localization, while increasing stiffness causes a precocious MET and a 50% increase in localization. Perturbing endoderm-generated tensile forces disrupts the timing of MET. Both delayed and precocious MET result in a failure of cardiomyocytes to incorporate into the vertebrate heart. We conclude that MET in HPCs is regulated by mechanics. Our findings expose the role played by biomechanics during early heart organogenesis and provide deeper insights into the etiology of congenital heart defects and developing biomimetic methods of heart regeneration.

Poster number 107 *Category: Tissue Engineering*

Tracking Scleral Collagen Uncrimping During Stretch Using a Novel Method

Ning-Jiun Jan (1,2), Michael Iasella (2), Mason Lester (2), Danielle Hu (1), Kira L. Lathrop (1), Andrew P. Voorhees (1), Huong Tran (1,2), and Ian A. Sigal (1,2)

(1) Ophthalmology, University of Pittsburgh School of Medicine, UPMC Eye Center, Eye and Ear Institute, Ophthalmology and Visual Science Research Center, Pittsburgh, PA, United States, (2) Bioengineering, Swanson School of Engineering, University of Pittsburgh, Pittsburgh, PA, United States

Collagen has a natural waviness called crimp that largely determines the nonlinear mechanical behavior of many ocular tissues. This is important in the study of many ocular diseases, including myopia, keratoconus, and glaucoma. However, almost nothing is known about how crimp changes in eye tissue with stretch. We have developed a novel method for measuring collagen crimp changes during stretch. Our goal was to quantify the effects of macro-scale stretch on the micro-scale stretch and waviness of collagen fiber bundles of sclera.

Three sheep eyes were cryosectioned axially (30 micron). Six samples of equatorial sclera were mounted to a uniaxial stretcher and imaged with polarized light microscopy at various levels of macro-scale stretch and analyzed for collagen fiber orientation. Using manual markings, the local micro-scale stretch and waviness were tracked through different levels of stretch for several bundles of each section. Waviness was defined as the normalized SD of the fiber orientations along the bundle. Linear mixed effect models were used to test the association between bundle stretch and loss of waviness, or uncrimping.

We tracked the stretch and waviness of 21 bundles over an average of 9 stretch levels. Waviness decreased significantly with increasing micro-scale stretch ($P<0.0001$). Bundles had variable initial waviness, though all bundles uncrimped with sufficient stretch. Even within the same sample under homogeneous macro-scale stretch, bundles uncrimped at different levels of stretch and at different rates. Our results indicate that the sclera response to macro-scale stretch is highly heterogeneous at the micro-scale, in both the stretch level at which fiber bundles uncrimp and their uncrimping rate. Understanding the micro-scale response to mechanical loading is important for determining the role of collagen architecture on the macro-scale tissue biomechanics, and for understanding the biomechanical environment of scleral cells and their contributions to tissue growth and remodeling.

Poster number 108 *Category: Tissue Engineering*

Functionalization of Proteins using Sulfur(VI) Fluoride Exchange Chemistry

Shaohua Li (1), Laura Beringer (1), Changgong Wu (2), Ethan Kallick (1,2), Howard Edington (2), Michael Passineau (3), Saadyah Averick (1)

(1) Laboratory for Biomolecular Medicine, Allegheny Health Network Research Institute, (2) Allegheny Health Network Department of Surgery, (3) Gene Therapy Program, Allegheny Health Network Research Institute.

The modification of proteins with reactive handles has facilitated the use of these biomolecules in diverse fields including drug delivery, diagnostics, environmental remediation, and cell culture matrices. Recently, a new “click” type reaction, sulfur(VI) fluoride exchange (SuFEx), was reported between sulfuryl fluoride and amines to a yield sulfamoyl fluoride (–NSO2F) moiety. The model protein bovine serum albumin (BSA) was reacted with SO2F2 gas under biphasic conditions to form BSA-SO2F. The resultant BSA-SO2F was characterized using gel electrophoresis, mass spectroscopy and Fourier transmission infrared spectroscopy to confirm the addition of the sulfamoyl fluoride functional group. SuFEx modification of BSA caused a marked change in the pH dependent size and zeta potential of the protein as well as increased the proteins melting temperature. Crucially, BSA-SO2F was demonstrated to be biocompatible after a 72-hour incubation with A549 lung endothelial cells. Due to the unique and elective reactivity of the SuFEx reaction with amines under high temperature, BSA-SO2F could be self-condensed to form a biocompatible hydrogel that was used to culture HEK 293 cells. The characterization of this reaction as well as the biocompatibility of the hydrogel upon completion has significant implications for the field of regenerative biology and medicine. This communication, to our knowledge, is the first report of the application of the SuFEx in the field of bioconjugates.

Enhancement of angiogenesis by bioactive elastic scaffold for in situ tissue engineering of the vascular system

Soo Hyon Lee (1), Kee-Won Lee (1), Jin Gao (1) and Yadong Wang (1,2,3)

(1) Department of Bioengineering and McGowan Institute for Regenerative Medicine, (2) Department of Chemical and Petroleum Engineering, (3) Department of Surgery, University of Pittsburgh, Pittsburgh, PA

Biodegradable synthetic polymers have been widely explored for their great potential in soft tissue engineering. In particular, over the last two decades, polyester family such as polygylcolide or polylactide has been developed to be used as scaffolds for tissue regeneration owing to its bio mimetic mechanical properties and its tunable biodegradability. In this study, we focus on attributing an angiogenic bioactivity to a biodegradable polyester scaffold to achieve an efficient in situ tissue regeneration for vascular disease. We synthesized a new PGS-derived polymer, poly(glycerol sebacate linoleate) (PGSL) by polycondensation of three biomolecules, glycerol, sebacic acid, and linoleic acid. The mechanical properities of PGSL has been conserved to be similar to the previous PGS polymer by controlling the ratio of three molecules in the polymer. The biocompatibility of PGSL was determined by in vitro cell viability assay and by implanting the material into sub-cutaneous of mice. Biodegradability tests were also carried out in physiological condition in presence of enzyme and in Dulbecco's phosphate buffered saline (DPBS). Furthermore, vascular endothelial cell migration was monitored after implantation of material in mice, through the release of the angiogenic fatty acid units during degradation of the material. The biodegradability and enhanced angiogenesis of PGSL together can be a promising candidate to apply as new class of scaffold for blood vessel regeneration.

Poster number 110 *Category: Tissue Engineering*

Evaluation of a Long-Term Survivor after Whole Eye Transplantation

Chiaki Komatsu (1), Yang Li (1,2), Maxine R. Miller (1,3), Bo Wang (3),Yolandi van der Merwe (4), Hongkun Wang (1), Liwei Dong (1,2) , Lin He (1,2), Huamin Tang (1,2), Ian Rosner (1), Nataliya Kostereva (1), Mario G. Solari (1), Shuzhong Guo (2), Gadi Wollstein (3), Joel S. Schuman (5), Valeria Fu (3), Kevin C. Chan (3,4), Vijay S. Gorantla (1), Kia M. Washington (1,6)

(1) Dept. of Plastic Surgery, UPMC, Pittsburgh, PA, (2) Dept. of Plastic and Reconstructive Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, China, (3) UPMC Eye Center, Eye and Ear Institute, Ophthalmology and Visual Science Research Center, Univ. of Pittsburgh SOM, Pittsburgh, PA, (4) Dept. of Bioengineering, Swanson School of Engineering, Univ. of Pittsburgh, PA, (5) Dept. of Ophthalmology, New York Univ. School of Medicine, New York, NY, (6) VA Pittsburgh Healthcare System, Pittsburgh, PA

Purpose: Approximately 39 million people worldwide suffer from blindness. Whole eye transplantation gives the opportunity to provide viable retinal ganglion cells and an entire optical system to recipients with vision loss. We previously established a functional face transplant model in the rat, and have expanded our model to include the whole eye, optic nerve and its blood supply. The purpose of our study is to evaluate gross morphology, viability and structural integrity in a long-term survivor after orthotopic whole eye transplantation.

Methods: A syngeneic transplant was performed in a Lewis (RT1l) rat. The graft was transplanted to the recipient and vascular anastomoses were performed, as were nerve appositions between donor and recipient optic nerves. A similar aged Lewis rat served as the non-transplanted control. Slit lamp examination, optical coherence tomography (OCT), gadolinium-enhanced magnetic resonance imaging (Gd-enhanced MRI) and electroretinography (ERG) were performed to evaluate the viability and structural integrity of the eyes of the transplanted rat and the naïve eyes of the control.

Results: The long-term survivor was at least 482 days PO at the time of OCT, MRI and ERG testing. Although corneal opacification prohibited OCT imaging of the retina of the transplanted eye, the transparency of the cornea and lens of the transplanted rat's native eye was confirmed. It correlated with the naive eyes of the control long-term survivor. Qualitative analysis of the retina of the transplanted rat's native eye revealed maintenance of structural integrity. Gd-enhanced MRI imaging revealed that aqueous humor dynamics in the native eye were comparable to eyes of the control. ERG revealed normal electrical responses to light stimuli in the native eye as in the control eyes.

Conclusion: We have established a viable orthotopic model for vascularized whole eye transplantation in the rat. Preliminary longitudinal study of our model reveals that structural integrity and function are maintained in the native eye after 482 days after whole eye transplantation.

Platelet Rich Plasma Impairs Chondrogenesis of Infrapatellar Fat Pad Derived Adipose Stem Cells In Vitro

Jr-Jiun Jean Liou (1,2), Benjamin B. Rothrauff (2), Peter G. Alexander (2), Rocky S. Tuan (1,2,3)

(1) Department of Bioengineering, Swanson School of Engineering, (2) Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, School of Medicine, (3) McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Post-traumatic focal degenerative cartilage defects of the knee affect >3 million Americans annually. Autologous cell-based cartilage repair, e.g., matrix assisted autologous chondrocyte implantation (MACI), is challenged by the need for ex vivo cell expansion and donor cartilage tissue site morbidity. Chondrogenic mesenchymal stem cells (MSCs) represent a promising cell type for cartilage repair. We have tested here the use of platelet-rich plasma (PRP), previously shown to promote stem cell proliferation and tissue healing, to enhance chondrogenic differentiation of human MSCs derived from infrapatellar fat pad (IFP-ASCs). IFP-ASCs were cultured as high-density pellets or encapsulated in photocrosslinked gelatin hydrogels in serum-free chondrogenic medium supplemented with 1, 5, 10, or 20% PRP for different durations (1-,3-, 7-, and 21-day pulses from beginning of culture). On day 21, cultures were analyzed for chondrogenic gene expression (SOX9/COL2/ACAN/COL10/MMP13) by qRT-PCR. Histological sections were stained with Safranin O/Fast Green and Alcian Blue/Nuclear Fast Red and immunostained for collagen type II. Sulfated glycosaminoglycan (GAG) contents were normalized to DNA. In 21-day pellet cultures, qRT-PCR and histological analysis of pellet cultures revealed that high PRP concentrations delivered over 7-21 days increased catabolic gene expression (MMP13), decreased chondrogenic gene expression (COL2 and ACAN), and decreased proteoglycan deposition, compared to TGFβ+/PRP- controls. In contrast, short and low concentration PRP treatment (1-day pulse, 1% PRP) resulted in increased cartilage matrix production and collagen type II deposition, although gene expression levels were not significantly different. Similar results were observed in gelatin hydrogel cultures: PRP treatment highly downregulated SOX9, COL2, and ACAN and decreased histologically detectable cartilage matrix deposition. These results showed that high concentration PRP treatment for prolonged period, while enhancing cell proliferation, impairs MSC chondrogenesis in vitro. Ongoing mechanistic studies aim to probe the mechanisms underlying the effects of PRP and the involvement of BMP-Smad2/3 signaling. (Funding: US Dept of Defense)

Macrophage Response to Lipid Accumulation with Increased ECM Source Animal Age

Samuel T. LoPresti (1,2), Bryan Brown (1,2,3)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine and (3) Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh PA

Macrophage polarization has been demonstrated to be a critical factor in the successful remodeling of extracellular matrix-based biomaterials (ECM). Differences in host response to tissue injury and disease have been shown to be deficient with age, but only in macrophages derived from tissues not the bone marrow. Our hypothesis is that ECM microenvironment components from aged donors contribute to dysfunctional host responses and could be conserved in ECM scaffolds fabricated from aged animal or cadaver tissue. One specific age-related change is the accumulation of lipids in aged tissues. The objective of the present study is to evaluate differences in the macrophage phenotype and function in response to lipid content of different age ECM.

Porcine small intestinal submucosa ECM was produced from 12, 26 and 52 week-old animals. Lipids were extracted using a chloroform:methanol solution. Lipids were quantified by a Free Fatty Acid Quantitation Assay. Bone marrow derived macrophages from 8 week-old (young) and 18-month old (aged) mice were exposed to M1 (IFN-γ/LPS) or M2 (IL-4)-polarizing cytokines or ECM lipid extracts for 24h. Macrophage phenotype was assessed using immunolabeling for F4/80, iNOS, Arginase, Fizz1, CCR2, CX3CR1 and MHC-II and Taqman gene expression for IL-1b, IL-12b, TNFa, IL-10, and PPARg. Macrophage function was assessed for phagocytosis using Vybrant Phagocytosis Kit and nitric oxide production using Greiss reagent system.

ECM scaffolds increased in fatty acid content with source animal age. ECM lipid extract exposure caused increased pro-inflammatory surface marker labeling, gene expression, and nitric oxide production with increased ECM age while decreasing phagocytosis.

These results show a compromised ability of ECM microenvironments to support the polarization of macrophages to anti-inflammatory phenotype with age-related lipid accumulation. These suggest implications for sourcing of biological ECM scaffolds and potential treatments to remove age-related changes such as lipids and glycation from these scaffolds during fabrication.

Controlled delivery of PRP proteins accelerate healing in a porcine wound model

Daniel Long (1), Noah Johnson (1), Hidetaka Hara (2) and Yadong Wang (1,3,4,5)

(1) Department of Bioengineering, (2) Thomas E Starzl Transplantation Institute, (3) McGowan Institute for Regenerative Medicine, (4) Department of Surgery, (5) Department of Chemical and Petroleum Engineering, University of Pittsburgh

Skin injuries such as trauma, burns, and surgical incisions are among the most prevalent issues in healthcare, and the annual market for wound care products exceeds $15 billion in the United States alone. Due to an aging population, widespread obesity, and increasing incidence of diabetes, the size of the market is only expected to increase. Wound healing is a complex process coordinated by the expression of many proteins such as Vascular Endothelial Growth Factor, Keratinocyte Growth Factor, and Platelet-Derived Growth Factor. Rather than applying these growth factors individually, we selected platelet-rich plasma (PRP) for its clinical availability and high concentration of numerous therapeutic proteins. However, the short half-life of PRP proteins requires that high doses be used and has led to inconsistent results in prior wound healing studies. There is also a deficit of systematic large animal studies assessing the efficacy of PRP treatments. To address these issues, we used a heparin-based coacervate delivery system to maintain the bioactivity of these proteins in the wound and slowly release them over time. When applied to full-thickness skin wounds on pigs, the controlled release of PRP proteins significantly accelerated healing as measured by the rate of epithelialization, wound contraction, and angiogenesis in the wound bed. Furthermore, the same dosage of PRP without the coacervate system had no effect on healing. These results demonstrate the importance of a delivery system for PRP proteins and reveal a promising and clinically-relevant therapy for skin wound healing.

Validating Microspheres for Use in Porous Scaffolds

Katherine Lorentz (1), Jeffrey Krawiec (1), Justin Weinbaum (1,7), Morgan Fedorchak (2,3), Antonio D'Amore (1,5,7), William R. Wagner (1,5,6,7), Steven Little (1,3,7), David Vorp (1,4,5,6,7)

Departments of (1) Bioengineering, (2) Ophthalmology, (3) Chemical and Petroleum Engineering, (4) Cardiothoracic Surgery, (5) Surgery, (6) Center for Vascular Remodeling and Regeneration and (7) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Significance

Cardiovascular disease is the number one cause of death in the US and treatment of this disease often requires the use of a vascular graft. In response, the burgeoning field of vascular tissue engineering has been working towards the development of a clinically-viable tissue engineered vascular graft (TEVG). Despite the promising results of previous studies utilizing autologous cell-based TEVGs, the use of this cell type poses two problems: regulatory concerns with regards to in-vitro cell expansion, and cell variability within different patient demographics (e.g., diabetics) precluding their use in TEVGs.

Objective

The purpose of this study was to determine if the pro-remodeling and anti-thrombotic properties of TEVGs could be replicated in a biodegradable, synthetic graft seeded with microspheres that release bioactive factors.

Methods

Bioactive factors known to promote remodeling were loaded into biodegradable microspheres and seeded into polyurethane urea (PEUU) scaffolds. The seeded scaffolds were incubated in a saline solution for 10 days, and samples of supernatant were withdrawn daily. Total protein in each supernatant sample was then measured with a commercial bicinchoninic acid assay. To test for uniform loading and retention in vivo, microspheres were loaded with fluorescein isothiocyanate (FITC), a fluorescent marker, and seeded into tubular PEUU scaffolds; the scaffolds were then implanted interpositionally within the abdominal aorta of a rat. The scaffolds were then explanted after 3 days, sectioned, and imaged for FITC fluorescence.

Results

A linear release of cargo from the scaffolds over a 10 day period was observed. Preliminary studies also showed that the microspheres remain within the graft after exposure to physiological flow in vivo. FITC-loaded microspheres alone were insufficient to prevent clotting, validating the need for a pro-remodeling agents within synthetic scaffolds.

Conclusion

These pilot studies suggest that our microsphere approach to TEVGs could be a viable alternative to current cell-based TEVGs.

Uncovering the Actin Network in Cardiomyocytes

Chelsea D Merkel (1), Donna Beer-Stolz (1,2), Adam V Kwiatkowski (1)

(1) Department of Cell Biology, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Cardiomyocytes must work in concert to produce the cyclical beating of the heart. This requires that individual cardiomyocytes interlock actin cytoskeletons for structural support and coordinate myofibrils for mechanical continuity. Yet little is known about how the cardiomyocyte cytoskeleton is organized to establish and maintain cohesion and form a highly functional contractile system.

Cardiomyocytes are joined end-to-end by a complex adhesive structure known as the intercalated disc (ICD). The ICD contains both adherens junctions (AJs) and desmosomes that link actin and intermediate filaments, respectively to the plasma membrane. The ICD is also commonly referred to as the “terminal Z-disc” because myofibrils, and their terminal barbed ends, are coupled to the plasma membrane there, presumably at adherens junctions. However, the organization of individual actin filaments along the adherens junction and the topology of the adherens junction in cardiomyocytes are unclear.

We sought to investigate actin cytoskeleton and myofibril organization in cardiomyocytes, specifically at the ICD, using super-resolution microscopy, scanning electron microscopy (SEM), and platinum-replica transmission electron microscopy (PREM). Super-resolution structured illumination microscopy (SIM) of mouse neonatal cardiomyocytes revealed organized myofibrils terminating at adherens junctions perpendicular to the ICD membrane. We then labeled the barbed ends of actin filaments in cardiomyocytes and imaged by stimulated emission depletion (STED) super-resolution microscopy. We observed G-actin incorporation at cell-cell junctions indicating that barbed ends of myofibrils are concentrated at AJs. Next, we used PREM to visualize actin ultrastructural organization. We found that cardiomyocytes possess a dense cortical actin cytoskeleton that enshrouds the underlying myofibrils unless physically removed. At contacts, cortical actin networks and underlying myofibrils from opposing cells are organized and linked. To visualize the myofibril network and AJs, we used hypotonic solution and physical force to “unroof” the apical plasma membrane and cortical cytoskeleton. Unroofing reveals myofibrils running parallel through the myocytes and terminating at junctional complexes. We are now combining immunolabeling with SEM and PREM to localize components of the AJ within the actin network at the ICD.

Our studies have begun to define the actin organization at the cardiomyocyte ICD. Knowledge of actin filament geometry, space, and integration at the cardiomyocyte AJ is needed to gain insight into the mechanics of cardiomyocyte adhesion and maintenance of the functional syncytium formed between cells.

Adipose Stem Cell Proliferation in Extracellular Matrix Derived Thermo-sensitive Hydrogel

Christopher Mahoney (1), Malik Snowden (1), J. Peter Rubin (1,2,3,4), Kacey G. Marra (1,2,3,4)

(1) Department of Bioengineering, (2) Department of Plastic Surgery, (3) School of Medicine, (4) The McGowan Institute of Regenerative, University of Pittsburgh, Pittsburgh, PA

Standards of care for soft tissue reconstruction for the repair of congenital deformities or defects from tumor resections/trauma include vascularized flaps or prosthetic implants consisting of silicone or saline. Although tissue flaps can have favorable results, complications may lead to flap failure, infections, pulmonary embolisms, and morbidity of the donor site. Autologous fat grafting using lipoaspirate is minimally invasive in reconstructive surgery but results are unpredictable due to resorption up to 10% volume retention. These limitations serve as motivation for developing therapies to regenerate adipose tissue. Abdomen whole fat was donated from a non-diabetic female (age: 41, BMI: 26.3) undergoing elective cosmetic surgery at the University of Pittsburgh Medical Center. The decellularization process includes four main phases consisting of alcohol rinses, delipidization, and disinfection of the adipose matrix. After processing, the matrix was snap frozen using liquid nitrogen and then lyophilized. A Mini Wiley Mill breaks down the lyophilized matrix into a powder for pepsin digest and hydrogel formation. PLGA (50:50) was used as the base polymer to encapsulate fluorescent dexamethasone in microscopheres using a single emulsion mixing technique. Adipose-derived stems cells (ASCs) were acquired using an isolation protocol on abdominal fat donated from a non-diabetic female (age: 38, BMI: 24.8) undergoing elective cosmetic surgery. Adipocyte quantification of ASCs study was conducted in a 12-well tissue culture plate along with Transwell tissue culture inserts to suspend the composite hydrogel above the cells in culture medium. The following culture conditions were used for comparison: cells with adipogenic medium, cells + composite hydrogel with adipogenic medium, cells + composite hydrogel without adipogenic medium, and cells in maintenance medium. At day 7 and 14, mature adipocytes were stained using the AdipoRed™ Reagent Assay (Lonza Brand). SEM images of the lyophilized hydrogel indicates porosity throughout the structure. As expected, higher concentrations of MS in hydrogel displayed a lower presence of porosity which may generate challenges for progenitor adipocytes migration into the scaffold. The differentiation study demonstrated higher amounts of adipogenesis in groups containing hydrogel and hydrogel with microspheres when compared to the positive control of ASCs alone. It is also important to note that adding the hydrogel to ASCs in maintenance medium resulted increased differentiation compared to the ASC in maintenance medium.

Long term performance of PEDOT/MWCNT/Dexamethasone coated electrode implanted in visual cortex of rats

Asiyeh Golabchi (1), Zhanhong Jeff Du (1,2,3), Xinyan Tracy Cui (1,2,3)

(1) Department of Bioengineering, (2) Center for Neural Basis of Cognition, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Implantable neural electrode arrays are an effective tool for restoring functions to people suffering from neurological disorders. These arrays can be implanted into cortex to provide single cell resolution electrophysiology with higher recording stability compare to the surface EEG recording. However, chronic neural recording is complicated by progressive decline in signal quality. The performance reliability is dependent on the interactions between tissue and the electrode itself. In long-term recording studies, the temporal degradation in signal quality such as single-unit yield and signal-to-noise ratio due to development of encapsulating glial scar and neuronal death has been reported. These limitations can be addressed by using organic electrode coatings which provide a combination of recording and stimulation advantages, including lowered impedance and increased charge transfer and ability to incorporate and release anti-inflammatory and neuroprotective drugs. Multi-walled carbon nanotubes (MWCNTs) loaded with dexamethasone can be incorporated into poly (3, 4-ethylendioxythiophene) (PEDOT) as electrode coatings to improve chronic stimulation. Previously, we have reported dexamethasone-loaded PEDOT/MWCNT-coated electrodes showed lowered impedance and reduced inflammation after 14 days of implantation into rat dorsal root ganglion compared to uncoated electrodes. Here, we report the tissue response and electrochemical behavior of the coated electrode/tissue interface during prolonged implantation period (>12 months). The coated electrodes performed very well in recording visually evoked neural response from rat visual cortex even at the chronic time points, showing great promise in advancing the quality and stability of chronic neural recording.

Total Human Eye Allotransplantation (THEA): Preclinical Cadaveric Studies

Maxine R. Miller (1,2), Edward H. Davidson (1), Eric W. Wang (3), Jenny Y. Yu (1), Juan C. Fernandez-Miranda (4), Dawn J. Wang (1), Joel S. Schuman (5), Kia M. Washington (1,6)

(1) Department of Plastic Surgery, (2) Department of Ophthalmology, (3) Department of Otolaryngology, (4) Department of Neurosurgery, University of Pittsburgh, Pittsburgh, PA, (5) New York University School of Medicine, (6) VA Pittsburgh Healthcare System, Pittsburgh, PA

Purpose: It is estimated that 40 million suffer from blindness globally. Whole eye transplantation offers the opportunity to provide viable retinal ganglion cells and an entire optical system to recipients with vision loss. Our group has established the first orthotopic model for eye transplantation in the rat. With advancements in immunomodulation strategies together with new therapies in neuroregeneration, parallel development of human surgical protocols is vital in ensuring momentum towards eye transplantation in patients.

Methods: Preserved injected human cadaveric heads (n=8) underwent donor and recipient procedures. Bilateral transplants were performed between two cadavers in each surgical session, for a total of 4 transplants between 2 cadavers. A globe and periorbita model was adopted. Donor procurement required orbital exenteration with combined endonasal and transcranial approach to decompress the orbital apex. Transection of cranial nerves II-VI and superior ophthalmic vein was performed at the cavernous sinus transcranially and the ophthalmic artery with carotid artery stem was ligated in the paraclival space to deliver the donor specimen. Candidate recipient vessels (superficial temporal, internal maxillary and facial artery and superficial temporal and facial vein) were exposed. All required vein grafting. Donor tissue was secured in recipient orbits followed by sequential arterial and venous anastomoses and nerve coaptation with standard microsurgical techniques. Pedicle lengths and calibers were measured. All steps were timed, photographed, video recorded and analyzed after each operative session.

Results: Technical feasibility of cadaveric donor procurement and transplantation to cadaveric recipient was established. Mean donor ophthalmic artery pedicle length and caliber were 13.5 and 1mm but with a stem of paraclival internal carotid artery were 33 and 2mm. Mean optic nerve was 25mm from orbital apex to annulus of Zinn and 14 mm from annulus of Zinn to optic chiasm. Cranial nerves III-VI had mobile pedicle lengths of 10 -14mm. Candidate recipient vessels required vein grafting.

Conclusion: This surgical protocol serves as a benchmark for optimization of technique, large animal model development, and ultimately potentiating the possibility of vision restoration transplantation surgery.

Extracellular Matrix and Spermatogonial Stem Cell Culture

Mark Murdock (1), Ilea Swinehart (1), Sherin David (2), Kathrin Gassei (2), Stephen Badylak (1), Kyle Orwig (2)

(1) McGowan Institute for Regenerative Medicine, University of Pittsburgh, (2) Magee-Womens Research Institute , University of Pittsburgh

Many factors contribute to or cause male infertility including infection, ductal defects, hormonal imbalances, and chemoradiation treatment. In some patients antibiotics, surgery, and hormonal medication can restore fertility. Other patients, especially those undergoing chemoradiation, have a compromised ability to produce sperm and currently no treatments are able to restore or enhance spermatogenesis. One potential therapeutic approach is the in vitro expansion of autologous spermatogonial stem cells (SSCs) with subsequent transplantation into the testes. However, cell culture of human SSCs has proven difficult and satisfactory conditions have yet to be identified that preserve stemness while promoting proliferation. The most common culture methods utilize STO feeder cells or murine laminin but do not yield satisfactory proliferation of human SSCs beyond the first passage.

Signaling molecules within mammalian extracellular matrix (ECM) have been shown to promote mitogenesis of stem/progenitor cells. ECM was obtained from porcine small intestinal submucosa (SIS) by mechanical and chemical decellularization, lyophilization, comminution, and sterilization. SIS powder was digested in pepsin for 1, 2, 3, or 4 days (SIS-1, SIS-2, etc.) for use in cell culture experiments. SSCs were cultured for five days in SIS coated wells (300ug/mL), SIS-spiked media (200ug/mL), or with STO feeder cells and expression of the SSC marker Sall4 was measured. There was a trend of decreasing Sall4 expression with increasing digestion time with SIS-1 coated wells, SIS-2 spiked media outperforming STO cells alone.

Additionally, SSCs were cultured for seven days in SIS coated wells, SIS-spiked media, or human laminin coated wells and stained for the SSC marker UTF-1. Cells grown on SIS-1, SIS-2, SIS-3, and SIS-4 coated wells had the following average percentages positive for UTF-1, respectively: 25.6%, 28.8%, 23.7%, and 15.5%. SSCs given media spiked with SIS digested for 1, 2, and 4 days had the following UTF-1-positive percentages, respectively: 30.4%, 21.5%, and 20.6%. SSCs grown on human laminin had 55.0% of cells positive for UTF-1. Our data suggest that while SIS provides superior culture conditions compared to STO feeder cells, human laminin is preferred. Experiments are in progress to evaluate the effects of human testicular ECM as a culture supplement.

In vivo ultrasound monitoring of cell-free, fast-degrading vascular grafts implanted in rat carotid artery

Muhammad Umer Nisar (1), Keewon Lee (2), Liwei Dong (3), Nadim Farhat (1,2), Yadong Wang (2,4,5), Kang Kim (1,2,5)

(1) Center for Ultrasound Molecular Imaging and Therapeutics, Department of Medicine and Heart and Vascular Institute, University of Pittsburgh and UPMC, Pittsburgh, PA, USA, (2) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA, (3) Department of Plastic Surgery, University of Pittsburgh and UPMC, Pittsburgh, PA, USA, (4) Departments of Surgery, Chemical Engineering, Mechanical Engineering and Material Science, Pittsburgh, PA, USA, (5) McGowan Institute of Regenerative Medicine, University of Pittsburgh and UPMC, Pittsburgh, PA, USA

Background: There are unmet clinical needs for vascular grafts with increasing cardiovascular diseases and aging population. Autologous grafts are current gold standard, but have donor site morbidity and limited availability. Tissue-engineered grafts have been proposed as an alternative, but have not shown clinical effectiveness yet, especially in small-diameter artery. We propose cell-free, fast-degrading vascular grafts for enhanced cell infiltration and in–host remodeling. Fast-degrading grafts should minimize the host's exposure to foreign material, thereby causing less chronic inflammation and thrombosis. Thus, timely and affordable assessment is necessary to evaluate these grafts post-implantation in preclinical animal model. The key monitoring factors for the graft include thrombogenic responses and patency.

Hypothesis: The patency and developed complications of vascular grafts can be monitored non-invasively by ultrasound imaging in animal model.

Methods: Grafts were fabricated using fast-degrading elastomer, poly(glycerol sebacate) (PGS) for a core and electrospun polycaprolactone (PCL) fibers for an outer sheath. Grafts were implanted into rat common carotid artery by end-to-end anastomosis (n = 4 per time point). Patency was checked using ultrasound B mode, Color Doppler mode and Pulsed Wave mode at 2, 4, 8 weeks post-implantation. Graft explantation was done if there was stenosis, dilation or infection seen by ultrasound imaging. Explanted grafts were analyzed by histology and immunofluorescence staining.

Results: Ultrasound successfully monitored the patency of the grafts in the carotid arteries. Graft complications such as thrombosis and dilation post-implantation were seen on ultrasound B mode, Color Doppler mode and Pulse Wave mode, later confirmed by explantation.

Conclusion: Ultrasound imaging enabled monitoring the patency of the graft and measuring the flow velocity non-invasively, without sacrificing animals at each time point. These results suggest that longitudinal monitoring of the grafts using ultrasound imaging might reduce variations between animals, therefore minimizing required number of animals and study time.

Poster number 121 *Category: Tissue Engineering*

Precisely sequenced poly(lactic-co-glycolic acid)s via entropy-driven ring-opening metathesis for bioengineering applications

Jamie A. Nowalk (1), Amy L. Short (1), Ryan M. Weiss (1), Tara Y. Meyer (1,2)

(1) Department of Chemistry, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Poly(lactic-co-glycolic acid)s (PLGAs) are a widely-used class of biodegradable polymers in regenerative medicine used to make osteofixation devices, cell scaffolds, and drug delivery matrices. Traditionally made from a random sequence of lactic and glycolic acid units, it has been found that controlling monomer sequence has a drastic effect on properties such as hydrolytic degradation, acidic microclimates, and immune response. Nature demonstrates the dynamic structure-function relationship that appears from polymer sequence, yet this concept is widely understudied in synthetic polymers largely due to the fact that sequenced polymers are synthetically challenging to prepare and methods to produce controlled molecular weights are lacking. This study focuses on the development of a method of preparing sequenced copolymers with controlled molecular weights using entropy-driven ring-opening metathesis polymerization (ED-ROMP), a polymerization that traditionally yields precise molecular weights. Using this novel synthetic method, we have demonstrated that sequenced PLGAs can be prepared with controlled molecular weight, high monomer conversion, and have the ability to undergo chain extension. Preparing these polymers will enable, for the first time, the study of sequence variation's effect on properties of PLGAs without a variable molecular weight. This synthetic methodology will also allow sequenced polymers to be prepared with reproducibility and on a larger scale than ever before.

Poster number 122 *Category: Tissue Engineering*

Augmented Repair of Radial Meniscus Tear with Biomimetic Electrospun Scaffold:An In Vitro Mechanical Analysis

Piya-on Numpaisal (1,5), Benjamin B. Rothrauff (1,4), Brian B. Lauro (1,3), Peter G. Alexander (1), Richard E. Debski (2,3,4), Volker Musahl (2,3) and Rocky S. Tuan (1,3,4)

(1) Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, (2) Orthopaedic Robotics Laboratory, Department of Orthopaedic Surgery, (3) Department of Bioengineering, Swanson School of Engineering, (4) McGowan Institute for Regenerative Medicine , University of Pittsburgh, Pittsburgh, PA, (5) Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan

Background: Extensive radial tear reduces meniscal function and increases risk of joint degeneration. Electrospun-nanofibrous scaffolds can mimic the topography and mechanics of meniscus, yet their incorporation into clinically relevant suture-repair is unexplored.Hypothesis: A biomimetic scaffold can be incorporated into standard meniscus suture repair without compromising repair mechanics, as compared to suture repair alone. Methods: Aligned, random, and biomimetic (aligned longitudinal-transverse-longitudinal and random) electrospun scaffolds were fabricated. Material properties of the scaffolds were determined in the parallel and perpendicular directions, as was suture retention strength. Complete radial tears of lateral bovine meniscus were repaired with a double horizontal mattress suture, with or without scaffold sheath. Mechanical testing (500 cycles of 5-20 N load and load to failure) was performed to determine. clamp-to-clamp, ultimate load, ultimate elongation, and stiffness. Results: Aligned scaffolds possessed the most anisotropic properties, whereas random scaffolds showed uniform properties in parallel and perpendicular directions. In comparison, the biomimetic scaffold possessed moduli in the parallel (68.7 ± 14.7 MPa) and perpendicular (39.4 ± 11.6 MPa) directions that respectively approximate the reported circumferential and radial tensile properties of native menisci. The ultimate suture retention load of the biomimetic scaffold in the parallel direction (7.2 ± 1.6 N) was significantly higher than all other conditions ($p < 0.001$). Biomimetic scaffold augmentation did not compromise mechanical properties when compared against suture repair in terms of residual elongation after 500 cycles (scaffold: 5.05 ± 0.89 mm vs. repair: 4.78 ± 1.24 mm), ultimate failure load (137.1 ± 31.0 N vs. 124.4 ± 21.4 N), ultimate elongation (12.09 ± 5.89 mm vs. 10.14 ± 4.61 mm), and stiffness (20.8 ± 3.6 vs. 18.4 ± 4.7 N/mm). Conclusion: A multilayer-electrospun nanofibrous scaffold can be incorporated within standard suture repair of radial meniscal tears without compromising repair integrity.

Poster number 123 *Category: Tissue Engineering*

Synthesis and Characterization of Doped Amorphous Calcium Phosphate based Cements (ACPC)

John Ohodnicki (1,4), Abhijit Roy (1,4), Boeun Lee (1,4), Emma Mcbride (1,4), Prashant N. Kumta (1,2,3,4,5)

(1) Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA, (2) Dept. of Chemical and Petroleum Engineering, Univ. of Pittsburgh, Pittsburgh, PA, (3) Mechanical Engineering and Materials Science, Univ. of Pittsburgh, Pittsburgh, PA, (4) Center for Complex Engineered Multifunctional Materials, Univ. of Pittsburgh, Pittsburgh, PA, (5) McGowan Institute of Regenerative Medicine, Univ. of Pittsburgh, Pittsburgh, PA

Amorphous calcium phosphate based cement (ACPC) scaffolds known for application in bone tissue regeneration have been shown to exhibit poor compressive strengths as well as inferior cytocompatability compared to other calcium phosphate cement (CPC) based scaffolds. Therefore, the goal of this study is to increase both the mechanical properties and cytocompatability of ACPC materials by the addition of a suitable dopant into the final hydroxyapatite lattice structure. In this study, amorphous calcium phosphate (ACP) and dicalcium phosphate dihydrate (DCPD) doped with 0-15 wt% of dopant were synthesized via a simple co-precipitation reaction. The scaffolds were prepared by mixing a solid powder component consisting of a 3:1 weight ratio mixture of ACP: DCPD and an aqueous phosphate solution.

In-vitro crystalline phase and morphological evaluations of the cement scaffolds were conducted using a plethora of materials characterization techniques after immersion and aging of the samples in phosphate buffered saline (PBS) for 0, 3, 7, and 14 days, respectively. X-ray diffraction (XRD) patterns revealed that higher levels of dopant substitutions facilitated inducement of the phase transformation reaction of the scaffolds to hydroxyapatite (HA) at earlier time points. Compression test analysis also revealed that scaffolds prepared with DCPD doped with 5 wt% and 10 wt% of the dopant exhibited higher compressive strengths compared to the un-doped scaffolds. On the other hand, scaffolds prepared with DCPD doped with 15 wt% dopant displayed significantly lower compressive strengths compared to the un-doped scaffolds. Additionally, the incorporation of DCPD doped with 15wt% dopant within the cement leads to extended initial (12 mins) and final setting (30 mins) times at 37 C, compared to cements made without the incorporation of dopant exhibiting an initial setting time of 6 mins and final setting time of 14 minutes. The use of pore formers and plasticizers were also studied to explore generation of porous and injectable cements.

In-vitro cell viability was assessed using an MTT assay utilizing MC3T3 cells via direct and indirect cell seeding methods at days 1, 4, and 7 of incubation while cell adhesion was assessed using live/dead staining. The results conclude that the doped cement scaffolds exhibit increased initial cell attachment as well as cell viability showing their likely potential as bone tissue engineering scaffolds. Results of these studies will be presented and discussed.

Poster number 124 *Category: Tissue Engineering*

Engineering Basement Membrane Micro-Scaffolds for Repair of the Corneal Endothelium

Rachelle Palchesko (1,2), Adam Feinberg (1,2,3)

(1) Department of Biomedical Engineering, Carnegie Mellon University, (2) Louis J Fox Center for Vision Restoration, (3) Department of Materials Science & Engineering, Carnegie Mellon University

Disease or injury to the corneal endothelium (CE) is one of the leading causes of corneal blindness worldwide. While corneal transplants are effective at restoring CE function, donor corneas are limited. Additionally, during transplantation, the donor CE cells are often damaged leading to premature graft failure. Thus, there is a need to develop new methods to treat corneal blindness due to CE failure. One promising therapy is the injection of cultured CE cells into the anterior chamber; however it is unknown whether single cells are capable of surviving injection, attaching to the posterior surface of the cornea and repairing the damaged monolayer. To address this problem, we have developed an ECM protein micro-scaffold that can be used to engineer CE cell rafts for cell delivery and repair of the CE by injection into the anterior chamber of the eye. These scaffolds mimic the ECM composition of Descemet's membrane, the native basement membrane of the CE cells in vivo, and are fabricated via surface-imitated assembly.

First, 200 micron square laminin and collagen IV micro-scaffolds were fabricated by microcontact printing onto the thermo-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm). The micropatterned samples were heated to 40ºC, seeded with bovine CE cells, and cultured for 24 hours so the cells could form monolayers on the squares. After 24 hours, the temperature was decreased below 32ºC to trigger the dissolution of the PIPAAm and release of the CE rafts. Upon release, the rafts folded up, with the ECM on the outside and the monolayer of cells on the inside. The rafts were collected via centrifugation, resuspended, and injected onto a collagen type I gel to mimic an anterior chamber injection of the cells onto a denuded stroma. The cells were cultured on the collagen gel for 6, 24 or 48 hours and fixed and stained for the nucleus, ZO-1, laminin and F-actin. The CE cells in the rafts were viable after injection and able to form a confluent monolayer that maintained their expression of ZO-1 through release and reseeding. In contrast, CE single cell suspensions seeded onto a collagen gel do not express ZO-1 during release and reattachment and only express ZO-1 once a confluent monolayer has formed.

These studies indicate that our CE cell rafts would be useful in therapeutic applications where a donor CE is unavailable. Future studies include testing the ability of the CE cell rafts to attach to a damaged or denuded cornea and restore corneal clarity in a rabbit model.

Self-assembled graphene nanocomposite hydrogels improve multinucleated myotube formation

Akhil Patel (1), Yingfei Xue (1), Rebecca Hartley (1), Shilpaa Mukundan (1), Vinayak Sant (1), Shilpa Sant (1,2,3)

(1) Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, (2) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, (3) McGowan Institute for Regenerative Medicine, Pittsburgh, PA

Trauma, cancer and genetic diseases damage skeletal muscle beyond its regenerative potential. Hydrogels hold a great promise for skeletal muscle regeneration due to their features similar to the native extracellular matrix (ECM). Additionally, biophysical stimuli such as nanoroughness, aligned microscale architecture, multiscale hierarchy conductivity and mechanotransduction are known to influence the myoblast differentiation. Recent skeletal muscle tissue engineering strategies focus on incorporation of these biophysical features individually, however, their combination remain an attractive approach to engineer a regenerative microenvironment. In this study, we designed a self-assembled nanocomposite hydrogel possessing multiple biophysical and nanoscale features to create interactive cell-material microenvironment.
Self-assembled hydrogel showed hierarchical fibrous structure from nano- to macroscale similar to myofibrils and fiber bundles in skeletal muscle. When interfaced with conductive graphene nanosheets, nanocomposite fibrous hydrogel showed uniform distribution of graphene throughout the scaffolds. Such arrangement throughout the scaffold is likely to provide conductive nanoplatforms facilitating better cell-cell communication as well as cell-material interaction due to increased nanoroughness. Compared to self-assembled hydrogels (without graphene), nanocomposite hydrogels resulted in increased ultimate tensile strength, reaching near 2.5 MPa. Morphological assessment of the fractured scaffolds revealed a more uniform stress distribution throughout the scaffold and prevention of deformation of the fibrous structure. Moreover, immobilization of graphene as nano-composites exhibited excellent cytocompatibility over a period of 14 days. Importantly, these composites promoted good cell spreading, evident by decreased aggregate formation. These composite hydrogels promoted myoblast differentiation and guided the formation of multinucleated continuous and fused myotube formation along the direction of fibers.

These results demonstrate that self-assembled graphene nanocomposite hydrogels with skeletal muscle-like multiscale hierarchy, aligned fibrous structure and conductivity is able to recapitulate the native skeletal muscle microenvironment. Synergistic influence of these biophysical features guide the myoblast cells to differentiate into multinucleated myotubes along the direction of fibers.

Modulation of Innate Immune Host Response and Antimicrobial Peptide Expression by Poly(4-Hydroxy Butyrate)

Catalina Pineda Molina (1,2), Brian M. Sicari (1,3), Ricardo Londono (4), Ross Giglio (1,2), Derek Wang (1), Riddhi M. Gandhi (1,2), Jenna Dziki (1,2), Shahab Shaffiey (4) and Stephen F. Badylak (1,2,3)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Bioengineering, (3) Department of Surgery, (4) School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

The bioactive metabolite, Butyrate, found mainly in the gastrointestinal tract has been associated with seemingly paradoxical effects; specifically, both an anti-inflammatory effect and antimicrobial effect (1). Poly(4-hydroxy butyrate) (P4HB), a hydroxylated form of butyrate, is a byproduct of bacterial metabolism, that has been commercially produced and used as an FDA approved 'biosynthetic' material for regenerative medicine and tissue engineering applications (2). Surgical mesh materials composed of P4HB degrade by hydrolysis in vivo and have been shown to promote resistance to deliberate surgical site bacterial contamination (3). The mechanism(s) behind the bacterial resistance remains only partially understood and is known to involve the host innate immune system. The present study evaluates the effects of biosynthetic mesh materials composed of P4HB upon host innate immunomodulation and antimicrobial peptide (AMP) expression.

Under in vitro conditions, an accelerated hydrolysis method (4) was used to obtain the degradation byproducts of P4HB. The AMP expression elicited by these hydrolysis byproducts, and the activation of the pro-inflammatory NF-kB pathway, were evaluated using mice bone marrow-derived macrophages. Surgical mesh materials composed of P4HB were evaluated using a rat bilateral partial thickness abdominal wall defect model in vivo (5). Immunolabeling quantification was used to determine the immunomodulatory effects of P4HB upon macrophage phenotype and AMP expression, at 3, 7, 14, 21, and 35 days post-implantation. The results suggest that surgical mesh materials composed of P4HB are associated with a predominant anti-inflammatory M2 phenotype, and increased expression of AMPs. Furthermore, the mechanism by which P4HB influences macrophage phenotype involves transcriptional regulation of NF-kB activation. Bioscaffolds composed of P4HB have the potential to provide the constructive regulatory and tissue "rebuilding" effects commonly seen with alternatively activated macrophages and the advantage of a reproducibly manufactured "synthetic" bioscaffold.

Acknowledgements
This work was supported as part of an industrial partnership with CR Bard, Inc.

References
1. Donohoe DR, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. Molecular Cell 48: 612, 2012.
2. Williams SF, Rizk S, Martin DP. Biomed Tech 58: 439, 2013.
3. Faulk DM, Londono R, Turner NJ, van der Merwe Y, Loneker AE, Sicari BM, Badylak SF. Manuscript in preparation.
4. Martin DP, et al. Journal of Surgical Research 184: 766, 2013.
5. Sicari B, Turner N, and Badylak SF. Methods Mol Biol 1037: 3, 2013.

An in vitro Chondro-Osteo-Vascular Model of the Osteochondral Complex for Studying Osteochondral Biology and for Drug Screening

Alessandro Pirosa (1), Riccardo Gottardi (1,2), Peter Alexander (1), Dario Puppi (3), Federica Chiellini (3) and Rocky Tuan (1)

(1) Center for Cellular and Molecular Engineering, University of Pittsburgh, Pittsburgh, PA, (2) Ri.MED Foundation, Palermo, Italy, (3) BIOLab Research Group, University of Pisa, Pisa, Italy

The development of veritable in vitro models of the osteochondral (OC) unit is essential in understanding the biology of cartilage/bone and in the development of high throughput screening approaches for drug testing (1). In this work, we have developed an OC model by combining a chondral construct based on photocrosslinkable methacrylated gelatin (gelMA), seeded with human bone marrow mesenchymal stem cells (MSCs), with a vascularized bone construct based on a poly(ε-caprolactone) (PCL) porous scaffold seeded with MSCs and GFP-transfected human umbilical vein endothelial cells (HUVECs). We used a 3D printed microphysiological tissue system bioreactor (2) that allows the simultaneous yet separate flow of specific media to the chondral and osseous components while maintaining them in contact and allowing tissue-tissue communication (3). After 4 weeks of differentiation, histology revealed chondrogenic differentiation (alcian blue staining) in the gelMA construct and osteogenic differentiation (alizarin red) in the PCL construct. The HUVECs formed clear interconnected networks of tube-like structures inside the scaffolds pores, as assessed by Live/Dead assay (using Calcein Blue AM). Stronger alizarin red staining was observed in the constructs containing HUVECs vs. no-HUVECs controls. RT-PCR of individual parts of the OC constructs showed up-regulation (vs. day 0) of chondral genes (COL2, ACAN, SOX9) and osseous genes (RUNX2, BSPII, OPN) in the gelMA and PCL construct, respectively. Presence of the HUVECs enhanced osseous gene expression compared to no-HUVECs control. These results strongly suggest that the engineered construct mimics native OC tissue in terms of structural architecture and gene expression profile.

Support: Commonwealth of Pennsylvania, NIH (1U18TR000532), Ri.MED Foundation, PhD grant (DM186/2008) by the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR).

References

(1) Alexander P.G., et al Experimental Biology and Medicine. 2014, 239: 1080-1095.
(2) Lozito T.P. et al. Stem Cell Research & Therapy. 2013, 4(Suppl 1):S6.
(3) Lin H. et al. Molecular Pharmaceutics. 2014, 11(7):2203-12.

Adipose-Derived Mesenchymal Stem Cell Secreted Factors Attenuate Elastin Expression with Rat Fetal Lung Fibroblasts and human aortic Smooth Muscle Cells in 2D and 3D Cultures

Aneesh K. Ramaswamy (1), Justin S. Weinbaum (1,5) and David A. Vorp (1,2,3,4,5)

(1) Department of Bioengineering, (2) Department of Cardiothoracic Surgery, (3) Department of Surgery, (4) Center for Vascular Remodeling and Regeneration, (5) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Abdominal aortic aneurysms (AAA) are enlargements of the abdominal aorta, characterized by the fragmentation of extracellular matrix (ECM) protein elastin resulting in decreased vascular wall strength. Currently, patients with AAA diameters larger than 5.5cm are treated with endovascular aneurysm repair (EVAR). Treatment options for patients with AAA dilations between 1.5- and 3-times average abdominal aortic diameter are limited. AAA rupture risk among patients with smaller-sized dilations can be significantly elevated given additional pathologies, such as obesity, diabetes or obstructive lung disease. Therefore, this abstract focuses on the need for an early therapeutic option in many elastin-deficient cardiovascular tissues. This report describes initial experiments to investigate transforming growth factor-β1 (TGF-β1) and secreted factors from adipose-derived mesenchymal stem cells (ADMSC CM), and their possible use in promoting elastin expression and organization in regenerative medicine therapies.

Rat fetal lung fibroblasts were cultured in 2D and 3D microenvironments to examine any changes in elastin expression. 2D cultures saw an increase in elastin fiber concentration, mean fibril diameter, and average fibril segment length between 8 and 13 days post-confluence, all of which decreased to initial seeding levels after 15 days. This leads to the hypothesis that an upper limit of elastin (or extracellular matrix in general) before the cells prioritize organization and equal tropoelastin dispersion over secretion.

3D cultures saw an increase in fibril concentration. Low treatments of Transforming Growth Factor-β1 addition the gels did not reach the previously seen limit that caused a switch from tropoelastin secretion to organization, even though an increase in elastin secretion through protein assays was detected. Cyclic uniaxial loading increases elastin expression, but additional TGF-β1 treatment does not attenuate this increase.

The conclusions of these studies, in addition to the development work on an elastin reporter gene's transfection into these cells, can establish a non-destructive monitoring method to evaluate elastin-targeted therapeutics for abdominal aortic aneurysms and other elastin-deficient diseases.

Toward Whole Liver Engineering: Liver Extracellular Matrix Promotes the Phenotype and Function of Hepatocytes and Liver Sinusoidal Endothelial Cells

Michelle Scarritt (1), Abigail Loniker (1), George Hussey (1), Scott Johnson (1), Li Zhang (1), Lisa White (1), Stephen Badylak (1)

(1) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Whole organ engineering offers an alternative to traditional liver transplantation that may address the critical shortage of donor organs and eliminate the need for recipient immunosuppression. Through perfusion decellularization of livers, biocompatible xenogeneic extracellular matrix (ECM) scaffolds can be produced that retain the liver's structural complexity. Subsequent recellularization of these scaffolds requires hepatocytes and liver sinusoidal endothelial cells (LSECs). However, these two cell types are notoriously difficult to culture in vitro—displaying a rapid loss of phenotype and function as well as limited proliferation—which restricts their application in liver engineering. We have previously shown that liver ECM can be used to enhance LSEC stability in 2D culture. Therefore, we hypothesized that liver ECM could also promote hepatocyte stability. Liver ECM was derived from rat, canine, porcine, and human livers decellularized by agitation with 0.02% tryspin/0.05% EGTA followed by 3% triton X-100. Liver ECM was then sterilized with 0.1% peracetic acid, rinsed with PBS, lyophilized, powdered, and digested with pepsin. Pepsin-digested liver ECM was added directly to culture media as a supplement or was mixed with PBS for use as a culture vessel coating solution. Supplementation of primary rat hepatocyte culture medium with 50 µg/mL liver ECM maintained hepatocyte phenotype as shown by the retention of cell morphology and the appearance of bile canaliculi. Canine liver ECM increased hepatocyte albumin production by 472±70% in comparison to controls, and porcine liver ECM increased albumin by 427±79%. In moving toward clinical translation, utilization of patient-derived cells for recellularization of liver scaffolds will minimize or eliminate graft rejection. One of the most promising cell types for this purpose is induced pluripotent stem cells (iPSCs). It has been shown that iPSCs can be differentiated into endothelial cells and hepatocytes. However, they have yet to be evaluated for liver recellularization. In preliminary work using human iPSC-derived hepatocytes (iPSC-Heps; Cellular Dynamics International, Madison, WI), cells grown in culture vessels coated with porcine liver ECM displayed better retention of hepatocyte morphology than vessels coated with collagen. When porcine liver ECM was used as a coating or as a media supplement, iPSC-Heps exhibited significantly more albumin and urea production than un-supplemented cells. To better understand the microenvironment necessary for the regulation and differentiation of LSECs, an ex vivo whole-organ culture system was used to investigate the application of human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs; Cellular Dynamics International) as a clinically-relevant source of cells for reconstitution of the sinusoids of liver scaffolds. Decellularized whole rat liver ECM scaffolds were mounted in a custom bioreactor and iPSC-ECs were seeded into both the portal vein and hepatic veins. Tissue sections stained with H&E showed that iPSC-ECs attached throughout the 3D liver scaffolds. Terminal deoxynucleotidyl transferase dUTP nick end labeling of tissue sections showed that iPSC-ECs were viable after 7 days of ex vivo culture in our perfusion-bioreactor. Overall, the data suggests that liver ECM, even in a solubilized form, promotes hepatocyte phenotypic stability in culture.

Poster number 130 *Category: Tissue Engineering*

Enhancement of human mesenchymal stem cell chondrogenesis by sustained release of TGF beta3 within a graphene oxide-incorporated hydrogel

He Shen (1), Hang Lin (2), Aaron Sun (1,2), and Rocky S. Tuan (1,2)

(1) Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, (2) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

The limited reparative and regenerative potential of diseased or injured articular cartilage, which contributes to the prevalence of degenerative joint diseases, necessitates the development of tissue engineering approaches to facilitate cartilage repair, such as the differentiation of human mesenchymal stem cells (hMSCs) into mature chondrocytes within scaffolds. Graphene-based nanomaterials, with unique properties, including large surface area, mechanical stability, and good biocompatibility, have received increasing recent attention for use as a biologics delivery vehicle for tissue engineering applications. In this study, we have incorporated graphene oxide nanoparticles (GO) within a hybrid hydrogel, composed of photopolymerizable poly-D, L-lactic acid/polyethylene glycol (PDLLA-PEG) and hyaluronic acid (HA), to test its applicability in sustained release of the chondroinductive growth factor, TGF-β3. With GO incorporation, the hydrogel scaffold (GO/PDLLA-PEG/HA) exhibited enhanced mechanical property, assessed by mechanical testing, and supported long-term release of TGF-β3 in a controlled manner for up to 4 weeks. hMSCs derived from bone marrow (hBMSCs) seeded within TGF-β3 loaded GO/PDLLA-PEG/HA hydrogel displayed high viability (>90%) and underwent robust chondrogenesis. In comparison with cultures maintained in GO-free scaffold containing equivalent amount of TGF-β3, hBMSCs cultured in GO/PDLLA-PEG/HA showed significantly higher chondrogenic gene expression (aggrecan, collagen type II, and Sox9). Moreover, our results also showed that, with the use of GO, less similar level of hBMSCs chondrogenesis could be achieved with lower amount of TGF-β3. Taken together, these findings support the application of GO in optimizing TGF-β3 induced hBMSCs chondrogenesis, and its potential utility in cartilage tissue engineering. (Support: US Dept. of Defense, Collaborative Academic Training Program for Post-doctoral Fellows of NANO-CIC China)

Mesenchymal Stem Cells Drive Cell Repopulation In An In Vivo Model Of Lung Regeneration

John Sembrat (1,2), Chandler Caufield (1), Nayra Cardenes (1,2), Rebecca Vanderpool (1), Margaret Benewitz (2), Sergei Snovida, Prithu Sundd (2), Mauricio Rojas (1,2)

(1) Dorothy P. & Richard P. Simmons Center for Interstitial Lung Disease, University of Pittsburgh School of Medicine, Pittsburgh, PA, (2) Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

Methods: Lungs were decellularized in situ by perfusion of the pulmonary vasculature with SDS. Following decellularization, matrix was seeded with GFP B-MSCs and heterotopically transplanted into the dorsum of C57BL/6 or BALB/c mice for 4 weeks. Sham lungs were also placed in the dorsum of mice to serve as an internal control. Alternatively, conditioned media was used in place of cells. Revascularization of implanted lungs was imaged using two-photon microscopy prior to tissue retrieval. To determine the cellular makeup of the recellularized tissue, histological staining, immunofluorescent staining, qPCR, flow cytometry and western blotting were used.

Results: Lungs seeded with GFP B-MSCs exhibited macroscopic re-vascularization confirmed by two-photon microscopy compared to control lungs. IF confirms the presence of endothelial, epithelial and smooth muscle cells as well as macrophages in lungs seeded with GFP B-MSCs compared to control lungs. A lack of co-localized GFP signal with cells indicates cells where recruited from the recipient mouse, not differentiated GFP B-MSCs. Lungs injected with CM appear to show greater recellularization compared to control lungs.

Conclusions: These results indicate that decellularized lung matrix reseeded with B-MSCs or conditional media, serves as a viable scaffold for the recruitment of specific types of cells that will generate a functional and viable organ for transplant. Lack of co-localization of the GFP signal with cell markers suggests that cells staining positive for CD31, CD45, SMA and Cytokeratin cell markers are most likely endogenous cells recruited via signaling mechanisms of the B-MSCs.

The influence of cholesterol/caveolin-1/caveolae homeostasis on human mesenchymal stem cell membrane properties and adhesive characteristics

Jihee Sohn (1), Natasha Baker (2) and Rocky S. Tuan (1)

(1) Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh, PA, (2) Kenneth P. Dietrich School of Arts and Sciences, Department of Biological Sciences, University of Pittsburgh, PA

Adult mesenchymal stem cells (MSCs) are considered an important resource for tissue repair and regeneration. Their utilization in regenerative medicine depends on an understanding of the mechanisms driving their responsiveness to external stimuli. It is likely that an initial determinant of stem cell responsiveness to external stimuli is the organization of signaling molecules in cell membrane rafts. Membrane rafts fall into two broad categories, non-caveolar and caveolar, based on the absence or presence, respectively, of caveolin proteins. We have previously demonstrated that expression of caveolin-1 (Cav-1) increases in MSCs induced to undergo osteogenic differentiation, and knockdown of Cav-1 expression enhances MSC proliferation and osteogenic differentiation. These results suggested that Cav-1 normally acts to regulate the differentiation and renewal of MSCs. In this study, we investigated the effects of perturbations in cholesterol/Cav-1/caveolae homeostasis on MSCs membrane properties and adhesive characteristics. We have generated 5 different MSC sub-populations: (i) control MSCs, (ii) cholesterol-depleted MSCs, (iii) cholesterol-enriched MSCs, (iv) MSCs transfected with control siRNA, and (v) MSCs transfected with Cav-1 siRNA. Each cell group generated was analyzed for perturbation of cholesterol status and Cav-1 expression. Our results demonstrated that perturbations in cholesterol/Cav-1 levels significantly affected the membrane properties of MSCs. Cholesterol supplementation resulted in increased cell membrane cholesterol, thus localizing elevated numbers of caveolae and Cav-1 to the cell membrane, and as a result, these cells showed decreased membrane fluidity, with changes in surface levels of some integrins. Conversely, knockdown of Cav -1 expression caused a parallel decrease in the number of cell surface caveolae, and therefore also decreased delivery of cholesterol to the cell membrane, thus increasing membrane fluidity. Cells with depleted Cav-1 expression exhibited elevated cell surface integrins. Our preliminary results suggest that manipulation of cholesterol/Cav-1/caveolae may impact aspects of the MSC cell membrane important to substrate sensing and cell adhesion.

Surface Mediated Polymer Electrolyte and Nanostructured Ceramic Composite Layers: New Approach to Non-Viral Gene Delivery from Degradable and Non-degradable Substrates

Sudhanshu Shekhar (1,4), Abhijit Roy (1,4), Pavithra S Murugavel (2) and Prashant N Kumta(1,2,3,4,5)

(1) Department of Bioengineering, (2) Department of Chemical and Petroleum Engineering, (3) Mechanical Engineering and Materials Science, (4) Center for Complex Engineered Multifunctional Materials and (5) McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Non-viral gene delivery techniques are very much needed due to their convenience, manufacturing simplicity, overall economy, and lastly, safety aspects. We have thus far developed nano-sized nanostructured calcium phosphates aptly called “NanoCaPs” as a novel, highly efficient gene delivery agent for plasmid DNA (pDNA) transfection. Surface mediation using multilayer polyelectrolyte assemblies (MPA) using Layer by Layer assembly (LbL) approach to bind the DNA is a nascent but viable approach still largely limited in genetic payloads. We have accordingly developed novel MPA-NanoCaPs-pDNA composites that display excellent in vitro cellular compatibility and gene transfection potential. In the current study, we have demonstrated gene delivery by surface mediation using a set of biodegradable synthetic polymers; polycationic and polyanionic polyelectrolytes generated on degradable (PLGA and Magnesium based alloys) and non-degradable (Glass and Titanium) substrate platforms. SEM, AFM, FTIR and Ellipsometry were employed to explore the layer build up process as well as the specific adsorption of NanoCaPs-pDNA complexes. Substrates coated with MPA-NanoCaPs-pDNA showed threefold increase in gene transfection of human embryonic kidney cells (HEK) even up to a week compared to substrates loaded with MPA-pDNA (sans NanoCaPs). The extent of transfection could also be tailored by controlling the bilayer numbers in the MPA assemblies. Results indicate that MPA are an encouraging platform for achieving controlled release of NanoCaPs-pDNA offering a potential for providing new gene-stimulating biomaterials that can also augment implant technology and likely heralding in a new generation of implants.

Poster number 134 *Category: Tissue Engineering*

Host cells infiltrate electrospun poly(glycerol sebacate) vascular grafts in a porcine vascular access model

Chelsea E.T. Stowell (1), Begona Campos-Naciff (2), Diego Celdran (3), Richard Hoehn (2), Peter Jernigan (2), Robert A. Allen (1), Jennifer J. Zhuang (1), Prabir Roy-Chaudhury* (3), Yadong Wang* (1,4)

(1) University of Pittsburgh Department of Bioengineering, (2) University of Cincinnati, (3) University of Arizona at Tuscon, (4) McGowan Institute for Regenerative Medicine

Synthetic vascular grafts implanted as hemodialysis access sites display poor patency and require frequent reinterventions. As a potential alternative, we previously developed a solvent-cast, salt-leached poly(glycerol sebacate) (PGS) graft that rapidly remodeled into a living neovessel in rats. In this study, clinically sized composite grafts were constructed of electrospun PGS/PVA cores and a bonded electrospun PCL reinforcing wrap. Cores had a dry porosity of 72±1%. PCL reinforcement improved the incremental circumferential modulus from 0.20±0.04 to 1.83±0.03 MPa. The suture retention load was increased from 45±7 to 280±40 gf. Composite grafts were implanted in four pigs as femoral-femoral or carotid-jugular arteriovenous shunts (L = 2 cm and ID = 6 mm for two pigs euthanized at 28 d, and L = 2.5 or 4.5 cm and ID = 5 mm for two pigs euthanized at 15 and 14 d). H&E staining indicated a cellular infiltration/reorganization and ECM deposition at 14 d, proceeding radially outwards from the lumen through approximately 2/3 of the graft wall. This degree of early infiltration is markedly greater than that seen with less porous electrospun PGS/PVA grafts recently implanted in mice. Possible endothelial, intimal and medial layers developed around the graft scaffold without causing stenosis. In addition, very preliminary data suggests superior closure of cannulation sites as compared to standard ePTFE grafts, which could have a significant positive impact on dialysis unit process of care/workflow and patient quality of life. Future work will evaluate the phenotypes of the remodeling cells, characterize the neotissue quality, and extend the implants to later timepoints.

Age-related changes in skeletal muscle matrix remodeling affect muscle stem cell fate

S. Shinde (1), L. Winter (2), A. Sahu (1,3), K. Stearns-Reider (1), A. D'Amore (2), A. Barchowsky (3), R. Tuan (2,4), F. Ambrosio (1,4)

(1) Department of Physical Medicine and Rehabilitation,(2) Department of Bioengineering, (3) Environmental and Occupational Health, (4)McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

One common problem that older individuals face is the decreased regenerative and healing capacity of skeletal muscles after an acute muscle injury. Work from our laboratory has demonstrated that the decline in age-related regenerative capacity is linked to differences in the architecture and composition of the skeletal muscle extracellular matrix. The purpose of this study was to test our hypothesis that differences in matrix deposition between young and aged myofibroblasts directly affect cell tendency to differentiate toward a myogenic or fibrogenic lineage.

In vitro studies were conducted with myofibroblasts isolated from general skeletal muscle from the legs of young and old animals (Mice). These myofibroblasts were allowed to proliferate and deposit matrix before they were lysed and removed, at which point collagen composition of the matrix was analyzed using confocal microscopy.

When compared to young counterparts, myofibroblasts from old muscles produce extracellular matrix that has a lower expression of Collagen III and VI protein as measured by immunofluorescence intensity analysis. It was also observed that matrix deposited by old myofibroblasts contains a higher expression of collagen IV. In a second set of experiments, human muscle stem cells were seeded on top of the matrix derived from young or old myofibroblasts, and stem cell differentiation and protein expression was assessed using confocal microscopy. Interestingly, whereas matrix isolated from young muscle promotes muscle stem cell myogenicity and regeneration, the aged matrix drives muscle stem cells toward a fibrogenic lineage.

These data show that there are distinct differences between the extracellular matrix composition of young and old animals, and these differences influence muscle stem cell fate toward a myogenic or fibrogenic lineage.

Poster number 136 *Category: Tissue Engineering*

Localized FK506 delivery for optic nerve regeneration

Yolandi van der Merwe (1-3,6), Kia M. Washington (4), Anne E. Faust (1,2), Ian Conner (2,3,6), Xinzhu Gu (1), Chris Kaufman (1,2), Fardeen Mehdi (1,2), Asma Naqvi (1), Tanchen Ren (1,2), Kevin C. Chan (1-3), Raman Venkataramanan (7), William R. Wagner (1,3,5), Michael B. Steketee (1,2,6,8)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Ophthalmology, (3) Department of Bioengineering, (4) Department of Plastic Surgery, (5) Department of Surgery, (6) Louis J Fox Center for Vision Restoration, (7) Department of Pharmaceutical Sciences and Pathology, (8) Center for Neuroscience, University of Pittsburgh

Trauma to the retina or to the optic nerve often leads to an inflammatory innate immune response that contributes to retinal ganglion cell (RGC) death and irreversible vision loss. We currently lack a therapy that can modulate inflammation in the CNS locally while minimizing adverse side-effects globally. FK506 (tacrolimus) is a clinically used immunosuppressive and neuroprotectant agent used after organ transplantation, however, high systemic levels of FK506 can have adverse side effects that lead to organ failure among other complications. To address this problem, we developed a nerve wrap that locally and sustainably releases FK506 over 14 days by electrospinning FK506 into a poly(ester urethane) urea (PEUU) sheet. Here we show that FK506 significantly increased RGC axon regeneration in vitro. In vivo after optic nerve crush in rat, the FK506-PEUU nerve wrap sustainably released FK506 for up to 14 days, and locally released FK506 to the injured optic nerve, with negligible amounts of FK506 present in the retina and contralateral optic nerve and retina. Following optic nerve crush in rat, the FK506-PEUU wrap decreased GFAP activation at the injury site, while increasing GAP43 positive staining compared to controls, suggesting FK506 decreased astrocyte activation and increased neuronal growth and regeneration, respectively. These studies show locally delivered FK506 promotes RGC axon regeneration after injury and reduces scarring. Thus, FK506 scaffolds hold promise for ameliorating RGC death and axon degeneration after ocular trauma in a highly translatable platform.

In-vivo effects on the eye of acute modulations of intraocular and intracranial pressures

Huong Tran (1,2), Andrew Voorhees (1), Bo Wang (1,2), Ning-Jiun Jan (1,2), Elizabeth Tyler-Kabara (3) , Larry Kagemann (1,2), Hiroshi Ishikawa (1,2), Joel Schuman (1,2), Matthew Smith (1,2), Gadi Wollstein (1), Ian Sigal (1,2)

(1) UPMC Eye Center, Eye and Ear Institute, Ophthalmology and Visual Science Research Center, Department of Ophthalmology, University of Pittsburgh School of Medicine, (2) Department of Bioengineering, University of Pittsburgh, (3) Department of Neurosurgery, University of Pittsburgh

Elevated intraocular pressure (IOP) is the main risk factor for glaucoma, the second leading cause of blindness worldwide. Despite the importance of IOP in glaucoma, it remains poorly understood how the biomechanical effects of IOP vary depending on the level of intracranial pressure (ICP). Our goal was to measure the in-vivo effects of acute modulation of IOP and ICP on the posterior pole of the eye.

In 4 eyes of 3 monkeys, IOP and ICP were each set at 4 levels (low, baseline, high, very high), and the ONHs imaged with a SD-OCT. The anterior lamina cribrosa (ALC) and scleral canal opening at Bruch membrane (BMO) were manually marked in 18 radial sections per scan. Custom code was used to reconstruct 3D ALC surfaces and computed ALC depths relative to the BMO best-fit plane within regions visible in all scans of an eye and normalized to baseline in each monkey.

Acute modulation of either IOP or ICP above or below baseline caused substantial non-linear and non-monotonic deformations of the ALC, with strong interactions between IOP and ICP. The ranges for normalized median ALC depth were 78-116%, 36-122%, 65-105%, and 66-104% in eyes 1R, 2R, 3R and 3L. In all 4 eyes, the most anterior LCs occurred with IOPs below baseline (15mmHg) and very high ICP (20-45mmHg).

Poster number 138 *Category: Tissue Engineering*

Kupffer cell subsets differ between young and aged murine livers

Elizabeth C. Stahl (1,2) and Bryan N. Brown (2,3)

(1) Cellular and Molecular Pathology Training Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, (2) McGowan Institute for Regenerative Medicine, Pittsburgh, PA, (3) Department of Bioengineering, Swanson School of Engineering, Pittsburgh, PA

The immune system, and in particular macrophages, are heavily implicated in wound healing, response to infection, and cancer progression, all of which are linked to mortality in the elderly population. Recently, it has been shown that tissue-resident macrophages from spleen and peritoneum become dysfunctional with aging, likely due to the aged microenvironment. The liver contains approximately 80% of total mammalian tissue-resident macrophages, known as Kupffer cells. Kupffer cells can be divided into two F4/80+ macrophage subsets: CD68+ and CD11b+ cells, which have different origins and functions. Currently, it is unclear how these macrophage subsets are affected by aging and the implications for tissue engineered constructs and regenerative medicine in the vulnerable elderly population.

We sought to characterize differences in the Kupffer cell compartment from young (8-10 week) and aged (18 -20 month) C57/Bl6 wild-type mice using immunofluorescent staining on tissue sections as well as flow cytometry. Preliminary histological analyses showed that the area of F4/80+ staining did not differ between young and aged mice. Interestingly the area of CD68+ staining was significantly higher in the aged murine livers. In addition, the area of CD32+ staining, which marks both macrophage progenitor and endothelial cells, was significantly greater in aged mice, consistent with previous reports of blood vessel thickening in aged livers. Flow cytometry analysis confirmed differences in the macrophage subsets between young and aged murine livers. Characterizing the differences in the subsets of tissue-resident macrophages during aging will increase our understanding of the host response to bioengineered tissue constructs and cellular therapeutics in the elderly population.

bFGF-induced Akt/mTOR activation protects the ischemic Heart via attenuation ATG7-dependent autophagy and ubiquitinated protein accumulation

Zhouguang Wang (1), Yan Huang (1), Yadong Wang (1)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

Autophagy is involved in the development and/or progression of many diseases, including myocardial ischemia/reperfusion (I/R). In this study, we hypothesized a protective role of basic fibroblast growth factor (bFGF) both in vivo and in vitro and demonstrated that excessive autophagy and ubiquitinated protein accumulation is involved in the myocardial I/R model. Our results showed that bFGF improved heart function recovery and increased the survival of cardiomyocytes in myocardial I/R model. The protective effect of bFGF is related to the inhibition of LC3II levels. Additionally, bFGF enhances the clearance of Ub by p62 and increases the survival of H9C2 cells. Moreover, silencing of p62 partially blocks the clearance of Ub and abolishes the anti-apoptosis effect of bFGF. An shRNA against the autophagic machinery Atg7 increased the survival of H9C2 cells co-treated with bFGF and rapamycin. bFGF activates the downstream signaling of the PI3K/Akt/mTOR pathway. These results indicate that the role of bFGF in myocardial I/R recovery is related to the inhibition of excessive autophagy and increased ubiquitinated protein clearance via the activation of PI3K/Akt/mTOR signaling. Overall, our study suggests a new direction for bFGF drug development for heart disease and identifies protein signaling pathways involved in bFGF action.

Poster number 140 *Category: Tissue Engineering*

Effect of Cell Density and Material Stiffness on Human Bone Marrow Stem Cell Chondrogenesis

Aaron X. Sun (1,2,3), Hang Lin (1), Madalyn Fritch (1,3), He Shen (1), Pete Alexander (1), Rocky S. Tuan (1,3)

(1) Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, (2) Medical Scientist Training Program, University of Pittsburgh School of Medicine, (3) Department of Bioengineering, University of Pittsburgh Swanson School of Engineering, Pittsburgh, PA

Live stem cell-incorporated three-dimensional hydrogels that support chondrogenic differentiation and maintenance offer a promising regenerative route towards addressing the limited self-repair capabilities of articular cartilage. In particular, hydrogels that augment chondrogenesis and recapitulate the native physical properties of cartilage, such as compressive strength, can potentially be applied in point-of-care procedures. In this study, we develop two new materials, [poly-L-lactic acid/polyethylene glycol/poly-L-lactic acid] (PLLA-PEG 1000) and [poly-D,L-lactic acid/polyethylene glycol/poly-D,L-lactic acid] (PDLLA-PEG 1000), that are biodegradable, biocompatible (>80% viability post fabrication), and possess high mechanical strength (~1500 to 1800 kPa). In addition, we utilize them with previously characterized PDLLA-PEG 4000 to examine the effect of physiologically relevant cell densities (4, 8, 20, and 50 million/mL) and hydrogel stiffnesses at different concentrations (~150kPa to ~1500 kPa Young's moduli and 20% to 30% w/v) on human bone marrow mesenchymal stem cell chondrogenesis. Results show that 20 million/mL was the most efficient cell concentration for extracellular matrix (ECM) production on the basis of hydroxyproline and glycosaminoglycan content. Interestingly, material stiffness did not significantly affect chondrogenesis, but rather material concentration was correlated to chondrogenesis with increasing levels at lower concentrations based on ECM production, chondrogenic gene expression, and histological analysis. These findings establish optimal cell densities for 3D cell-incorporated hydrogels, inform hydrogel material development, and demonstrate the efficacy and utility of PDLLA-PEG 1000 for cartilage tissue engineering. (Support: NIH, Department of Defense)

Nanosized hydroxyapatite particles/poly(glycerol sebacate) composite scaffold for bone tissue engineering

Yefei Wang (1), Jin Gao (1), Xiaochu Ding (1), Kee-Won Lee (1), Hongshuai Li (1), Yadong Wang (1)

(1) Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

Poly (glycerol sebacate) (PGS) scaffolds show great promise for tissue engineering. In this study, different amount (5 wt.%, 10 wt.% and 20 wt.%) of nanosized hydroxyapatite (nHA) particles are blended to PGS solution to construct osteoconductive composite. The uniform dispersion of nHA in the polymer matrix was confirmed. Along with increasing the amount of nHA particles in the scaffold, the mechanical properties were dramatically enhanced in the group of 20 wt.% nHA. Compared to pure PGS group, cell adhesion, proliferation and differentiation of rat bone marrow stromal cells (BMSCs) were improved with addition of nHA in the composite. nHA/PGS composite scaffolds have great potential as osteoconductive constructs for bone tissue engineering.

Poster number 142 *Category: Tissue Engineering*

Mechanical Regulation of Cell Behaviors During Convergent Extension of the Xenopus Laevis Neural Plate

Deepthi S. Vijayraghavan (1), Joseph H. Shawky (1), Lance A. Davidson (1,2,3)

(1) Department of Bioengineering, (2) Department of Developmental Biology, (3) Department of Systems and Computational Biology, University of Pittsburgh, Pittsburgh, PA

The formation of the embryonic neural tube, a precursor of the central nervous system, is one of the earliest morphogenetic events in development. Failure of neurulation results in congenital disorders. Understanding the mechanism of neural tube formation may give more insight as to how these defects occur. At the onset of neurulation, the neural plate begins to undergo a process of convergent-extension that narrows the tissue in the mediolateral direction while elongating in the perpendicular anterior-posterior direction. Planar polarized cell intercalations and shape change are thought to drive convergent extension. Cells may be able to transduce tissue polarity from molecular signaling or other patterning signals such as anisotropic tissue tension. In this study, we sought to describe how neuroepithelial cells within Xenopus laevis neural plates respond to altered tissue strain patterns.

To alter the tissue strain field we excised dorsal tissue explants from the embryo that contain the neural plate at the beginning of neuralation. These explants continue to undergo convergent extension when cultured in isolation. We prevented explants from extending by placing polyester blockades on their anterior and posterior. We assessed changes in tissue strain rate by creating stereoscopic time-lapse image sequences of both constrained explants and unconstrained explants. We found that unconstrained explants extended at a rate of ~15% per hour and converged at about ~10% per hour. Constrained explants elongated ~0.2% per hour and converged at a rate of 2% per hour. Fixing and staining explants for F-actin after 3 hours of tissue confinement showed revealed that cells near the neural plate midline maintained similar apical area in both unconstrained and constrained isolates. Constrained cells at the neural plate border had a small but significant increase in apical area and were ~25% less elongated than unconfined cells. We found a slight decrease in percentage ofnvertices where 4 or more cells meet, a hallmark of cell rearrangement, in constrained (18.9% ± 2.2%) versus controls (22.2% ± 2.0%). Overall, these results suggest that a population of cells at the border of the neural plate may be sensitive to the tissue mechanical environment. Although we find that cells may continue to rearrange, whether they do so in a polarized manner must be assessed in future experiments on live explants. Thus, tissue tension may play a role in neural plate morphogenesis.

Poster number 143 *Category: Tissue Engineering*

Using Nature's Strategies to Design Materials for Bioengineering Applications

Michael A. Washington (1), Morgan V. Fedorchak (2,3,8), Steven R. Little (2,4,5,8), Simon C. Watkins (6,7), Tara Y. Meyer (1,8)

(1) Department of Chemistry, (2) Department of Chemical and Petroleum Engineering, (3) Department of Ophthalmology, (4) Department of Bioengineering, (5) Department of Immunology, (6) Department of Cell Biology and Physiology, (7) Centers for Biological Imaging, (8) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh PA

Poly(lactic-co-glycolic acid) (PLGA) and its copolymer derivatives are utilized extensively as bioabsorbable materials; currently, many clinical applications employ a random, racemic, or unsequenced form of PLGA. Although these materials have been well studied and are readily available, there are few applications which exploit the use of monomer sequence to tailor a polymers properties for a specific therapeutic application. This study focuses on evaluating the properties of a new set of precisely sequenced PLGAs compared to commonly used random analogs. Changes in sequence, stereochemistry, and monomeric ratios were shown to have a profound effect on such properties as in vitro erosion and swelling and in vivo microparticle stability and foreign body response. Two-photon microscopy studies of PLGA microparticles also illustrate the profound influence of backbone sequence on the hydrolysis profile and acidic microclimate distribution within PLGA microparticles. These discoveries establish a greater understanding of the role of sequence in controlling the properties of PLGA for bioengineering applications.

Poster number 144 *Category: Tissue Engineering*

Cell-instructive graphene-PCL nanocomposite promotes myoblast differentiation

Akhil Patel (1), Yingfei Xue (1), Shilpaa Mukundan (1), Vinayak Sant (1), Shilpa Sant (1,2,3)

(1) Department of Pharmaceutical Sciences, (2) Department of Bioengineering, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Introduction: Myoblast differentiation is a key step in myogenesis and has long been considered to be controlled mainly by biochemical cues such as soluble growth factors. However, the tissue engineering approaches based on biochemical cues suffer from high cost and low reproducibility due to challenge in achieving a precise spatial and temporal control over their bioactivity. Recently, substrate micro/nano-structure and electro-responsive properties are recognized for their important roles in myoblast differentiation. In this study, we hypothesized that engineering biophysical features such as nano/micro- fibrous structure and conductive properties into a nanocomposite scaffold will guide myoblasts differentiation into multinucleated myotubes even in the absence of differentiation media.

Methods: Blends of polycaprolactone (PCL) and different graphene concentrations were subjected to electrospinning. Scanning electron microscopy was performed to study the fiber morphology. Electro-responsive properties were measured by impedance of scaffolds. Scaffold mechanical properties and degradability were examined by uniaxial tensile testing. Mouse myoblast C2C12 were seeded on scaffolds and the cytocompatibility of the scaffold was assessed. C2C12 differentiation study was carried out using either growth media or differentiation media. Myosin heavy chain was used as marker for differentiation.

Results and Discussion: Graphene-PCL nanocomposite scaffolds showed fibrous and porous structure similar to PCL scaffold. The resulting graphene-PCL scaffolds possess excellent conductivity due to the addition of graphene nanosheets. Additionally, physicochemical and mechanical properties of nanocomposite scaffolds can be tuned by varying graphene concentration. Graphene-PCL nanocomposites and their 8-week degradation products exhibited remarkable cytocompatibility and promoted adhesion and proliferation of C2C12 mouse myoblast cells. Importantly, these nanocomposite scaffolds induced graphene concentration-dependent differentiation of C2C12 cells into multinucleated myotubes even in normal growth media. Interestingly, 1% and 2% graphene-PCL showed greater bioactivity for myoblast differentiation over the rest of the nanocomposites. Overall, these composite scaffolds showed to be a promising class of cell-instructive scaffolds for skeletal muscle tissue engineering.

A novel therapy using localized delivery of biopatterned CTLA4/Fc fusion proteins to promote immune regulation and pancreatic islet allograft survival

Wensheng Zhang (1), Yang Li (1), Yang Yang (1), Chiaki Komatsu (1), Maxine Miller (1), Kia M Washington (1), Phil G Campbell (2), Vijay Gorantla (1), Xin Xiao Zheng (1,3), Mario Solari (1)

(1) Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, (2) Department of Biomedical Engineering, Materials Science and Engineering and Biology, Carnegie Mellon University, Pittsburgh, PA, (3) Research Center of Translation Medicine, Shanghai East Hospital, Tongji University, Shanghai, P.R. China

The success of islet cell transplantation remains limited by the lack of donor islets and the cytotoxicity of chronic immunosuppression. In this study, we employed a novel biopatterning technology to bioprint a small dose of murine CTLA4/Fc fusion proteins within human acellular dermal matrix (ADM) and create an immunoregulatory microenvironment around the islet allograft, with the goal of regulating alloimmunity, sustaining islet allograft survival and preserving glucose homeostasis in the absence of systemic immunosuppression.

Methods: 1) Murine CTLA-4/Fc were constructed and bioprinted onto an ADM using our 2D inkjet deposition system. 2) 300 DBA/2 islets were transplanted into streptozotocin-induced diabetic C57BL/6 mice. CTLA4/Fc-biopatterned ADM disks were placed on top of the islet allograft under the renal capsule of the recipient. 3) Blood glucose levels were monitored and the expression of regulatory T cells (Treg) and T-cell derived cytokines were analyzed by flow cytometry, ELISA, real-time PCR, MLR, and immunohistology.

Results: 1) Localized delivery of CTLA4/Fc disk significantly prolonged islet allograft survival (MST: 71d; n=10) with 40% of allografts surviving infinitely (>150d), as compared with CTLA4/Fc single dose i.p. of treatment and no treatment groups. 2) Local CTLA4/Fc disk treatment resulted in beneficial changes of serum levels of T-cell derived cytokines (IFN-r, IL-4, IL-6, IL-17) and gene expression of CTLA4, Foxp3, Gzmb in islet allografts. 3) Foxp3+ Treg were expanded in CTLA4/Fc disk-treated recipients and exhibited a potent ability to suppress efftive T cell responses in MLR.

Conclusion: Localized delivery of CTLA4/Fc biopatterned ADM disk promotes long-term engraftment of a minimal load of allogeneic islet cells and allows for glucose homeostasis without additional systemic therapy in the murine diabetic model. Our findings demonstrate a new paradigm of transplant immunotherapy that local immunomodulation of the allograft could be achieved via immune alterations of the microenvironment with the biopatterning technology.

Treatment for periodontitis by inducing M2 macrophage polarization

Zhe Zhuang (1), Sayuri Yoshizawa (1,2), Steve Little (2), Charles Sfeir (1,2)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine

Periodontal diseases are characterized by the progressive destruction of tooth-supporting alveolar bone and gingival soft tissues, which result from infections and chronic inflammation. Host's persistent high level inflammatory response is the prior factor in periodontitis pathogenesis, while bacterial infection second in line. Different subsets of macrophages serve diverse roles in the development of periodontitis, also macrophage subset polarization profile transits as disease proceeds. Generally speaking classically activated macrophages or M1 macrophages contribute to gingival tissue destruction and bone resorption in periodontitis progression, while alternatively activated macrophages or M2 macrophages are considered as anti-inflammatory, associated with resolution of pro-inflammatory immune reaction and tissue regeneration. The cytokines and chemokines in disease gingival tissues create a M1 polarization prone environment, and adjust the imbalance ratio of M1/M2 in periodontitis might be an effective insight to pursue innovative treatment for the disease.

In our study, the goal is to use different formulations of cytokines and chemokines to recruit marcophages and adjust M1/M2 ratio as a potential treatment for periodontitis.
We created a macrophage M1-like /M2-like polarization modulating model in vitro with mouse bone marrow derived macrophages (mBMDM) and mouse macrophage cell line RAW264.7. Flow cytometry (FACs) analysis data shows that our cytokine formulation can skew a proportion of BMDM population towards M2-like phenotype, detected by M2 specific marker CD206. Additionally, we simulate the challenges which macrophages are facing in periodontitis condition by stimulating mBMDM and RAW264.7 with LPS. The pro-inflammatory cytokine TNF-a secretion shows marked reduction when rescued our cytokine formulation. Animal experiments were conducted with murine periodontitis model, disease induced by oral infection of Porphyromonas gingivalis (Pg). Our cytokine formulation was administered into gingival tissue. This ongoing animal experiment aims to investigate whether changes in M1/M2 ratio would have effects on periodontitis phenotype.

Posters
Corporate and Partners

Cyfuse Biomedical K.K.
McGowan Wound Healing Seminar Series
GLP Consortium
Pediatric Clinical Challenges

Sunday, 3/6/2016, 5:00 – 7:00 pm: Presentation of ***odd*** numbered posters
Monday, 3/7/2016, 5:00 – 7:00 pm: Presentation of ***even*** numbered posters

Bio 3D Printer "Regenova"

Cyfuse Biomedical K.K.

Cyfuse Biomedical K.K. develop, manufacture and sell Bio 3D Printer "Regenova" which is totally new way of bioprinting. Our system enable 3D Tissue construction without Gel or Scaffold. Advantage is enhanced cell to cell interaction which lead to higher functionality and longer viability. Blood vessel, Cartilage, Liver and Beating heart structure have been successfully printed. Application will not be only for Transplantation, but also Micro Tissue for research purpose. Please visit our booth at Exhibit area.

Poster number

Category: Corporate and Partners