College of Engineering Biomedical Research Day
Poster Presentation Abstracts

139 Abstract Title:	**Delivery of Doxycycline from PLGA-Coated Hernia Meshes**
Author(s):	T.A. Phan, Department of Biosystems & Agricultural Engineering, U of Kentucky S.K. Ramineni, Department of Biomedical Engineering, U of Kentucky M.E. Brown, Department of Biomedical Engineering, U of Kentucky P.D. Fisher, Department of Biomedical Engineering, U of Kentucky D.A. Puleo, Department of Biomedical Engineering, U of Kentucky

Abstract: Statement of Purpose Worldwide, over 20 million hernias are repaired each year, with approximately 700,000 of those occurring in the U.S. alone.1 The high recurrence of hernias, from 32-63%, has been linked to overexpression of matrix metalloproteinases (MMPs).2 Doxycycline inhibits the expression of MMPs and can aid healing of incisional hernias.3,4 The goal of the present studies was to develop coated surgical meshes for local and sustained delivery of doxycycline over a period of two months. Two types of poly(lactic-co-glycolic acid) (PLGA) coatings were examined, and retention of bioactivity was verified. Methods Polypropylene surgical meshes were cut into 2.5 x 2.5 cm squares and dip-coated in a mixture of 10 mL of acetone, 20 mg of doxycycline, and 2 or 5 g of PLGA. Two types of PLGA were tested: 50:50 (ester terminated, IV:0.55-0.75 dL/g, 30-40 kDa) and 75:25 (ester terminated, IV: 0.55-0.75 dL/g). Dip-coatings were repeated, with drying in between, until each dry mesh was 0.35-0.4 g. Coated and dried meshes were placed in 6 mL tubes with 5 mL of phosphate-buffered saline, pH 7.4, (PBS) and gently shaken during incubation at 37°C. At selected time points, meshes were then transferred to new tubes with fresh PBS and the supernatants stored. Supernatants were analyzed using HPLC to quantify the amount of doxycycline released. At selected time points, in a separate experiment, samples were cut from meshes, dried, and analyzed using Kirby-Bauer tests. Staphylococcus aureus were plated on blood-agar plates at a density according to the 0.5 McFarland standard. Meshes were placed on the plates and incubated at 37°C for 24 hours prior to measuring the zones of inhibition. Results 75:25 PLGA coated meshes released doxycycline in a small initial burst and then had a steady release of approximately 20 µg per week for 5 weeks. For the 50:50 PLGA meshes, there was also a small initial burst of doxycycline released within 4 days and then a major release from two weeks until one month. Over the course of 43 days, 62% of the doxycycline loaded into the 50:50 PLGA mesh was recovered, while only 8.5% of the drug was recovered from the 75:25 PLGA coated mesh. This may be due to the greater hydrophobicity and slower degradation of the 75:25 polymer. The Kirby-Bauer tests revealed that the 75:25 PLGA meshes retained their activity, as reflected by antimicrobial properties, over the course of a month. Conclusions Both types of coated meshes are viable options for local and sustained release of doxycycline. With additional mechanical testing of different PLGA coatings, we will be able to identify a coated mesh that can be used for delivery of doxycycline for enhancing healing of hernias.

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Primary Presenter / email: **Phan, T. A.** / andrew.phan@uky.edu
Student
Undergrad

Mentor / e-mail: Puleo, D.A. / dave.puleo@uky.edu

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Poster Presentation Abstracts

140 Abstract Title:	**Characterization of poly(simvastatin)-containing copolymers and blends**
Author(s):	T.A. Asafo-Adjei, Department of Biomedical Engineering, U of Kentucky T.D. Dziubla, Department of Biomedical Engineering, U of Kentucky D.A. Puleo, Department of Chemical and Materials Engineering, U of Kentucky

Abstract: Polymerizing the bioactive agent counteracts disadvantage of current drug delivery systems by controlling loading via drug:initiator ratios chosen for synthesis. Simvastatin, which has osteogenic, anti-inflammatory, and angiogenic properties, was copolymerized because its lactone ring is amendable to ring-opening polymerization. Poly(lactic acid) (PLA) copolymers were then blended with poly(simvastatin), and different methyl-terminated poly(ethylene glycol) (mPEG) initiators were used to assess their effect on degradation. Simvastatin or D,L-lactide was copolymerized with 5000, 2000, or 550 Da mPEG at a 100:1 molar ratio. Poly(simvastatin)-mPEG (5 kDa) was mixed with each PLA-mPEG copolymer at 80:20 or 60:40 w/w with PLA-mPEG (5 kDa). Samples were degraded by incubated at 37 ˚C in phosphate-buffered saline, pH 7.4. Mass loss was measured gravimetrically, and simvastatin release was analyzed via high performance liquid chromatography. At 60 days, the 80:20 blend with 5000 and 550 Da mPEG retained the most mass at 55±3.1%, followed by poly(simvastatin), poly(simvastatin) blended with PLA-mPEG (2 kDa) or PLA-mPEG (5 kDa) with 50±5.6, 39±2.6, and 37±3.6% of mass remaining, respectively. The 60:40 blend had the lowest mass remaining (25±2.5%) and simvastatin amount released. The 80:20 blend, with 5 kDa mPEG, released the most simvastatin. At 44 days, poly(simvastatin) copolymers retained 71±7.6, 72±8.1, and 93±6.5% of initial mass as the mPEG molecular weight (MW) decreased, correlating with a decreasing simvastatin amount released. The more hydrophilic samples exhibited faster degradation, leading to altered rates achieved by blending with PLA-mPEG and copolymerization with mPEG. Tunable poly(simvastatin)-based copolymers and blends may be useful in tissue regenerative applications.

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Primary Presenter / email: **Asafo-Adjei, T.A.** / theodora.asafo-adjei@uky.edu
Student
PhD

Mentor / e-mail: Puleo, D.A. / dave.puleo@uky.edu

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141 Abstract Title:	**Temperature and pH Dependent Degradation of AH6 3:1 and AH6 5:1 Poly(beta-amino ester) Polymers**
Author(s):	A.J. Chen, Department of Biomedical Engineering, U of Kentucky D.A. Puleo, Department of Biomedical Engineering, U of Kentucky

Abstract: Introduction: Poly(beta-amino esters) (PBAEs) are a class of hydrogel polymers that are of interest as degradable cell scaffolding and drug delivery biomaterials. PBAEs have material properties and degradation periods that are tunable through their macromer composition. This study's purpose is to evaluate the effect of elevated temperature and decreased pH on PBAE degradation alone and in combination to enable accelerated testing protocols to be used with longer lasting PBAEs. Methods: AH6 3:1 and AH6 5:1 single macromer hydrogels were prepared in accordance with previous studies, immersed in 2ml of PBS solution at pH 7.4, pH 6, and pH 5 and incubated at 37ºC, 50ºC, and 60ºC on an orbital shaker in individually sealed polyethylene tubes. Samples were removed, weighed and dried at predetermined intervals to observe mass changesAt each time point all samples that were not removed received a solution change to avoid accumulation of degradation products. Results and Discussion: This study shows significant differences between degradation profiles of PBAE samples in elevated temperature environments; AH6 3:1 polymer samples degraded 32-69% faster in elevated temperature conditions, and 27-99% faster. In decreased pH conditions Samples did not display significant differences in peak swelling, but did show significant differences in swelling profile and degradation rate. AH5 5:1 samples degraded 77-99% faster in lower pH conditions, and 92-145% faster in elevated temperature conditions. Significant differences were seen in degradation rate and swelling profile, but not peak swelling amount. Conclusions: Preliminary data indicates that incubation temperature has an influence on the rate of degradation of AH6 3:1 and 5:1 PBAEs. Elevated temperature appears to accelerate degradation without impacting the swelling characteristics of the sample.

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Primary Presenter / email: **Chen, A.J.** / ajch222@g.uky.edu
Student
PhD

Mentor / e-mail: Puleo, D.A. / dave.puleo@uky.edu

College of Engineering Biomedical Research Day
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142 Abstract Title:	**Concentrically and Axially Graded Hybrid Polymeric Scaffold**
Author(s):	A. Najarzadeh, Department for Biomedical Engineering, U of Kentucky D.A. Puleo, Department for Biomedical Engineering, U of Kentucky

Abstract: Introduction: The ultimate goal of this research was to fabricate and characterize hybrid polymeric scaffolds composed of at least two components to mimic natural tissue structure at specific defect sites. Using degradable hydrogel particles as porogens allows for controlled pore opening after implantation as well as the potential for drug release during degradation. The controlled pore opening also allows the scaffolds to withstand the necessary mechanical properties at the implant site while degrading at a rate consistent with tissue regeneration. The system comprised poly(lactic-co-glycolic acid) (PLGA) and poly(β-amino ester) (PBAE). In the present study, concentrically and axially graded systems were examined to determine the compositional relationship, mass loss, and pattern of porosity development to design application-based scaffolds. Materials and Methods: PLGA (50:50, IV: 0.55-0.75 dL/g, acid-terminated; Durect Corporation) microspheres (MS) were fabricated using a water/oil/water double emulsion technique. The resultant microspheres were sieved to <250 μm. PBAE macromer was synthesized through a step-wise reaction between poly(ethylene glycol) diacrylate (H; Polysciences), diethylene glycol diacrylate (A; Polysciences), and isobutylamine (Sigma-Aldrich). Macromers were made with A:H molar ratios of 1:1 and 0:1 and with a 1:1.2 ratio of amine to total diacrylate. A cryogrinder was used to produce a homogenous mixture of PLGA and PBAE. The mixture was poured in a novel compression mold device and exposed to 49ºC for 1 day to sinter the MS around the porogens. Once individual concentric and axially graded samples were fabricated, they were assembled and placed in another compression mold system for an additional day of sintering. Scaffolds were placed in 4 mL phosphate-buffered saline (PBS), pH 7.4, on a plate shaker at 37°C for almost 40 days. Samples were removed at predetermined time points to conduct nondestructive mass loss measurements and examine swelling and degradation of PBAE porogen and PLGA matrix. Results and Discussion: Once samples were placed in PBS, PBAE particles used as porogens embedded within the PLGA matrix underwent hydrolysis leaving a porous structure behind. Systems with H6 particles exhibited faster degradation rates due to the increased hydrophilic nature compared to the AH6 porogens, thus allowing more water into the network structure resulting in accelerated hydrolysis. It was observed that after the hydrogel porogen was degraded, the residual PLGA matrix degraded at a much slower rate (Figure 1). Degradation of PLGA matrix continues as long chains cleave into smaller chains. This creates a more acidic and reactive environment most concentrated at the bulk center of the scaffold where the water cannot flow freely, which results in autocatalysis effect that can further break down the ester bonds and accelerate degradation. Conclusions: The combined effect of a quick-degrading porogen and a porosity that allows for rapid aqueous infiltration is advantageous to design functionally concentric graded scaffolds with application based degradation profile that can maintain the initial physiological mechanical properties needed while degrading away with the rate commensurate the new intended tissue formation.

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Primary Presenter / email: **Najarzadeh, A.** / ahnaja2@g.uky.edu
Student
PhD

Mentor / e-mail: Puleo, D.A. / dave.puleo@uky.edu

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143 Abstract Title:	**Evaluation of Poly(curcumin) Microparticle Degradation and Activity in the Presence of Free Radical Generating Systems**
Author(s):	C.T. Jordan, Department of Chemical and Materials Engineering, U of Kentucky J.Z. Hilt, Department of Chemical and Materials Engineering, U of Kentucky T.D. Dziubla, Department of Chemical and Materials Engineering, U of Kentucky

Abstract: Curcumin, the bioactive component of curcuma long (turmeric), has been found to have antioxidant, anti-inflammatory characteristics, and recently used in combinatorial cancer therapy treatments. However, due to its hydrophobicity, poor solubility and bioavailability, it has possessed a low therapeutic effect in vivo. To overcome these limitations, our group has previously synthesized poly(curcumin beta amino ester), referred to as poly(curcumin) to increase curcumin's bioavailability utilizing a controlled release mechanism, which gives great potential to show positive efficacy on oxidative stress treatment and cellular protection. We hypothesize that the driving force of accelerated microparticle degradation is the concentration of free radicals within the system, further accelerating hydrolytic cleavage to release active curcumin, which will also reduce antioxidant activity due to its scavenging capabilities. The overall goal of this project is to characterize poly(curcumin) microparticles degradation and activity in the presence of different reactive oxygen species environments, ultimately observing the efficacy and microparticle response to injury models closely related to the development of oral mucositis in vitro. Curcumin's stability in both mass and antioxidant activity over 24 hours was studied to observe the properties of the free drug in its natural state. Upon full degradation of the microparticles, the recovered curcumin has the same stability characteristics as free curcumin. This verifies the acrylation of curcumin, synthesis of poly(curcumin) crosslinked networks, and cyromilling to result in microparticles has little to no effect on its antioxidant behavior. As free radical generators, such as AAPH, are added to microparticle systems during incubation, accelerated degradation of the particles and curcumin as well as activity significantly decreases due to free radical scavenging, where as a low activity of alpha amylase, a prominent enzyme in the oral environment, shows no effect on the release or activity of released curcumin.

Supported by: Bluegrass Advanced Materials, LLC

Primary Presenter / email: **Jordan, C.T.** / ctsc226@g.uky.edu
Student
PhD

Mentor / e-mail: Dziubla, T.D. / dziubla@engr.uky.edu

College of Engineering Biomedical Research Day
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144 Abstract Title:	**Quantitative expression of cancer associated surface biomarkers for circulating tumor cell detection and enumeration**
Author(s):	C.F. Cahall, Department of Chemical and Materials Engineering, U of Kentucky J.L. Lilly, Department of Chemical and Materials Engineering, U of Kentucky E.A. Hirschowitz, Div. of Pulmonary & Critical Care Medicine, Dept. of Internal Medicine, U of Kentucky B.J. Berron, Department of Chemical and Materials Engineering, U of Kentucky

Abstract: Cancerous tumor cells circulating in the blood were first observed in 18691 and have since become a large focus in cancer research. Circulating tumor cells (CTC's) in the blood are the most common cause of cancer metastasizing to form distant secondary tumors.2 Here we review the role of nine commonly studied surface biomarkers that play key roles in cancer progression and metastasis. We then seek to provide quantitative reference data for relative surface marker density between three breast cancer cell lines, two non small cell lung cancer (NSCLC) cell lines and a healthy peripheral blood sample. Surface density of target biomarkers are largely important in the current detection and viable cell isolation methods of CTC's. Epithelial cell adhesion molecule (EpCAM) is the most commonly targeted antigen for targeting cells of epithelial nature in circulation in the blood. However, in most invasive cancers the expression of most key cell adhesion molecules, such as EpCAM and E-cadherin, is known to be down regulated, decreasing the number of binding sites for fluorescent tagging or immobilization and therefore, decreasing the potential of a cell with epithelial nature to be detected. This is consistent with our data of surface expression in comparing the more invasive cell lines, such as MDA-MB-231's to the less invasive cell lines such as MCF-7's and T-47D's.3 CTC's are also known to undergo an epithelial to mesenchymal transition (EMT) while in circulation. This transition causes the cell to even further down regulate epithelial specific antigens causing differentiation between normal blood and CTC's to be even more challenging. For future work, we propose an antigen specific fluorescent amplification method that will increase the fluorescent intensity per binding event binding to further distinguish between positive and negative events. Fluorescent polymerization based amplification (PBA) is an antigen specific polymerization technique that coats cells expressing the target antigen in a hydrogel infused with fluorescent nanoparticles. This technique turns one fluorescent event of traditional staining into many fluorescent events through the entanglement fluorescent nanoparticles in the polymer network to amplify the signal associated with one primary binding event. This amplification will allow for a greater separation in fluorescent intensity from auto-fluorescence of cells and non specific staining that is intrinsic to all staining methods. Fluorescent PBA will be compared to the traditional staining immunostaining method using Alexa 488, and an enzymatic fluorescent amplification method, tyramide signal amplification (TSA). Each of these methods of will then be tested using epithelial cells spiked into a healthy blood sample to determine the sensitivity and specificity their ability to positively identify cells of epithelial nature.

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Primary Presenter / email: **Cahall, C.F.** / calvin.cahall@uky.edu
Student
PhD

Mentor / e-mail: Berron, B.J. / brad.berron@uky.edu

College of Engineering Biomedical Research Day
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145 Abstract Title:	**Photopolymerization and photolithography of polyethylene glycol-based hydrogels on biotinylated glass and A549 cell surfaces**
Author(s):	P-J Wu, Department of Chemical and Material Engineering, U of Kentucky J.L. Lilly, Department of Chemical and Material Engineering, U of Kentucky B.J. Berron, Department of Chemical and Material Engineering, U of Kentucky

Abstract: Cell surface patches provide a potential approach to deliver therapeutics to targeted tissues. Prevailing techniques to manufacture cell surface patches focus on the synthesis of multilayered polymer prior to their attachment to the cells. The application of photolithography on the production of cellular patches has not been fully studied. The goal of this work is to establish an efficient approach to synthesize polymer patches on cell surface via polyethylene (glycol) diacrylate (PEGDA) photopolymerization and photomask-mediated photolithography. PEGDA photo-polymerization triggered by 530nm green light was modeled by Matlab software and experimentally characterized on epoxy microarray, with results showing elevated polymer production with the illuminating duration and light intensity. Furthermore, A549 cell surfaces were biotinylated by sulfo-NHS-LC-biotin or EpCAM primary antibody / biotinylated secondary antibody. PEGDA hydrogels were successfully photo-polymerized on biotinylated cell surface. Moreover, photolithographic patterning of PEGDA hydrogel was characterized on an epoxy microarray. PEGDA polymer formation detected by fluorescein signal showed the stronger and shorter illumination had the best chance to accurately generate the desired PEGDA pattern. Finally, photomask-patterned PEGDA polymer production was successfully applied to produce cell surface patches. To sum up, our work first demonstrated the feasibility to pattern the desired size and thickness of cellular patches onto adhesive cells such as cancer cell line or stem cells. Further exploration is necessary to optimize the effects of these patches on cell function, and conjugate therapeutic drug or nanoparticles into these cell patches to commit their therapeutic application.

Supported by: NSF-CBET-1351531

Primary Presenter / email: **Wu, P-J** / pwu224@g.uky.edu
Student
PhD

Mentor / e-mail: Berron, B.J. / brad.berron@uky.edu

College of Engineering Biomedical Research Day
Poster Presentation Abstracts

146 Abstract Title:	**Characterization techniques for biological specimens at the UK Electron Microscopy Center**
Author(s):	A. Akbari, Department of Chemical and Materials Engineering, U of Kentucky N. Briot, Department of Chemical and Materials Engineering, U of Kentucky T. J. Balk, Department of Chemical and Materials Engineering, U of Kentucky

Abstract: The Electron Microscopy Center (EMC) at the University of Kentucky (UK) is a multi-user shared equipment center that serves the entire university community and industries locally and nationwide. Electron microscopy is a powerful tool to investigate materials at micro and nano scale. The EMC offers services and training on scanning electron microscopy (SEM), environmental SEM (ESEM) and transmission electron microscopy (TEM). These systems are capable of performing different characterization techniques for imaging, elemental analysis and micro-structural studies. These techniques include but are not limited to: secondary electron imaging (SE), back-scatter electron imaging (BSE), energy dispersive X-ray spectroscopy (EDS), electron backscatter diffraction (EBSD), focused ion beam (FIB) and scanning transmission electron microscopy (STEM). This presentation briefly introduces the applications of these characterization techniques to study bio materials and biological samples. Concrete examples will be presented to showcase the capabilities and expertise in characterizing challenging biomedical and biological samples at the EMC. We wish to invite the biomedical research community at the University of Kentucky to use the facilities available at EMC in their research.

Supported by: Collage of Engineering

Primary Presenter / email: **Akbari, A.** / azin.akbari@uky.edu
Student
PhD

Mentor / e-mail: Balk, T.J. / john.balk@uky.edu

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147 Abstract Title:	**Inhibition of Mammalian Glycoprotein YKL-40: Identification of the Physiological Ligand**
Author(s):	A.A. Kognole, Department of Chemical and Materials Engineering, U of Kentucky C.M. Payne, Department of Chemical and Materials Engineering, U of Kentucky

Abstract: YKL-40 is a non-catalytic mammalian glycoprotein and known biomarker associated with progression, severity, and prognosis of chronic inflammatory diseases and a multitude of cancers. Despite this well-documented association, conclusive identification of the lectin's physiological ligand and, accordingly, biological function, has proven experimentally difficult. From experiments, YKL-40 has been shown to bind chito-oligosaccharides; however, the natural production of chitin by the human body has not yet been documented. Possible alternative ligands include proteoglycans, polysaccharides, and fibers such as collagen, all of which make up the mesh comprising the extracellular matrix. It is likely that YKL-40 is interacting with these alternative polysaccharides or proteins within the body, extending its function to cell biological roles such as mediating cellular receptors and cell adhesion and migration. Here, we consider the feasibility of polysaccharides including cello-oligosaccharides, hyaluronan, heparan sulfate, heparin, and chondroitin sulfate, and collagen-like peptides as potential physiological ligands for YKL-40. Molecular dynamics (MD) simulations resolve the molecular-level recognition mechanisms, as several of these potential ligands appear to bind YKL-40 in modes analogous to chito-oligosaccharides. Further, we calculate the free energy of binding of the hypothesized ligands to YKL-40 to address the thermodynamic preference relative to chito-oligosaccharides. Our results suggest that chitohexaose and hyaluronan preferentially bind to YKL-40 over collagen, and hyaluronan is likely the preferred physiological ligand as the negatively charged hyaluronan shows enhanced affinity for YKL-40 over electrically neutral chitohexaose. Collagen binds in two locations at the surface of YKL-40, which may be related to a role in fibrillar formation. Finally, heparin non-specifically binds at the surface of YKL-40 as predicted from structural studies. Overall, YKL-40 likely binds many natural ligands in vivo, but its concurrence with physical maladies may be related to associated increases in hyaluronan.

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Primary Presenter / email:	**Kognole, A.A.** / abhishek.kognole@uky.edu Student PhD
Mentor / e-mail:	Payne, C.M. / christy.payne@uky.edu

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148 Abstract Title:	**Quantification of White Matter Hyperintensity and Cerebral Blood Flow in Older Adults with Low or High Risk for Cerebrovascular Disease using MRI**
Author(s):	A.A. Bahrani, Department of Biomedical, U of Kentucky and U of Baghdad W. Kong, Department of Biomedical, U of Kentucky Y. Shang, Department of Biomedical, U of Kentucky C. Huang, Department of Biomedical, U of Kentucky M. Zhao, Department of Biomedical, U of Kentucky C.D. Smith, MRI Center, U of Kentucky D. K. Power MRI Center, U of Kentucky A. Rayapati, Department of Psychiatry, U of Kentucky Y. Jiang, Department of Behavioral Science, U of Kentucky R. J Kryscio, Department of Statistics, U of Kentucky P. T. Nelson, Sanders-Brown Center on Aging, U of Kentucky F. Schmitt, Sanders-Brown Center on Aging, U of Kentucky G. A. Jicha, Sanders-Brown Center on Aging, U of Kentucky G. Yu, Department of Biomedical, U of Kentucky

Abstract: Background: Cerebrovascular disease (CVD) refers to a set of pathological or physiological conditions that affect blood circulation to the brain. CVD is prevalent in the older population. Early detection of CVD in asymptomatic patients is the key for prophylactic interventions that can efficiently prevent stroke. Methods: The study goal is to use MRI with arterial spin labeling (ASL) and T2-fluid attenuation inversion recovery (T2-FLAIR) sequences to quantify cerebral blood flows (CBF) and deep and periventricular white matter hyperintensities (dWMH and pWMH) volumes in elder subjects. Based on Framingham criterion for the estimation of risk to develop CVD, older adults (66 – 88 years old) were classified into low-risk (n = 14) and high-risk (n = 12) groups. The correlations between the CBF and neighboring WMH volume in each lobe and over the whole brain are investigated to evaluate the usefulness of these measurements for distinguishing subjects with high or low risk to develop CVD. Results: Mostly, there are high WMH volume in subjects who are classified a high-risk to develop CVD. Total pWMH volume correlated (negatively) with total CBF and with posterior frontal, parietal, temporal and occipital CBF. Notably parietal pWMH volume was correlated with CBF in posterior frontal, parietal temporal and occipital grey-matter. Significant correlations are interpreted to mean that grey-matter CBF decreases with increasing pWMH volume. Discussion and Conclusions: Quantification of WMH and CBF using MRI with multiple functional sequences show potential as biomarkers for the early diagnosis of CVD. The number of subjects maybe is not enough to get a very clear picture for the relation of WMH and CBF with the risk score.

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Primary Presenter / email:	**Bahrani, A.A.** / ahmed.bahrani@uky.edu Student PhD
Mentor / e-mail:	Yu, G. / gyu2@uky.edu

College of Engineering Biomedical Research Day
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149 Abstract Title:	**Intraoperative Optical Assessment of Blood Flow Changes in Mastectomy Skin Flaps in Patients with Breast Cancer**
Author(s):	C. Huang, Department of Biomedical Engineering, U of Kentucky N. B Agochukwu, Division of Plastic Surgery, U of Kentucky M. Zhao, Department of Biomedical Engineering, U of Kentucky A.A. Bahrani, Department of Biomedical Engineering, U of Kentucky L. Wong, Division of Plastic Surgery, U of Kentucky G. Yu, Department of Biomedical Engineering, U of Kentucky

Abstract: Background: The most common complication following prosthesis-based breast reconstruction after removal of breast tumors is mastectomy skin flap necrosis. There are currently no noninvasive methods to assess mastectomy skin flap perfusion. A new portable and inexpensive technology, noncontact near-infrared diffuse correlation spectroscopy (DCS), has been recently developed in our group for noninvasive measurement of tissue blood flow by analyzing the motions of moving red blood cells in deep tissues (~1.5 cm). In this prospective study, we aimed to validate the use of this noncontact device in the prediction of mastectomy skin flap necrosis. Methods: The device was used to continually measure tissue blood flow at three time points: before and immediately after the mastectomy, and after the reconstruction. Flow measurements were done at 2-3 locations and at four depths in each patient along the mastectomy incision. All patients were tracked for the development of complications including skin necrosis and need for further surgery. Results: Eleven patients have been enrolled in the ongoing study. Two patients developed skin necrosis and one of which required re-intervention. The difference in relative blood flow levels after mastectomy in patients who did, and did not develop necrosis was statistically significant, with values of 24.3% ± 17.7% and 64.1% ± 24.2% of pre-mastectomy baselines (assigning 100%) respectively ($p = 0.02$, paired t-test). Conclusions: Noncontact DCS is a promising tool that may provide objective information regarding mastectomy skin flap viability intraoperatively, thus allowing surgeons early identification of those compromised and ischemic flaps with the hope of salvaging them.

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Primary Presenter / email: **Huang, C.** / chong.huang@uky.edu
Postdoc

Mentor / e-mail: Yu, G. / guoqiang.yu@uky.edu

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150 Abstract Title:	**Comparison of Post-occlusive Reactive Hyperemia Responses in Thigh Muscles and Tails of Mice Measured by Diffuse Correlation Spectroscopy**
Author(s):	M. Zhao, Department of Biomedical Engineering, U of Kentucky M. Akenhead, Department of Biomedical Engineering, U of Kentucky H. Shin, Department of Biomedical Engineering, U of Kentucky G. Yu, Department of Biomedical Engineering, U of Kentucky

Abstract: Background and Objective: Post-occlusive reactive hyperemia (PORH) protocol is often used to evaluate tissue microvascular function. This study compares blood flow responses in thigh muscles and tails of mice during PORH measured by two custom-designed fiber-optic probes for diffuse correlation spectroscopy (DCS) flow measurements. Methods: We used reflection and transmission probes to measure blood flow responses in thigh muscles and tails of mice (n = 16), sequentially. A 5-min arterial occlusion was created by tying a thin PVC tube around the thigh or tail. Tissue blood flow was continuously measured before, during and after occlusion. Relative changes of blood flow (rBF) were calculated by normalizing time-course data to its pre-occlusion baseline values. In order to characterize flow recovery after occlusion we quantify the time from the releasing of occlusion to the time that rBF reaches half of its peak value (i.e., T50). Results: We observed similar PORH response trends in both thigh muscles and tails of mice. The mean values of T50 between the thighs (5.38 ± 2.42 s) and tails (20.00 ± 21.87 s) were significantly different (p = 0.01). The linear regression suggested a significant correlation between these two measurements [R2 = 0.30, p = 0.029, T50 (thigh) = 0.079 × T50 (tail) + 4.059]. Discussion and Conclusions: Slower reperfusion following the occlusion was observed in the tail as compared to the leg muscle, which is expected due to the smaller amount of vessels in the tail. However, the measurement (probe installation and occlusion procedure) on the tail is much easier than that on the thigh muscle of a mouse.

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Primary Presenter / email: **Zhao, M.** / mingjun.zhao@uky.edu
Student
PhD

Mentor / e-mail: Yu, G. / guoqiang.yu@uky.edu

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151 Abstract Title:	**An Interactive Videogame Designed to Optimize Respiratory Navigator Efficiency in Children Undergoing Cardiac Magnetic Resonance**
Author(s):	S.M. Hamlet, Depts. of Electrical Engineering and Pediatrics, U of Kentucky C.M. Haggerty, Dept. of Pediatrics, U of Kentucky; Institute for Advanced Application, Geisinger Health System, Danville, PA J.D. Suever, Dept. of Pediatrics, U of Kentucky; Institute for Advanced Application, Geisinger Health System, Danville, PA G.J. Wehner, Depts. of Biomedical Engineering and Pediatrics, U of Kentucky J.D. Grabau, Dept. of Pediatrics, U of Kentucky K.N. Andres, Dept. of Pediatrics, U of Kentucky M.H. Vandsburger, Depts. of Biomedical Engineering and Physiology, U of Kentucky D.K. Powell, Dept. of Biomedical Engineering, U of Kentucky V.L. Sorrell, Dept. of Medicine, U of Kentucky B.K. Fornwalt, Depts. of Pediatrics, Biomedical Engineering, Physiology, and Medicine, U of Kentucky; Institute for Advanced Application, Geisinger Health System, Danville, PA

Abstract: Background- Advanced cardiac magnetic resonance (CMR) acquisitions often require long scan durations that necessitate respiratory navigator gating. This is particularly important in children with limited ability to hold their breath. We hypothesized that visual feedback of the diaphragm position using an interactive videogame during CMR would increase respiratory navigator efficiency and improve image quality in children. Methods- A feedback videogame was developed using MATLAB. The navigator image provided within the Siemens Syngo user-interface was processed in real-time to yield a kid-friendly representation of diaphragm position which was then projected to the subject in the scanner. The game used a point-based system to incentivize children to hold their diaphragm within the navigator acceptance window (±3 mm) throughout image acquisition. Using a 3T Siemens Tim Trio, 20 healthy children (Age: 13 ± 3, 35% female) underwent a navigator-gated 2D spiral cine displacement encoding with stimulated echoes (DENSE) acquisition first with no feedback and then with the videogame. Navigator efficiency and image quality signal-to-noise ratio (SNR) were determined for each participant and compared using a paired student's t-test. Results- The videogame improved navigator efficiency by 50% ($p < 0.0001$) and improved SNR by 7% compared to scans without feedback ($p = 0.006$). Conclusions- Use of a diaphragmatic feedback videogame during navigator-gated DENSE CMR can improve navigator efficiency by 50% in children. The videogame also has a slight positive effect on image quality with a 7% increase in SNR. These findings should be generalizable to all CMR acquisition sequences which utilize a respiratory navigator.

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Primary Presenter / email: **Hamlet, S.M.** / sean.hamlet@uky.edu
Student
PhD

Mentor / e-mail: Fornwalt, B.K. / b.f@uky.edu

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152 Abstract Title:	**Off-resonance Distorts Images, Corrupts Displacement Measurements, and Alters Quantifications of Cardiac Strains in 2D Spiral Cine DENSE MRI at 3T and 1.5T**
Author(s):	G.J. Wehner, Department of Biomedical Engineering, U of Kentucky J.D. Suever, Institute for Advanced Application, Geisinger Health System, Danville, PA D.K. Powell, Department of Biomedical Engineering, U of Kentucky S.M. Hamelt, Department of Electrical Engineerring, U of Kentucky M.H. Vandsburger, Departments of Biomedical Engineering and Physiology, U of Kentucky C.M. Haggerty, Institute for Advanced Application, Geisinger Health System, Danville, PA B.K. Fornwalt, Institute for Advanced Application, Geisinger Health System, Danville, PA

Abstract: Purpose: To assess off-resonance effects on cardiac strain from spiral cine Displacement Encoding with Stimulated Echoes (DENSE) MRI at 1.5T and 3T Methods: DENSE encodes tissue displacement into phase images, and spatial gradients within the phase images yield cardiac strains, which are valuable measures of cardiac function. When used with long readout durations, spiral acquisitions are prone to distortions and blurring due to off-resonance, which may yield dampened image gradients. Typical spiral cine DENSE acquisitions use 6 spiral interleaves with an 11.1 millisecond readout duration. Five healthy subjects underwent short-axis mid-ventricular 2D spiral cine DENSE at both 3.0 T and 1.5 T. The number of spiral interleaves was varied between 6 and 36 to assess a range of readout durations below 11.1 milliseconds. Magnitude images were visually assessed for blurring and distortions while radial and circumferential strains were quantified from phase images. Strains were correlated against the number of interleaves. Results: At 3.0 T and 1.5 T with the typical, 6-interleaf DENSE acquisition, blurring and distortions were present predominantly in the anterior and lateral left ventricular walls. Those artifacts were markedly reduced in acquisitions with shorter readout durations. Compared to the 36-interleaf acquisition, radial and circumferential strains were underestimated by the 6-interleaf acquisition in those cardiac segments at both field strengths by up to 18.9% and 1.0% (absolute), respectively. Conclusion: Due to off-resonance effects, image quality and measured cardiac strains are dependent on the readout duration of spiral cine DENSE at both 3.0 T and 1.5 T.

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Primary Presenter / email: **Wehner, G.J.** / gregory.wehner@uky.edu
Student
PhD

Mentor / e-mail: Fornwalt, B.K. / bkf@gatech.edu

College of Engineering Biomedical Research Day
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153 Abstract Title:	**The Effect of Hypertrophy in CardioCEST Magnetization Transfer Contrast**
Author(s):	S.W. Thalman, Department of Biomedical Engineering, U of Kentucky A. Pumphrey, Cardiovascular Research Center, U of Kentucky Z. Yang, Cardiovascular Research Center, U of Kentucky M.H. Vandsburger, Departments of Physiology and Biomedical Engineering, U of Kentucky

Abstract: Introduction: Cardiovascular disease is characterized by both increased interstitial fibrosis and extracellular volume, and by hypertrophic remodeling of individual cardiomyocytes. These structural changes are associated with increased risk of heart failure, arrhythmia, and sudden cardiac death. The endogenous contrast mechanism of magnetization transfer (MT) is influenced by changes in tissue structure, and recently MT-weighted CMR approaches have shown promise in identifying cardiac fibrosis. However, the impact of increased intracellular macromolecule concentration concomitant with hypertrophy on MT contrast remains unknown. In this study we use a murine model of Angiotensin-II (AngII) stimulation to demonstrate that hypertrophy has little effect on MT contrast generated using cardiac chemical exchange saturation transfer (cardioCEST). Methods: Adult male C57Bl/6 mice (n=12) received either constant infusion of AngII (1000ng/kg/min, BACHEM, n = 7) or saline (n = 5) via mini osmotic pump (Alzet). CardioCEST MRI was performed at two saturation frequency offsets (6 and 15 ppm) prior to and 10 days after pump implantation. Maps of the magnetization transfer ratio (MTR) were calculated on a voxel-wise base as MTR(ω)=[(SRef-S(ω))/SRef]*100 and analyzed over the entire myocardium. Following post-treatment scanning, all hearts were harvested, fixed, and stained with picrosirius red and wheat-germ agglutinin. Collagen volume fraction (CVF) and mean cellular cross-sectional area (MCA) were measured using ImageJ (NIH). Results: Angiotensin-II treatment provoked a perivascular pattern of fibrosis, however the CVF was not significantly increased (AngII= 2.86% ± 0.9, Sal= 1.63% ± 0.3) Angiotensin-II treatment provoked a significant increase in MCA when compared to saline infusion (AngII= 4825μm2 ± 717, Sal= 2372μm2 ± 158, p=0.01). Parametric maps reveal no significant changes in MTR at 6ppm in AngII-treated mice (30.8% ± 7.3) relative to saline-treated mice (27.2% ± 8.6) or pretreatment scans (25.8% ± 4.2). Linear regression revealed no correlation between regional MCA and MTR values (p=0.943).

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Primary Presenter / email: **Thalman, S.W.** / scott.thalman@uky.edu
Student
PhD

Mentor / e-mail: Vandsburger, M.H. / moriel.vandsburger@gmail.com

College of Engineering Biomedical Research Day
Poster Presentation Abstracts

154 Abstract Title:	**Electrical alternans and prediction of ventricular arrhythmic events.**
Author(s):	D.R. Wasemiller, Department of Biomedical Engineering, U of Kentucky P. Anaya, Division of Cardiovascular Medicine, U of Kentucky A. Patwardhan, Department of Biomedical Engineering, U of Kentucky

Abstract: Background: Alternans of the T wave in the ECG (TWA) has received considerable interest as a potential predictor of ventricular arrhythmia. However, large clinical trials show that while TWA has a very high negative predictive value, its positive predictive value is poor. Objective: To explore complementary predictors to TWA in order to improve its positive predictive value. Methods: Mathematical models and tissue level microelectrode recordings were used to show that the mechanisms linking TWA with arrhythmia onset are importantly affected by alternans of the depolarization phase of action potentials. These mechanisms, which include temporal and spatial alternans in myocytes and tissues change profoundly when the relationship between depolarization alternans changes from that of repolarization alternans which causes TWA. Based on these results we are now developing algorithms to quantify alternans of the amplitude of the R wave in patients who display TWA during stress tests but who do not have cardiac effusion. Results: The link between alternans of depolarization and that of repolarization may affect conduction and onset of arrhythmia. Conclusion: Exploration of complementary metrics to TWA in order to improve its positive predictive value will be clinically useful. The link between depolarization and repolarization alternans has the potential to be one of such metrics and warrants further exploration.

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Primary Presenter / email: **Wasemiller, D.R.** / david.wasemiller@uky.edu
Student
MS

Mentor / e-mail: Patwardhan, A. / abhijit.patwardhan@uky.edu

College of Engineering Biomedical Research Day
Poster Presentation Abstracts

155 Abstract Title:	**TraceLab: A Software Instrument Supporting Replication of Experiments**
Author(s):	D. Allison, Computer Science, U of Kentucky C. Woodson, Computer Science, U of Kentucky J. H. Hayes, Computer Science, U of Kentucky

Abstract: In software development, the term traceability is used to describe the ability to connect and understand the relationships between the various textual artifacts of a software project such as stakeholder's requirements, design, code; these relationships are called trace links (analogous to URLs retrieved by a search engine in response to a query). By capturing and maintaining trace links for a project, developers can more easily maintain code, ensure that specifications are met, analyze test case coverage, and more. In practice, trace links are not often captured or are captured but then not kept up to date. TraceLab is an instrument developed under NSF's Major Research Instrumentation (MRI) program to enable researchers to experiment with and evaluate traceability techniques. TraceLab is similar to other tools such as Weka, MatLab, and RapidMiner as it uses a precedence graph as its main interface and allows users to drag and drop components to compose experiments thus allowing researchers to share aspects of their work – components and datasets. This structure allows for development across multiple operating systems, and in many programming languages. TraceLab differs from the aforementioned tools in that it specifically supports traceability experiments; its latest instantiation is more broadly focused on software engineering experiments and computing experiments in general. TraceLab specifically facilitates replication of experiment results. In this poster and demo, we will show biomedical researchers how easy it is to build TraceLab experiments, by composing provided and/or custom-developed components and datasets, and package these into repeatable experiments to share with the community.

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Primary Presenter / email: **Allison, D.** / daylynn.allison@uky.edu
Student
Undergrad

Mentor / e-mail: Hayes, J.H. / hayes@cs.uky.edu

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156 Abstract Title:	**Incidence of Cortical High Frequency Oscillations is Modulated by Vigilance Changes in the Epileptic Brain**
Author(s):	A.F. Al-Bakri, Department of Biomedical Engineering, U of Kentucky F. Yaghouby, Department of Biomedical Engineering, U of Kentucky P. Modur, Department of Neurology, U of Texas Southwestern, Dallas, TX S. Sunderam, Department of Biomedical Engineering, U of Kentucky

Abstract: Recent studies show that high frequency oscillations (HFOs) can help delineate the epileptogenic zone in individuals with refractory epilepsy. However, HFO incidence could vary with vigilance state, which makes the choice of an appropriate diagnostic baseline for spatiotemporal analysis of HFO activity an important issue. Furthermore, since detection of individual HFOs requires a high data sample rate (> 2000 Hz) and computation on millisecond timescales, a surrogate measure is sought that is easier to compute and correlates with HFO incidence in both spatial and temporal domains. An obvious choice is an estimator of the instantaneous power in the frequency range in which HFOs reside (which we label the HFO index). In this study, we quantify HFO activity and then examine the consequences of vigilance state changes in overnight sleep. We acquired and analyzed ten good quality overnight interictal recordings (1000-2000 Hz sampling rate) from the electrocorticogram (ECoG) in five patients being evaluated for epilepsy surgery. The proportion of epochs containing HFOs varied significantly with vigilance state (Kruskal-Wallis test, $p = 0.0055$) and also appeared to increase with sleep depth. In addition, there were strong temporal and spatial correlations between HFO counts and HFO index (Spearman's rho of 0.6-0.8 and 0.78-0.88 respectively). HFOs show great promise as markers of epileptogenic tissue but analysis of their dynamics is complicated by changes in HFO activity with vigilance state and the lack of uniform guidelines for detecting and labeling them. Our results suggest that simple estimates of HF band power may reflect the relative numbers of HFOs in the ECoG and their variation with vigilance state.

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Primary Presenter / email: **Al-Bakri, A.F.** / amir.al-albakri@uky.edu
Student
PhD

Mentor / e-mail: Sunderam, S. / ssu223@uky.edu

College of Engineering Biomedical Research Day
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157 Abstract Title:	**Feasibility of Graded Somatosensory Stimulation for Selective Sleep Restriction in Rodents**
Author(s):	D.M. Huffman, Department of Biomedical Engineering, U of Kentucky K.E. Staggs, Department of Pharmacology and Nutritional Sciences, U of Kentucky F. Yaghouby, Department of Biomedical Engineering, U of Kentucky A. Agarwal, Signal Solutions, LLC, Lexington, KY B.F. O'Hara, Department of Biology, U of Kentucky K.D. Donohue, Department of Electrical Engineering, U of Kentucky E.M. Blalock, Department of Pharmacology and Nutritional Sciences, U of Kentucky S. Sunderam, Department of Biomedical Engineering, U of Kentucky

Abstract: Sleep is a complex, dynamic multi-stage process that serves a vital role in normal physiological function. Interruption of sleep, whether stage-specific or total, can negatively affect several aspects of daily life including memory and cognition. While factors such as injury, medication, and lifestyle can all contribute to reduced sleep quality, simulating this effect in a laboratory setting can be difficult. Most systems designed for sleep restriction in animals target total sleep deprivation. There are some devices that do produce stage-specific restriction (e.g. the “flower-pot”, stir bar, manual handling), but offer limited control over stimulus intensity and are often intrusive and induce added stress on the animal. In an attempt to restrict sleep in a controlled, non-intrusive manner we investigated the efficacy of somatosensory stimulation to interrupt sleep. Here, we outline the development of this system from a binary, “all-or-none” version into one that actively predicts and tracks sleep dynamics and applies stimulation on a logical basis. Through the course of its development, it was used in two preliminary studies: deep sleep restriction in rats and REM sleep restriction in mice.

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Primary Presenter / email: **Huffman, D.M.** / dillon.huffman@uky.edu
Student
PhD

Mentor / e-mail: Sunderam, S. / ssu223@uky.edu

College of Engineering Biomedical Research Day
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158 Abstract Title:	**Computational Investigation of Hydrogel Injection Characteristics for Myocardial Support**
Author(s):	H. Wang, Department of Mechanical Engineering, U of Kentucky C.B. Rodell, Department of Bioengineering, U of Pennsylvania M.E. Lee, Gorman Cardiovascular Research Group, U of Pennsylvania N.N. Dusaj, Department of Chemistry and Physics, U of Pennsylvania J.A. Burdick, Department of Bioengineering, U of Pennsylvania R.C. Gorman, Gorman Cardiovascular Research Group, U of Pennsylvania J.F. Wenk, Department of Mechanical Engineering, Department of Surgery, U of Kentucky

Abstract: The material properties of myocardium are an important determinant of global left ventricular (LV) function in both health and disease. In vivo studies have demonstrated that hydrogel injections can mitigate the adverse effects of myocardial infarction. More importantly, the stiffness of these injections can be tuned to minimize wall thinning and ventricular dilation. The current investigation combines experimental data and finite element (FE) modeling to correlate how injection stiffness and volume influence myocardial wall stress and wall thickness. In order to evaluate the effects, FE model of the LV with two different injection volumes were generated (150μL and 300μL). For the models with hydrogel injections, the control model was modified to include a 4 x 4 pattern of 16 injections around the free wall. The results indicate that increased injection stiffness reduced LV myofiber stress. Also stiffer hydrogel injections maintained an increased level of LV wall thickness. Overall, this study may predict that stiffer and bigger hydrogel injection can reduce MI bulging during LV remodeling.

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Primary Presenter / email: **Wang, H.** / hwa229@g.uky.edu
Student
PhD

Mentor / e-mail: Wenk, J.F. / jonathan.wenk@uky.edu

College of Engineering Biomedical Research Day
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159 Abstract Title:	**An in Vitro Investigation of Tubulogenic Sprout Formation by Endothelial Cells Under Pressure**
Author(s):	M. Song, Department of Biomedical Engineering, U of Kentucky J. Wallin, University Research Program, Lafayette High School H.Y. Shin, Department of Biomedical Engineering, U of Kentucky

Abstract: This study explored the use of hydrostatic pressure (HP) as a mechanobiological parameter to control in vitro endothelial cell (EC) tubulogenesis in 3-D hydrogels as a model microvascular tissue engineering approach. Recently, we showed that HP exposure is a magnitude-dependent stimulus of endothelial tubulogenic sprout formation that may involve VEGF-C/VEGFR-3 autocrine signaling. The present investigation sought to adapt to using endothelial spheroid cultures, which we believe is a more suitable tissue engineering strategy than the Cytodex® beads used in our previous work. At the same time, we also aimed to identify the operating magnitudes and exposure times for HP-sensitive sprout formation as well as verify the involvement of VEGFR-3 signaling. For this purpose, we used a custom-designed system and a 3-D bovine aortic EC spheroid model of sprouting angiogenesis. Using microscopy, we report that an exposure time of 3 days is the minimum duration required to increase EC sprout formation in response to 20 mmHg. Notably, exposure to a 5-mmHg stimulus for 3 days was inhibitory for endothelial spheroid lengths without affect sprout numbers. Moreover, EC spheroids exposed to 40 mmHg also inhibited sprouting activity, but, in this case, by reducing sprout numbers without affecting sprout lengths. Finally, blockade of VEGFR3 signaling abolished the effects of the 20-mmHg HP stimuli on sprout formation. Based on these results, VEGFR-3 dependent EC sprouting appears to exhibit a complex pressure dependence that one may exploit to control microvessel formation. More work, however, is needed to further support and rationalize this possibility.

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Primary Presenter / email: **Song, M.** / m.song4746@gmail.com
Student
MS

Mentor / e-mail: Shin, H.Y. / hy.shin@uky.edu

College of Engineering Biomedical Research Day
Poster Presentation Abstracts

160 Abstract Title:	**Quantifying the effects of hydrostatic pressure on fibroblast growth factor-2 binding by human endothelial cells**
Author(s):	T. McKenty, Department of Biomedical Engineering, U of Kentucky H.Y. Shin, Department of Biomedical Engineering, U of Kentucky

Abstract: There is a growing body of evidence indicating that fluid pressures regulate endothelial cell (EC) tubulogenic activity (e.g., cell proliferation) involving fibroblast growth factor 2 (FGF2) and its receptor, FGF receptor-2 (FGFR2). Our laboratory has previously reported that human ECs exposed to pressure exhibit enhanced tyrosine phosphorylation of FGFR2 in the presence of its ligand, FGF2. The underlying mechanism, however, remains unclear. We conducted a pilot study to test the novel hypothesis that pressure is facultative for FGF2-FGFR2 binding interactions on the EC membrane. For this purpose, we used a custom hydrostatic pressure system, immunofluorescence, and flow cytometric analysis to quantify the FGF2 binding efficiency of human microvascular ECs after exposure to 5 to 40 mmHg hydrostatic pressure (HP) for 30 min. These FGF2 binding studies were carried out using commercially-available FGF2-biotin conjugates (R & D Systems) and procedures adapted from the manufacturer's instructions. The recent results of the present study provide evidence that FGF2 binding efficiency of ECs is pressure sensitive. Compared to cells under control pressure conditions, ECs exposed to 20, but neither 5 nor 40 mmHg, displayed significantly increased binding of FGF2-biotin conjugates. Further work is under way to determine whether the observed increase in FGF2 binding by ECs subjected to 20 mmHg is due to upregulation of either FGFR2 surface expression or FGFR2 affinity for its ligand.

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Primary Presenter / email: **McKenty, T.** / tmckentybk@gmail.com
Student
MS

Mentor / e-mail: Shin, H.Y. / hy.shin@uky.edu

College of Engineering Biomedical Research Day
Poster Presentation Abstracts

161 Abstract Title:	**Microindentation Testing Protocols for Human Trabecular Bone**
Author(s):	D.A. Jones, Department of Biomedical Engineering, U of Kentucky D. Pienkowski, Department of Biomedical Engineering, U of Kentucky C. Wood, Department of Statistics, U of Kentucky H.H. Malluche, Division of Nephrology, Bone and Mineral Metabolism, U of Kentucky

Abstract: Reference point indentation (RPI), a form of micro-indentation, offers a novel approach for providing information regarding the mechanical properties of bone. Prior studies using the RPI method have been performed on cortical bone exclusively. RPI is not readily applied to trabecular bone because usable trabecular surface area is limited and it physically alters the substrate. Thus, RPI methods applied to trabecular bone have a single opportunity to effectively utilize available area. The objective of this study was to determine the optimum methodology that efficiently quantifies the resulting RPI parameters. Strain and stress fields produced by RPI were theoretically modeled using finite element analysis. A series of evenly spaced indentations were represented in a 3D model using 3 planes of symmetry. The indentation force was modeled as an axisymmetric non-uniform pressure distribution normal to the indentation surface. It was assumed that the substrate was 280 microns wide and 140 microns deep. Eight ex vivo trabecular bone samples from a homogeneous group of human subjects were indented 9 times (3 trabeculae per sample; 3 indents per trabecula) each using a BioDent RPI device. Variance among: a) bone samples (σb2), b) trabeculae within a bone sample (σt2), and c) indents within a trabecula (σi2) were estimated using standard variance component analysis for balanced hierarchical designs. Each candidate indentation protocol and biopsy sample size was represented by a single, averaged coefficient of variation. The protocol and consequential maximum sample size with the lowest calculated value was selected. The largest factor reducing theoretical variance involved maximizing the number of bone samples. The model showed an experimental indent depth of 50 microns placed at least 175 microns from of a second indent will avoid strain field interaction.

Supported by: NIH-NIAMS AR RO1061578 Kentucky Nephrology Research Trust

Primary Presenter / email: **Jones, D.A.** / drew.jones@uky.edu
Student
MS

Mentor / e-mail: Pienkowski, D. / pienkow@uky.edu

College of Engineering Biomedical Research Day
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162 Abstract Title:	**How Aging Affects Viscoelastic Response of the Human Lower Back to Passive Flexion**
Author(s):	I. Shojaei, Department of Biomedical engineering, U of Kentucky K. Allen-Bryant, College of Nursing, U of Kentucky B. Bazrgari, Department of Biomedical engineering, U of Kentucky

Abstract: Low back pain (LBP) has been suggested to be a leading cause for chronic health problems in older population. Structure and behavior of tissues constituting musculoskeletal subsystems (responsible for spinal stability) change with aging. Spinal instability and the risk for LBP with aging can indirectly be assessed using measures of viscoelastic behavior of lower back. Passive flexion tests were conducted in upright posture to account for the effects of gravity and corresponding muscle force responses. Viscoelastic response of lower back was characterized using measures of trunk stiffness, viscoelastic relaxation and dissipated energy. The outcome measures were found to be larger in older vs. younger population. Moreover, the trunk stiffness was found to decrease with lower back flexion angle such that the highest trunk stiffness was observed around the neutral posture. Although passive contribution of lower back tissues to trunk stiffness has been reported to be minimal around the neutral posture, the larger trunk stiffness in the neutral posture highlights the important role of active contribution to trunk stiffness and stability. Regarding the larger trunk stiffness in different lower back flexion angles (neutral standing to 40 degrees) in older population, a diminishing contribution of passive and active subsystems to spinal stability is unlikely to be a reason for higher reports of back pain in the elderly. Accordingly, the role of other contributor elements to spinal stability and equilibrium (reflexive response, muscle forces and spinal loads) in increasing the risk for LBP with aging should be further investigated.

Supported by: CDC award: R21OH010195

Primary Presenter / email: **Shojaei, I.** / shojaei.iman@uky.edu
Student
PhD

Mentor / e-mail: Bazrgari, B. / babak.bazrgari@uky.edu

College of Engineering Biomedical Research Day
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163 Abstract Title:	**How Activation of Trunk Muscles Affects Trunk Stiffness**
Author(s):	C. Suri, Department of Biosystems and Agricultural Engineering, U of Kentucky I. Shojaei, Department of Biomedical Engineering, U of Kentucky B. Bazrgari, Department of Biomedical Engineering, U of Kentucky

Abstract: Low back pain (LBP) is a leading cause of disability with annual estimates of ~ $100 billion cost in the U.S. Reduced trunk stability has been recognized as a risk factor for LBP. Trunk stability is provided by lower back stiffness including passive and active components. However, the exact relationship between muscle activity and trunk stiffness and stability is not well understood mainly due to experimental limitations and simplified modeling. The purpose of this study is to determine how different levels of trunk muscles activation influence the lower back stiffness and how this stiffness changes with different flexion postures. Twelve gender-balanced participants (18-40 years old) with no history of LBP will complete the study. Each participant will complete six passive flexion tests including two free style, two equilibrium (low load, high load; held in a lower level) based, and two stability (low load, high load; held in a higher level) based. Using a custom-made rigid frame, a flexed posture will be achieved by rotating participant's legs around their lower back in an upright standing posture while holding a load (repressing one of the six combinations above) in hand in each trial. Using a harness connected rod assembly including an in-line load cell, the rod's force and the lower back moment demand (force times lever arm) corresponding to each flexion angle are estimated and the instantaneous stiffness of the trunk is calculated. Activity of erector spinae and abdominal muscles for each trial are measured and controlled using surface EMG electrodes.

Supported by:

Primary Presenter / email:	**Suri, C.** / cazmon.suri@uky.edu Student Undergrad
Mentor / e-mail:	Shojaei, I. / shojaei.iman@uky.edu