Contributed Talks Abstracts

Arranged alphabetically by speaker's last name

The role of secretory phospholipase A_2 and PLA2 receptor expression in modulating the delivery and anticancer activity of lipid-nanomedicines in a xenograft model of human prostate cancer

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Nanoparticle drug carriers, such as pegylated liposomes, entrap drug stably, alter drug disposition, improve antitumor activity and minimize toxicity. However, control of their drug-release kinetics has limited their clinical potential. Secretory phospholipases A_2 ($sPLA_2$) are excreted and over expressed in a variety of tumors, *e.g*., up to 22-fold in prostate. These enzymes degrade phospholipids preferentially at the *sn-2* ester position and have been hypothesized as targets to control drug release from lipid-based nanoparticles, such as liposomes. We *hypothesized* that over expression of $sPLA_2$ and its receptor (PLA2R) can modulate the degradation and uptake of $sPLA_2$ responsive liposomes (SPRL), thereby increasing the rate and extent of drug release, enhancing their antitumor activity and limiting non-target tissue toxicity. Utilizing electrospray ionization mass spectrometry (ESI-MS) we determined the effect of $sPLA_2$ on the degradation of individual and combinations of lipids to their respective lysophospholipids and fatty acids. The *in vivo* specificity and antitumor activity of secretory phospholipase A_2 response liposomes (SPRL) in human prostate (PC-3) cancer cells and those where phospholipase A_2 receptor (PLA2R) expression was knocked down (PC3-PLA2R-KD) was determined in athymic mice. Mice were treated intravenously (5 mg/kg, Q1W x 5) with doxorubicin (DOX) containing SPRL or SSL formulations. Multispectral optoacoustic tomography (MSOT) and IVIS (bioluminescence, fluorescence and x-ray imaging) were used to monitor tumorigenesis, *i.e.,* blood flow, tumor/tissue oxygenation, tumor growth, and deposition of nanoparticles non-invasively. The distribution, deposition and degradation of SPRL and SSL labeled with deuterated lipids were determined by ultra-high performance liquid chromatography – tandem mass spectrometry (UHPC-MS/MS). A time-dependent increase in tumor deposition was observed following treatment with SPRL and SSL relative to free DOX. Although a significant increase in tumor deposition of SPRL *vs.* SSL was not observed, a decrease in rate of accumulation and an increase in antitumor activity (decrease tumor volume and increase in survival) were observed ($p \leq 0.05$). MOST was used to show alterations in oxygenation in tumor with growth and suggests deposition is increased in areas that were more normoxic. The effect of PLA2R-KD suggests increase in SPRL metabolism (total lipid, d70-DSPC and breakdown to lysophospholipid, d35-LPC and increased antitumor activity compared to SSL formulations; $p \leq 0.05$). Utilizing a combination of non-invasive imaging strategies combined with mass spectrometry we demonstrated that $sPLA_2$ and PLA2R alter the degradation and activity of SPRL formulations compared to SSL, but not overall tissue/tumor distribution. These data also suggest that PLA2R expression may be used as a marker to personalize treatment with different nanomedicines. These nanotherapeutics are representative of a variety of next generation targeted drug carriers. More importantly, similar approaches may be utilized to non-invasively identify disease, phenotype or grade tumors, and monitor treatment mediated effects. *This work was supported by NIH, R01EB016100, Auburn University Internal Grants Program, the Auburn Laboratory for Imaging Animal Systems (ALIAS), the Auburn Univeristy Specialized Pharmaceutical and Experimental Center for Tranlational Research and Analysis (SPECTRA) and Auburn University Initiative in Cancer Research (AURIC)*.

Smart nanomaterials for targeted drug delivery

Allan E. David

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Nanomaterials have drawn increasing attention in the biomedical field for their potential diagnostic and therapeutic applications. In addition to control of material dimensions on the nanometer length scale, functionalization of the surface with "smart" ligands that provide environment-responsive performance is also generating tremendous interest. Clinical translation of these technologies, however, has been low, primarily due to large variabilities in *in vivo* performance and safety. This is a significant barrier that can most effectively be overcome if the design of nanomaterials is based on an understanding of the structure-property relationship of nanomaterials and their interactions with biological systems. This talk will present some of the work done in developing "smart" nanoparticles and nanocomposites for application in drug delivery. We will examine the role of particle

size and surface modification on the cellular uptake and toxicity of superparamagnetic iron oxide nanoparticles (SPIONs), which have been used clinically as contrast agents for magnetic resonance imaging (MRI). We will also discuss the potential of microenvironment-responsive nanocomposites for controlled delivery of vaccines.

Polymeric Tissue Scaffolds and Biomaterials: Issues and Opportunities

Derrick Dean
Integrated Bioengineering and Advanced Materials (1-BEAM) Center, Alabama State University

Our laboratory is focused on the fabrication of polymeric tissue scaffolds. Our work seeks to understand how tissue scaffolds mimic the size scale, chemistry and functionality of the extra cellular matrix. This work is poised at the interface of nanotechnology, materials engineering and the life sciences. Our approach utilizes nano and microfabrication techniques to develop bioactive, multifunctional tissue scaffolds in which we control surface chemistry, spatiotemporal interactions, impart developmental cues and structural stability. The efficacy of our scaffolds is investigated through studies of the interactions of cells with our scaffolds. This talk will briefly describe the current issues and opportunities in the field as well as some of our recent efforts to elucidate the processing-structure-property relationships of some polymeric tissue scaffolds.

Bio and Nanophased Fiber Reinforced Composites for Structural Use

Mahesh Hosur, Shaik Zainuddin
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High performance lightweight materials are made of composites which constitute at least two different synergistic materials, a load bearing fiber normally made of glass, carbon or Kevlar® and the other one, a matrix (thermoset or thermoplastic polymer) which holds the fibers together to give unique properties that are different from the constituent materials. In recent years, a third phase consisting of nanofillers such as nanoclay, carbon nanotubes, nanoparticles made of metals and metal oxides, has been added to improve the properties of composites at very small weight loadings. While the composites give tremendous weight advantages and tailorability over conventional metallic materials, they are not sustainable or biodegradable. Hence, factors such as greater environmental awareness, societal concern and depletion of petrochemical resources together provide an impetus to develop new materials and products that are based on natural fibers, waste materials, and biopolymers. Biocomposite materials provide a significant competitive advantage for manufacturers over traditional reinforcing fibers such as glass and resins such as polyesters as product reuse or recycling at the end of life becomes the norm. The presentation will cover two prime areas of research work done at Tuskegee University: Structural Nanocomposites and Advanced Green Composites. Under Structural nanocomposites area, research work was carried out to introduce different types of nanoparticles in thermoset and thermoplastic polymers, which were subsequently used to fabricate fiber-reinforced composites and characterized. Under advanced green composites area, work was focused on developing bio-based polymers, use of fully degradable biopolymers in processing natural fiber reinforced composites, extraction of lignin and cellulose from different biomass and their use as reinforcements in polymers and synthesis of novel resin systems. This presentation address will present highlights of some of the research activities carried over in recent years. *Support from National Science Foundation through EPSCoR, CREST, HBCU-RIA grants is acknowledged*.

Molecular imaging of cells and tissues using spectral imaging approaches

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Cell physiology and pathophysiology fields have found increasing need to understand the complex, multiplexed, and spatially and temporally-localized nature of cell signaling events. Similarly, clinical diagnostic and imaging fields have found increasing need to understand the molecular basis for disease stratification and progression. Spectral imaging is a technique that was originally developed by NASA and the DoD for satellite and remote sensing applications that has potential to fulfill the molecular detection needs of both the biomedical research and clinical diagnostic communities. The technique combines aspects of spectroscopy and imaging by acquiring spectroscopic data for each pixel in an image and allowing spectroscopic analysis to be performed on individual pixels or regions. Spectral

imaging approaches hold great promise for a range of biomedical research and clinical diagnostic fields, where the ability to simultaneously analyze spectroscopic changes across many features in an image is invaluable. Such analysis can yield critical insight into the molecular composition of cells and tissues, molecule-molecule interactions, and nano-scale events. We have worked to develop spectral imaging technologies and analysis approaches to address a range of biomedical applications, from research microscope platforms to clinical endoscopy, from monitoring intracellular signaling in 5 dimensions to colorectal cancer detection. In this talk we will demonstrate new spectral imaging technologies we have developed as well as approaches to apply spectral imaging for molecule-specific studies in cells and clinical diagnosis of tissues.

Molecular engineering of myofibrils in vivo

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Purpose: The basic contractile unit of striated muscle, the sarcomere, self-organizes through the precise assembly of actin (thin) and myosin (thick) filaments. Comparative studies have shown that although the basic architecture of sarcomeres are highly conserved, evolution has generated a wide variety of specialized muscle types and contractile behaviors. To examine what governs sarcomere architecture, my lab uses the model organism *Caenorhabditis elegans* (*C. elegans*), which develop obliquely-striated, "single-sarcomere", and non-striated (smooth) muscles. By understanding this self-organization process and determining the molecules that specify sarcomere architecture in vivo, it will be possible to reconstitute and modify this process in vitro to make novel contractile systems. Methods: We are using CRISPR-Cas9 mediated homologous recombination to generate fluorescently-tagged and mutant myofibrillar proteins. The lab uses these transgenic strains to characterize myofibril assembly at the molecular level. To determine the role of myosin motors during sarcomere assembly, we generated a "headless" mutant of the muscle myosin isoform myo-3 where the ATPase motor domain is detached from the filament-forming tail domain. We used molecular and behavioral assays to verify the modification of genomic DNA and characterize muscle function within the transgenic strains and high-speed confocal and super-resolution light microscopy to observe the assembly and dynamics of myofibril components in intact, live worms. Results: Transgenic strains containing fluorescently-tagged alleles of thin filament (troponin I and tropomodulin) and thick filament (myosin isoforms myo-3 and unc-54) components show normal myofibril assembly and muscle function. In the "headless" myo-3 strain, the absence of the motor domain has minimal impact on thick filament assembly and muscle function; however, the organization of the actin filaments is altered, such that the thin filament lengths appear shorter, yet remain fully overlapped with the unc-54 myosin heads. Conclusion: CRISPR-Cas9 gene editing is a powerful new tool to observe *C. elegans* myofibril assembly and self-organization in fine detail. The appearance of shorter thin filaments in the absence of the myo-3 myosin motor domain shows that interactions between thin and thick filaments are important for the precise determination of thin filament lengths. Our results show that myosin contributes to thin filament arrangement during myofibril assembly.

Characterization of surface markers expressed on exosomes derived from tumor cells in varying culture conditions

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Introduction: Extracellular vesicles have become a major focus of study in many fields of both basic and clinical research. The smallest of these particles, exosomes range from 50-200nm in size. Traditional flow cytometers generally struggle to resolve individual exosomes. We have leveraged the precision of the acoustic focusing cytometer Attune™NxT to analyze individual exosomes for the expression of a variety of surface markers using multi-color staining. Methods: Exosomes were isolated via differential ultracentrifugation from several cancer cell lines as well as primary tumor cells cultured from patient samples. The cells were maintained in a variety of culture conditions (media with or without FBS, normoxia versus hypoxia) to compare the relative expression of surface markers. Directly labeled monoclonal antibodies were used in a variety of combinations to identify true lipid bi-layer exosomes from debris using markers such as CD9, CD63 and CD81 as well as the lipophilic styryl dye FM 1-43. Oncological related surface markers such as CD44, CD184, CD202b and EGFR were also evaluated. Particles

isolated at varying centrifugal forces were measured via dynamic light scattering, to attempt to relate measured size to forward and side scatter parameters. Results: The use of ultra-filtered sheath fluid (25nm pore size) resulted in extremely low background noise, facilitating the resolution of individual exosomes. Compared to conventional flow cytometers, the Attune™NxT uses far less sheath fluid making the time consuming task of ultra-filtration feasible. Narrow beam shaping optics (10mm X 50mm) along with the particle alignment precision afforded by acoustic focusing allow for optimal detection of small particles. Diluting exosome preparations far enough enables the measure of single particles, avoiding what is often referred to as the "swarm" effect of multiple particles traversing the laser beam spot simultaneously. Exosome preparations of varying size were able to be detected using traditional forward and side scatter parameters. 405nm side scatter excitation provided a moderate improvement over 488nm side scatter. Cells are routinely cultured in serum free media to prepare exosomes for western blot or mass spec proteomic analysis. We sought to characterize the expression of surface markers on exosomes prepared from both conditions and found very little variation between the two groups. Differences in marker expression were seen when comparing cancer cells grown in either normoxic or hypoxic conditions.

Testing novel antimicrobials using 3D printed scaffolds

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Many challenges remain in the development of new drugs and testing platforms for resistant bacterial infections. Although there are several 2D monolayer tissue models they cannot reproduce *in vivo* complex environments, with several studies reporting discrepancies in cell signaling pathways and drug responses between 2D or 3D culture conditions. Additive manufacturing (AM) technology is a potential solution for constructing complex 3D biocompatible structures via automated deposition of molecules on a substrate using computer-aided design/computer-aided manufacturing (CAD/CAM) technology.
In our previous studies, we have gained expertise in testing novel antimicrobial agents for the treatment of bacterial infections. In partnership with the University of Louisville, we are now exploring the development of 3D printed scaffolds for growing keratinocytes and epithelial cells. We are also exploring the use of commercially available scaffolds for the same purpose. Here, we will present some of our results on growing keratinocytes and epithelial cells on 3D scaffolds, followed by infection with bacterial pathogens and their inhibition by using novel nanomaterials.

Bioenergetics of the self-organizing forces across endothelial cells

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Background: Biological cells are no exception to the laws of physics. One such law is that of energy conservation. However, this fundamental law has not been assessed quantitatively in cells. As a result, it is unclear how much effort (or mechanical work) does a cell put into doing routine tasks such as (a) adhesion to the substrate, (b) adhesion to the neighbors, (c) migration and (d) cytoskeletal contraction. Enabling the measurement of such mechanical work is crucial to put the cellular energy conservation can be under the microscope. Innovation: We demonstrate that Monolayer Stress Microscopy (a novel in vitro platform to measure local mechanical forces around a cell [Ref. 1-2]) enables a novel and straightforward quantification of the mechanical work that each cell in an advancing monolayer does on its substrate, UT, and on its neighbors, US. Experimental system: We report mechanical work by individual cells within advancing monolayers of three cellular systems: pulmonary artery endothelial cells (AEC), pulmonary microvascular endothelial cells (MEC), and pulmonary vein endothelial cells (VEC). These three cellular systems are known to exhibit remarkable functional and molecular heterogeneity [Ref. 2-4]. Results and Conclusion: Although each cellular system had an advancing front, the AEC with their uniform cobblestone morphology and negligible motion were most quiescent and VEC with their non-uniform mesenchymal morphology and non-coherent motion were least quiescent. The forces that each cellular system exerted on the substrate and on neighbors were remarkably heterogeneous. Heterogeneity was also present in the patterns of mechanical work. But compared to the patterns of UT, the patterns of US appeared to have stronger spatial correlations. The farther cells were from being quiescent, the more strongly they were engaged in mechanical work. The mechanical work of the fastest and most coherently moving

cells – the MEC – were least sensitive to the cellular size, distance from the advancing front. Surprisingly, and again in contrast with AEC and VEC, the MEC with spatially homogeneous motion appeared to exert greater mechanical effort. This unanticipated behavior appeared to be steered by the unique ability of the MEC to orient the intercellular traction more strongly along the cell-cell boundary. Taken together, we demonstrate a straightforward method to quantify mechanical work in adherent cells. We discovered a unique physical behavior of MECs that may find applications in tissue engineering and drug discovery.

Silver shield against UVB induced skin carcinogenesis

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Exposure to ultraviolet (UV) radiation from sun remains the foremost epidemiological cause of skin malignancies, which account for more than a million new cases each year in the United States alone. Direct exposure of skin to UV radiation causes DNA damage, which if not corrected, leads to accumulation of carcinogenic mutations over time and results in transformation of cutaneous cells. Hence, there is a pressing need for the development of a novel, safe and effective preventive approach to combat UV radiation induced deleterious effects. In this regard, we have tested the chemoprotective role of silver nanoparticles (Ag NPs) against UV radiation-induced skin damage. AgNPs were synthesized by reduction-chemistry and characterized for their physicochemical properties. Synthesized AgNPs were well tolerated by human immortalized keratinocytes (HaCaT) cells and their pretreatment protected them from UVB-irradiation-induced apoptosis along with significant reduction in cyclobutane-pyrimidine-dimer formation. Moreover, AgNPs pre-treatment led to G1-phase cell-cycle arrest in UVB-irradiated HaCaT cells. AgNPs were efficiently internalized in UVB-irradiated cells and localized into cytoplasmic and nuclear compartments. Application of AgNPs on the skin of SKH-1 hairless mice drastically reduced (88.8 %) the incidence of squamous cell carcinoma formation upon repetitive UVB (180 mJ/cm^2) exposure for 29 weeks. Furthermore, in a comparative analysis of direct and indirect UVB-protection efficacy of AgNPs against known active ingredients of commercially available sunscreens viz. zinc-oxide (ZnO) and titanium-dioxide (TiO2) nanoparticles, we observed that UVB-reflective/absorption abilities was the highest for TiO_2-NPs followed by Ag- and ZnO-NPs. However, only Ag-NPs were active in protecting HaCaT cells against direct UVB-induced DNA-damage by repairing bulky-DNA lesions and also protecte HaCaT cells from UVB-induced oxidative DNA damage. In contrast, ZnO- and TiO_2-NPs not only failed in providing any direct protection from DNA-damage, but rather enhanced oxidative DNA-damage by increasing ROS production. Together, our findings raise concerns about safety of ZnO- and TiO_2-NPs and establish superior protective efficacy of Ag-NPs.

Economical Processing and Property Optimization of High Temperature Polymer and Fiber Nanocomposites

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High temperature carbon fiber reinforced bismaleimide composites (CFRBCs) have been used for making 30-50% airplane parts in the Air Force F-22, F-35, C-17 and Boeing 780. However, the autoclave mold processing method utilized for manufacturing these parts is not cost effective and energy efficient. Therefore, the goal of this joint effort between Tuskegee University, AFRL, and Grambling University is to develop alternate cost-effective and relatively energy efficient method/s to manufacture these parts and utilize the same for large scale production. Here, we present one such CFRBC processing method developed which is cost effective and relatively energy efficient. In addition, results obtained from the mechanical and thermal tests of the processed CFRBC will be discussed.

Undergraduate Student Poster Abstracts

Arranged alphabetically by presenting author's last name with names of presenting authors underlined

UP01 Micro-LIBS on NIST SRM 610 and 1831

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Laser-induced breakdown spectroscopy is a proven effective analytical tool for elemental analysis of a variety of materials. Although LIBS is considered a minimally destructive method, using less energy and reducing the spot size may further reduce damages caused during the analysis. This may be advantageous when working on precious materials or forensic samples. Typical Micro-LIBS produces craters that have diameters and depths that are much less than conventional LIBS. One of the smallest reported crater sizes was 3 □ m in diameter. The purpose of this experiment is to implement a procedure in which less material of the sample is destroyed in the LIBS process, but can still be used to get accurate elemental data for samples with a range of transparencies. In this study, a 10X microscope objective is used to create LIBS plasmas on 5 glass standard reference materials. NIST standard reference materials 610, 612, 614, 620 and 1831 were used in this study. The Nd:YAG laser is operated at 532 nm and experiments are performed at energies less than 1.50 mJ. In this experiment, the emission line from Strontium at 407.76 nm is used to optimize experimental conditions. We report on the influence that the lens to sample distance has on the intensity and percent relative standard deviation of the emission.

UP02 Analysis of Au Nanoparticles Using Laser Induced Breakdown Spectroscopy

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In the field of Nanotechnology, nanoparticles can be applied to various biomedical applications. Specifically, gold nanoparticles (GNPs) have been evaluated for applications in targeted drug delivery due to their size and chemical properties. In the present study, we investigate the analytical capabilities of Laser Induced Breakdown Spectroscopy (LIBS) for the determination of the concentration of GNPs in a biological matrix. Six concentrations of GNPs, ranging from .05 µg/µL to 1.5 µg/µL, were deposited in Hep-2 cells and after left to incubate for 24 hours at 37 °C. The cells are then deposited on pure silicon wafers and left to dry. LIBS was used to detect the GNPs using a gold emission line at 267.5 nm, Au I. To determine the concentration of GNPs in the Hep-2 cells, calibration curves were produced from the LIBS emission using standards. In this study, we used the second harmonic of the Nd:YAG at 532 nm as the irradiation source. The laser was delivered to the samples using a 10X microscope objective. Optimization of focusing conditions, gate width and gate delay are presented. In addition, we estimate the limit of detection of gold nanoparticles using the slope and three times the noise.

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UP03 Surveillance of *Salmonella* contamination using Quantum Dots

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Salmonella is a food borne bacterial pathogen that causes diarrhea, vomiting and fever on consumption of contaminated food. Hence, faster and efficient detection methods are required. We plan to conjugate quantum dots (QDs) with antibody (Ab) for detection of bacteria and magnetic nanoparticles (MNs) for capturing of the targeted bacteria in a solution. Various concentrations of Salmonella will be then plotted against Fluorescent intensity of QDs-Ab-MNs. This method is expected to rapidly detect bacteria in contaminated food and can be used for surveillance of Salmonella contamination.

UP04 Comparative analysis of cadmium and cadmium-free quantum dot interactions with boar spermatozoa

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Purpose: The typical core-shell of quantum dot nanoparticles (QD) contains cadmium/selenide-zinc sulfide (CdSe/ZnS). This semiconductor has a large spectrum and size-dependent fluorescence emission, varying from violet to deep-red and providing opportunities for bioimaging applications. However, cytotoxic effects of QD have been attributed to the cadmium and efforts for its replacement with non- or less-toxic materials such as copper indium sulfide (CuIS), while maintaining the fluorescence capability of the QD are still ongoing. Here we compared the effectiveness of copper (Cu) and cadmium (Cd) core-shell QDs for successful sperm labeling.
Methods: Extended fresh boar semen were centrifuged and sperm pellets were resuspended in phosphate buffered-solution (PBS). Sperm concentrations were adjusted to $2x10^8$/ml and 0.5 ml aliquots were mixed with various concentrations (0, 0.02, 0.05, and 0.1 nM) of QD (CuIS/ZnS or CdSe/ZnS). Immediately after co-incubation (30-45 minutes) at 37^{o}C, aliquots of sperm mixtures were evaluated for motility and morphology using a computer-assisted sperm analyzer (CASA). The excess of QDs in remaining sample mixtures were removed by two successive centrifugations. Resulting sperm pellets were imaged to detect sperm-bound QD fluorescence emission, using the IVIS-Imaging system and EVOS-FLAuto microscope. Sperm motility for 3 independent replicates was analyzed byANOVA-1 with significance at $P<0.05$)).
Results: Analyses of sperm motility characteristics indicated general and dose-dependent decreases in the proportions of motile and forward progressive spermatozoa, irrespective of the core QD material (Cd or Cu; $P<0.05$). At 0.02nM and 0.05nM, both QD-core materials significantly reduced the proportions of abnormal spermatozoa, displaying bent tail or distal cytoplasmic droplet ($P<0.05$). More specifically, all Cu-QD concentrations significantly reduced the proportion of spermatozoa with bent tail, as compared to the control ($P<0.5$). Fluorescence imaging revealed labelling throughout the entire spermatozoon with higher accumulation in the head region, but the signal intensity of the Cu-QD appeared weaker than that of Cd-QD.
Conclusion: Both Cu and Cd core QD nanoparticles showed potential toxicity at higher concentrations, and Cu-QD exhibited greater enhancement of sperm motility characteristics and morphology. Further ultrastructural analyses of will provide a better indication of QD-spermatozoa interactions.
Work supported by USDA-ARS Biophotonics Initiative #58-6402-3-018.

UP05 The *In Vitro* Effects of Alcohol on Exosome Biology

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Purpose: Exosomes are extracellular vesicles that are active in cell-to-cell communication, transferring macromolecules between cells, biological markers used to detect disease states. Exosomes are found in extracellular space and made up mostly of lipids and proteins. Exosomes can be affected by external factors such as alcohol and nicotine. Specifically, we will be focusing on the effect of alcohol on exosome biogenesis. Alcohol has factors that can be beneficial to the body in moderation. However, alcohol in high concentrations can negatively alter extracellular vesicles production and release. Our goal is to study the impact of alcohol exposure on kidney exosome biology.
Methods: Human kidney cells (293A) were subjected to (control), 50 mM, or 100 mM of Ethanol for 24 hours, 48 hours or 72 hours. Cell viability was observed at 72 hours post Ethanol treatment. 293A cell viability was significantly decreased with treatments of 50 mM or 100 mM of Ethanol. Exosomes were purified using an ultracentrifugation after dosing with alcohol or vehicle control. The protein quantity and quality was determined using standard protein quantitation methods. NanoSight technology was used to measure exosome count and characterize exosomes along with Enzyme-Linked immunosorbent assay (ELISA).
Results: We observed that when 293A cells were treated with alcohol, exosome biogenesis was impacted, we observed a decrease in exosome production over time as well as an increase in exosomes carrying HSP60 and HSP70 proteins.
Conclusion: Overall, these results suggest alcohol has a negative affect on cells leading to the downstream impact on exosomes. This supports the claim that alcohol can have detrimental effects

on the human body. Our future research includes investigating the effects of alcohol consumption on exosome production *in vivo*.

UP06 Simulation and Optimization of a Hyperspectral Imaging Light Source

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Purpose: The mechanical switching of tunable filters in a hyperspectral imaging system does not allow for imaging live cells. Combining multiple wavelengths using LEDs in a mirror array will improve acquisition time for data to be acquired. The goal of this work was to optimize the optical geometry of a led mirror array using Monte Carlo optical ray trace modelling.
Methods: We have simulated a prototype light source using a ray tracing software called TracePro (Lambda Research Cooperation). It includes two mirrors, a lens, a liquid light guide input, and LEDs. The flux percentage was measured at the liquid light guide input. A parametric study was performed where the model was optimized by varying the lens focal length, x and z position, and using one or two lenses. The power output was recorded via feedback from the program in an irradiance map. The irradiance map also displays the distribution of light from the LED.
Results: Initial results demonstrate that spacing of lenses and LEDs have a large effect of the optical power that can be coupled into the liquid light guide. For each type of configuration (1 lens,2 lens) an optimized lens and LED position was calculated.
Conclusions: Desired results for the power output are around ten percent. We will continue to optimize our current software model till power transition of ten percent is achieved and then we will prototype the current system on our optical bench and test it experimentally.

UP07 Liposomes for delivery of an anti-cancer drug genipin

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Purpose: Liposomes are tiny lipid vesicles with hydrophilic core. Liposomes due to their structural features can be used as a carrier of hydrophilic as well as lipophilic molecules. On the other hand genipin (GPN) is a naturally aglycon sourced from the gardenia plant (Yoo, Kim et al. 2011; Lin, Wu et al. 2013). In this project genipin encapsulated in liposomes will be tested against breast, colon and prostate cancer cells.
Method: The lipsoomes were produced using the conventional thin film hydration method (Bangham, Standish et al. 1965). The drug genipin was dissolved along with the lipid in ethanol and using the rotary evaporator the ethanol was then evaporated to form a thin lipid film. This lipid film was then hydrated using phosphate buffered saline (PBS) which resulted in formation of multilamellar vesicle (MLV). The MLV suspension was then subjected to probe sonication to produce small unilamellar vesicles (SUV). The size, size distribution and surface charge was analyzed using Malvern zetasizer-ZS. The percent encapsulation of genipin was then determined using the UV-Visible spectrophotometer at 242 nm wavelength. The efficacy of genipin alone and genipin encapsulated liposomes was tested against MDB-MA 231 cell lines.
Results: It was observed that upon sonication the size of empty liposomes was 130 nm (± 1 nm, n=3) and no significant difference was observed after encapsulation of genipin as the size of liposome found was 105 nm ± (10 nm, n=3). There was no charge observed on the liposomes (0.0 ± 10) before or after encapsulation of genipin. Passive encapsulation of lipophilic drug usually results in less than 50 % encapsulation and in case of genipin it was 28.2 % (± 0.2 %, n=3). During initial sturdies the efficacy of these genipin encapsulated liposomes was tested against MDB-MA 231 cell lines and it was found that the liposome encapsulated genipin is remarkably efficient than genipin alone.
Conclusion: The initial result suggest successful encapsulation of genipin into the liposomes. Also, the study performed against MDB-MA 231 cell lines suggest that liposomal encapsulation could bring remarkable difference in efficacy of genipin instead of using it alone. However, the further stage of this project would include testing these genipin encapsulated liposomes against other cancer cells such as breast, colon and prostate.
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UP08 Effects of Magnetic Nanoparticles conjugated to Annexin-V on the purification and cold storage of *Anaxyrus fowleri* sperm

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Purpose: To ensure the survival of amphibian populations, assisted reproductive strategies are essential. One strategy is the storage of genetic material for in-vitro fertilization (IVF); however, the degradation of healthy sperm due to contamination of byproducts from apoptotic sperm cells is a major issue when storing amphibian sperm. Magnetic nanoparticles have been used to purify mammalian semen, but nanopurfication of amphibian sperm has not been conducted. The purpose of this study is to determine if annexin-V conjugated magnetic nanoparticles could purify amphibian sperm by removing pre-apoptotic sperm cells, which contribute to apoptosis driven toxicity.
Methods: Spermic urine samples collected from Fowler's Toads (*Anaxyrus fowleri, n=5*) were stored at 4^{o}C throughout the sampling process. Annexin-V conjugated magnetic nanoparticles (8.8µl, 17.5µl or 35µl) were added to each 500µl of spermic urine after collection and incubated for 10 minutes. A magnet was applied to the samples to remove nanoparticle-bound apoptotic sperm. Sperm motility parameters were collected initially and after purification from samples. Nanoparticle effectiveness on sperm sample quality was further assessed by measuring sperm viability using SYBR 14/Propidium iodide (live/dead) stains as well as pre-apoptotic binding by annexin-V stain.
Results: Initial sperm samples were 74% viable, 61% motile and 16% apoptotic. After treatment using 17.5□l of magnetic nanoparticles sperm samples contained 68% viable, 50% motile and 41% apoptotic sperm. Thus the motility decreased while the fraction of sperm that became apoptotic increased upon exposure to the magnetic nanoparticles. The pellet of nanoparticle-bound sperm contained 97% necrotic sperm of which 79% were non-viable, demonstrating the effective targeting of existing apoptotic sperm by the magnetic nanoparticle purification process. However, these effects were not significant (p-value: < 0.05).
Conclusions: Magnetic nanoparticles did not statistically improve the quality of amphibian sperm samples; however, through observation of the pellet and concentration changes, the ability to remove apoptotic sperm cells is suggestive. Notably, the increase population of necrotic sperm in the sample post-purification procedure implies potential induced toxicity by the nanoparticles. The degree of toxicity may be determined through further experiments testing dose dependence of the nanoparticles and their effective decrease in sperm quality.

UP09 Green synthesis of silver nanoparticles using Beringia Ligulata plant extract: characterization and antimicrobial properties

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Purpose: This study was conducted to develop an environmentally safe, and cost-effective method for synthesis of silver nanoparticles (AgNPs) using Bergenia Ligulata plant extract. The inherent anti-microbial activity of Bergenia Ligulata may add or synergize the efficacy of silver nanoparticles and can be useful in many biomedical applications.
Method: A rapid, simple, one-step approach was applied for synthesis of silver nanoparticles using Bergenia Ligulata plant extract. The plant extract acts both as reducing agent as well as stabilizing agent. In brief, an aqueous solution of silver nitrate (0.1 mL, 0.1 M) mixed with freshly prepared aqueous plant extract (9 mL) and volume was made up to 10 mL with distilled water. The mixture was incubated at 30^{o}C in dark and continually stirred for 48 hrs. After 48 hours, the solution was

removed and stored for characterization and antimicrobial testing. Minimum bactericidal concentration (MBC) was determined by double dilution technique. MBC was recorded as the lowest concentration of AgNPs that showed no growth on agar plates after spot inoculation and incubation for 24 h. Assay was performed in triplicate with appropriate controls (uninoculated medium and medium without AgNPs).
Results: UV-Visible spectroscopic studies showed characteristic Surface Plasmon resonance (at 500 nm) for silver nanoparticles. TEM studies revealed that silver nanoparticles were spherical in shape and in the size range of 10-100 nm. Elemental analysis using Energy Dispersive X-ray (EDX) spectroscopy confirmed the presence of silver in nanoparticles. Minimum bactericidal concentration (MBC) for *Escherichia* coli and *Pseudomonas* aeruginosa was found to be 0.625µg/mL.
Conclusion: In this study, we have successfully developed a novel green method for synthesis of silver nanoparticles using aqueous extract of Bergenia Ligulata for the first time with desirable antimicrobial effects against gram-negative bacteria. Green synthesized AgNPs could be an effective and safer alternative to conventional antimicrobial agents.
This work was supported by National Science Foundation (NSF) Major Research Instrumentation (MRI) Program (NSF MRI 0817504)

UP10 In Vitro Characterization of the Gelatin and Extracellular Matrix Based Construct for Wound Dressing

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Purpose: During liver surgery, uncontrolled oozing from small vessels and sinusoids can be reduced by techniques such as electrocautery, laser technique, and vessel sealing with biodegradable hemostatic agents. Gelatin-based hemostats have been long known as an effective hemostatic agent and have been processed into a variety of forms for pharmaceutical and medical applications. To enhance robust tissue regeneration and reduce scarring and post-operative complications after liver surgery, the gelatin based agents can be combined with natural polymers to better mimic the native tissue microenvironment and to induce cell ingrowth and activities. Extracellular matrix (ECM) that derived from the decellularized native tissues have been broadly investigated by using many biomedical applications because they are cellular and antigen free as well as contain a complex mixture of biomolecules, growth factors, and biological properties.
Methods: In this study, macroporous ECM–gelatin (EG) sponges were prepared by integrating different proportions of liver ECM with gelatin. The sponges were then characterized by their in vitro digestion, mechanical, and thermogravimetry properties. The use of equipment and methods such as thermogravimetric analysis and BCA assay helped us to determine the percentage of weight loss at a specific temperature as well as the percentage of protein loss, respectively.
Results: Scaffolds that contained only gelatin had a higher rate of loss of protein than the scaffolds that contained liver ECM. Degradation of the scaffolds using collagenase was higher in the scaffolds that were composed of only gelatin as opposed to the scaffolds that were composed of gelatin and liver ECM.
Conclusion: Protein loss was almost 50% greater in the gelatin only scaffolds as opposed to those that contained gelatin and liver ECM, which indicates that the degradation, stability, and the mechanical properties of the gelatin scaffolds can be altered and predicted by adding addition natural ECM.
This work was supported by NSF-REU (DBI-1659166) to Dr. Komal Vig (PI) and by NSFCREST (HRD-1241701) to Dr. Shree S. Singh (PI)

UP11 Characterization of antibacterial effects of silver-coated magnetic nanoparticles against *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *S.* Anatum

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Purpose: The increasing resistance of many food borne pathogens to existing antibiotics continues to plague healthcare and food safety. The antibacterial effect of silver nanoparticles have shown promise in killing different species of pathogens. Further magnetic nanoparticles coating with silver offer capability for targeting and separation of antibacterial agents. Here we analyzed the antibacterial effect of silver-coated

magnetic nanoparticles on food borne pathogenic bacteria.

Methods: Three major foodborne pathogens, *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *S.* Anatum, underwent transfection of plasmids to create self-illuminating bioluminescent bacteria by electroporation. Transformed bacteria were cultured in the presence of various concentrations of silver magnetic nanoparticles to evaluate bacterial inactivation. Cultures were performed in triplicates, followed by the evaluation of bacterial growth kinetics by in *vivo* bioluminescence imaging and optical density (OD) measurements. Thereafter, culture media containing bacteria and nanoparticles were placed under a magnetic field to trap nanoparticles. While bacteria were eluted and incubated on solid agar media for direct colony forming unit (CFU) counts, trapped-nanoparticles were prepared for ultrastructural examinations of interactions with bacteria using transmission electron microscopy (TEM). Data were analyzed by ANOVA-1 and significance threshold set for $P < 0.05$.

Results: Bioluminescence intensities, OD measurements, and CFU revealed significant antibacterial effects of high concentrations of silver-coated magnetic nanoparticles (200 µg). In comparison to control cultures, lag phases of all bacteria cultured in the presence of silver-coated magnetic nanoparticles were significantly and dose-dependently increased ($P<0.05$). Among the three investigated bacteria, *E. coli* exhibited the strongest growth inhibition with a longer lag phase, as compared to *S.* Typhimurium and *S.* Anatum lag phases ($P>0.05$). Bacteria originated from higher nanoparticles exposure generated significantly lower CFU than the controls. The antibacterial effect of the silver-coated magnetic nanoparticles was confirmed by the TEM images, showing complete cell membrane ruptures.

Conclusion: The present study demonstrates the antibacterial capability of silver-coated magnetic nanoparticles through three different evaluation tools. These effects were stronger in *E. coli* and further studies are needed to extend the usefulness of silver-coated magnetic nanoparticles on other bacterial types.

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UP12 Endosomal Escape of Bacteriophage in MDA-MB 231

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Bacteriophage also known as “phage” is a virus that infects a bacteria and reproduces inside it. Phage display is important in cell targeting and therapy. Phage display targets selected proteins on the surface of phage. The purpose of this study was to select phages from the phage library which will specifically bind to MDA-MB 231 cells and can bypass endosomes. Chloroquine phosphate is the endosomal inhibitor that was used to disrupt the endosome in the MDA-MB 231 breast cancer cells. A series of phage selections on MDA-MB 231 breast cancer cells were performed on cells with the endosomal inhibitor, chloroquine. Binding assays were performed to investigate if the selected phages could bind specifically to the MDA-MB 231 cells. Three rounds of phage selection were performed. In the first round phages were sequentially selected to plastic, to serum and to MDA-MB 231 cells to acquire cancer cells binding specific phages. Cells were washed with elution buffer to wash unbound phages followed by lysis buffer to break the cells to release selected phages. Phage tittering was performed on all washes, input, eluate and lysate using E. coli and plaque colonies were counted. The selected phages in lysate and eluate were amplified using E. coli. Amplified phages were centrifuged and supernatant containing phages was precipitated using PEG NaCl. These steps were repeated in second and third round of phage selection in MDA-MB 231 cells. Binding of the phage to the target MDA-MB231 cells in the third round were tested by phage ELISA using phage specific M13 antibody. ELISA results show 3.8 times higher binding to the MDA-MB231 cells compared to untreated cells. Phages will be further tested for their ability to escape endosomes.

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UP13 Ethanol Exposure Impacts Exosome Biogenesis of HeLa Cell-derived Exosomes

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Purpose: Exosomes are microvesicles that range from 30 – 100 nm in size. They can be found in various bodily fluids (breast milk, blood, semen, urine) and function mainly in cell-to-cell communication. Exosomes allow intracellular communication via DNA, RNA, and protein trafficking. External stressors such as alcohol have been known to alter the biogenesis and release of these vesicles.
Methods: Currently, we are quantifying alcohol's effects on exosome biogenesis at varying time points and concentrations in HeLa (cervical cancer) cells. HeLa cell were treated with 50 mM and 100 mM of Ethanol, respectively, for 24, 48 and 72 hours. Cell viability was observed at 72 hours post Ethanol treatment. HeLa cell viability was significantly decreased with treatments of 50 mM and 100 mM of Ethanol. After treatment, exosomes were purified from media via high-speed centrifugation. Exosome quantity was determined by the Lowry protein quantitation method. NanoSight technology was used to measure exosome diameter and exosome count after alcohol exposure. Enzyme-Linked Immunosorbent Assay was performed on exosomes to detect various exosome-specific proteins.
Results: The presence of exosomes was confirmed by the detection of various tetraspanins (CD9, CD63, CD81), as well as chaperone proteins (HSP90, HSP70, HSP60). Our findings show that alcohol-derived exosomes contain significant quantities of HSP60, HSP70, Fas, and Caspase-9 when compared to control- derived exosomes.

UP14 Using CRISPR-Cas9 gene editing to fluorescently-tag myosins in *C. elegans* muscles and characterize their assembly

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Purpose: Understanding how actin and myosin filaments self-organize into contractile myofibrils is important for promoting and restoring normal muscle function during growth or after injury. The roundworm *Caenorhabditis elegans* (*C. elegans*) uses obliquely striated muscles for locomotion, and non-striated 'single-sarcomere' muscles for reproduction and digestion. Within its body muscles, two isoforms of myosin (myo-3 and unc-54) co-assemble and are responsible for the initiation and growth of thick filaments, respectively. To directly observe how thick filaments normally assembly into contractile structures, we generated transgenic strains that express fluorescently-tagged myo-3 and unc-54 myosins.
Methods: To fluorescently-tag muscle myosin in *C. elegans*, we targeted CRISPR-Cas9 to the myo-3 and unc-54 genes (on chromosomes V and I, respectively) and screened for individual worms that had fluorescent muscles, incorporated the mCherry (mCh) and GFP coding sequences into the endogenous chromosomal loci. We used spinning disk confocal microscopy to observe the organization of mCh-Myo3) and GFP-Unc54 into thick filaments within intact, live *C. elegans*.
Results: Homozygous strains expressing either mCh-Myo3 or GFP-Unc-54 were viable, motile, and formed thick filaments. In the obliquely-striated body wall muscle (BWM), narrow regular bands were visible in mCh-Myo3 homozygotes and wide double bands (doublets) were visible in GFP-Unc54 heterozygotes. In contract, irregular clusters of thick filaments were visible in GFP-Unc54 homozygotes, resulting in abnormal BWM myofibrils. In double heterozygous worms expressing both mCh-Myo3 and GFP-Unc54, the narrow mCh-Myo3 striations were located between the GFP-Unc54 doublets, consistent with the known spatial organization of the myosin isoforms within the *C. elegans* thick filaments.
Conclusion: CRISPR-Cas9 gene editing has enabled us to tag the endogenous muscle myosin genes of *C. elegans* with fluorescent proteins, which are well-tolerated during muscle assembly and appear to have minimal impact on contractile function. Although the GFP-Unc54 (homozygous) strains have slightly disrupted BWM striations which precludes their direct use to study normal myofibril assembly, they are relatively healthy and can be maintained to make heterozygotes that assemble normal BWM. Together, the fluorescent tags will allows us to use these strains in future studies to determine how the thick filaments assemble and organize into striated myofibrils.

UP15 The Effect of Simulated Body Fluid Exposure on the Structure and Properties of 3-D Printed Polymeric Scaffolds

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Additive manufacturing, also known as 3-D printing, is revolutionizing many fields, including the field of tissue engineering. The ability to fabricate tissue scaffolds and tissues with complex structures is an appealing feature of this technique. Polymeric scaffolds fabricated using 3-D printing are finding numerous applications for a wide range of tissues. Polymeric tissue scaffolds have shown great potential in tissue engineering and are gaining importance for a range of applications. Ideal scaffolds should possess interconnected pores and surface characteristics (e.g., hydrophilicity) to promote cell adhesion and growth. Scaffolds should also have adequate mechanical properties to maintain the 3-D structure as well as biodegradability. Our research investigates the effect of simulated body fluid (SBF) on the degradation behavior of polymeric tissue scaffolds. A number of scaffold materials, architectures and porosities are under investigation**,** including Polylactic acid (PLA) and Polyvinyl alcohol (PVA) based hydrogels. The effect of SBF exposure on crystallinity, modulus, mass gain and mechanical strength have been investigated. These properties were correlated with structural changes, as characterized by infrared spectroscopy. Studies of the crystallinity show that 3D printed samples exhibit lower crystallinity compared to conventional processing methods. This leads to slightly lower mechanical properties. The effect of the crystallinity on properties and its impact on degradation behavior will be presented.

UP16 Development of a novel liposome nanoparticle for sperm transfection

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Purpose: Liposomes are sphere-shaped vesicles with a membrane composed of at least one bilayer phospholipids. They have been successfully applied for intracellular drug or genetic material delivery in many biomedical areas. In agriculture however, their use to produce transgenic farm animals through exogenous DNA transfection of spermatozoa remains unsatisfactory. Here we tested a newly designed liposome preparation for effective and harmless interactions with boar spermatozoa.

Methods: Extended fresh boar semen were centrifuged and sperm pellets were resuspended in phosphate buffered-solution. Sperm concentrations were then adjusted to 2x 10^8 /ml and aliquoted in 0.5 ml for labeling with various concentrations of plain-fluorescent (Experiment 1) and fluorescence-loaded and modified (Experiment 2) liposomes. After co-incubation of 45-60 minutes at 37^{o}C, sperm aliquots were immediately evaluated for motility and morphology characteristics using a computer-assisted sperm analyzer. In experiment-1, the excess of liposomes in mixtures were removed by two successive centrifugations, followed by sperm fluorescence imaging, using the In vivo Imaging System (IVIS) and EVOS-FLAuto microscope. Sperm motility of three independent replicates was analyzed by ANOVA-1 with significance at $P<0.05$.

Results: In experiment 1, the presence of liposomes did not significantly affect the proportions of motile, forward progressive, and rapid spermatozoa that remained comparable to the control ($P>0.05$). In experiment 2, the presence of liposomes significantly improved the motility characteristics and velocity parameters of spermatozoa, as compared to the control ($P<0.05$). In parallel, the proportions of abnormal spermatozoa with bent tail or distal cytoplasmic droplet were significantly decreased when compared to the control ($P<0.05$). The successful interaction of spermatozoa with liposomes was confirmed by fluorescence imaging.

Conclusion: Fluorescence-loaded and modified liposomes appeared more effective than the plain liposome formulation to interact with boar spermatozoa while improving their motility and quality.

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UP17 Watching muscle thin filaments in live *C. elegans* to characterize their assembly

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Purpose: In striated muscle, calcium release allows precisely aligned myosin-containing (thick) and actin-containing (thin) filaments to interact and move relative to each other resulting in shortening and contraction. The troponin (Tn) complex regulates contraction by sterically blocking the myosin motor binding sites along the thin filaments

in the absence of calcium. Understanding how striated muscle myofibrils self-assemble into uniform, functional structures is important for preventing muscle disease progression, reversing declines in muscle contraction during aging, and promoting muscle regeneration during growth. To observe the process of myofibril assembly and growth, we are using gene editing techniques to observe thin filament components in the roundworm *Caenorhabditis elegans* (*C. elegans*). Here, we present our initial characterization of fluorescently-tagged tni-3, one of four troponin I (TnI) isoforms in *C. elegans*, which is expressed in a subset of muscles.
Methods: We used CRISPR-Cas9 gene editing to insert the coding sequence for the red fluorescent protein mCherry (mCh) in frame with the tni-3 gene and, based on the known expression pattern of tni-3, we screened for fluorescent body wall muscles near the head. We used spinning disk confocal microscopy to observe the incorporation of mCh-Tni3 into the thin filaments in live worms.
Results: We isolated four independent strains of transgenic worm that expressed mCh-Tni3 in body wall, excretory, and reproductive muscles within adult hermaphrodites. We also observed mCh-Tni3 expression within the specialized reproductive muscles of males. In each muscle, mCh-Tni3 incorporated into wide, well-organized bands along the thin filaments. Co-localization with other myofibril components was consistent with fully-assembled sarcomeres. Transgenic worms strains homozygous of mCh-Tni3 were motile, capable of laying eggs, able to defecate, and progressed through development normally.
Conclusion: Fluorescently-tagged troponin I is fully functional and incorporates normally along the thin filaments in *C. elegans* muscles. Because mCh-Tni3 is expressed under the control of the endogenous promoter, there is no observable defect in myofibril self-assembly. These transgenic strains will be useful for observing myofibril assembly in developing *C. elegans*, to determine how thin filaments self-organize into uniform arrays.

UP18 Gold nanoparticles decrease viability of *S. pneumoniae*

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Purpose: *Streptococcus pneumoniae* is the pathogenic bacterium that has been found to cause diseases including, ear and sinus infections, meningitis, and pneumonia. These infections are typically treated with antibiotics, but due to the highly adaptive nature of *S. pneumoniae* there is a need for new antimicrobials that can avoid resistance while effectively inhibiting the growth of the microbe. The purpose of this study was to investigate the efficacy of gold nanoparticles (AuNPs) as an inhibitor of *S. pneumoniae*.
Methods: Pneumococcal strains D39 and MTC553 (penicillin resistant) were grown in Todd Hewitt Media supplemented with 10% yeast extract. AuNPs (spherical, 10-20nm) were added at a final concentration of 1mg/ml. The samples were incubated at 37°C for 4 or 24 hours. Following incubation the number of live, non-adherent bacteria was measured using viable colony counts. A 0.1% crystal violet solution was used to stain the resulting biofilms and absorbance was read at OD600 nm.
Results: At the 4-hour timepoint the AuNP caused a statistically significant decrease in the viable non-adherent bacteria for D39 (P=0.0148). The data for MTC553 trended towards significance at the same point. Both strains had a statistically significant decrease in the viable non-adherent bacteria at the 24 hours (P<0.05).
Conclusion: The viability of *S. pnaumoniae* was decreased due to the AuNPs. Further studies are needed to investigate and quantitate the biofilm formation in detail.

UP19 3-D Articular Cartilage Scaffolds Based on PVA/PLA Composites

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Articular cartilage is a smooth, transparent tissue that covers the ends of bones in joints such as the knee and elbow. Defects and loss of cartilage can be caused by traumatic injuries and degenerative joint diseases. Unfortunately, because of its lack of vasculature, cartilage has a limited capacity to undergo self-repair. While some treatments are available to repair defective cartilage, they do not present a long term solution. Tissue engineering presents an approach to synthetically develop replacements for articular cartilage. The aim of this research is to fabricate 3-dimensional scaffolds that mimic the complex architecture and biochemical nature of articular cartilage. Methods: We have

fabricated scaffolds based on electro-spun nanofibers of poly lactic acid (PLA) infused with a hydrogel system based on polyvinyl alcohol (PVA) and sodium alginate (SA). A highly porous, nonwoven mat of PLA was prepared using electrospinning. A hydrogel coating was applied to the surface of the PLA mat using a syringe. The gel coating was then crosslinked by exposing it to a calcium chloride solution. The microstructure, chemical composition, mechanical properties and cell attachment and proliferation have been studied. Microscopy of the scaffolds reveals a two-phase, porous structure. A gradient in the structure and properties is also evident. The chemical composition of the gradient was characterized using infrared spectroscopy. The data confirms the presence of the PLA and the hydrogel system. The coated scaffold exhibited a modulus (i.e. stiffness) that was 20% higher than the uncoated scaffold. Therefore, the coating enhanced the mechanical properties of the electrospun scaffold.
We have fabricated a hybrid, three-dimensional scaffold that mimics the structure and of articular cartilage. The samples exhibit a phase system with interconnected porosity in each phase. The sample also exhibits a well-defined, diffuse interphase region. The impact of the interphase structure on the mechanical properties will be presented.

Graduate Student Poster Abstracts

Arranged alphabetically by presenting author's last name with names of presenting authors underlined

GP01 Impact of engineered nanoparticles on seedlings

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Purpose: As nanoparticles are widely becoming part of everyday consumer products, it is essential to assess their impact on the environment. Though organisms have evolved to cope with naturally occurring nanoparticles like nano-sized airborne particles from volcanic eruptions or components of aquatic natural organic matter, engineered nanoparticles can be a markedly different scenario. In particular, engineered nanoparticles are designed to have specific properties, distinct from the natural nanoparticles. Here, we study the effect of two model nanoparticles on the growth of embryonic roots.
Methods: In this study, iron oxide and hybrid Pt-attached iron oxide nanoparticles, home-made at UTC are used as models to assess the effect of engineered nanoparticles on different seedlings (*e.g.,* chickpea, green pea, green gram, red bean, and black bean). In specific, length of the embryonic roots are measured over a period of six days in the presence of different nanoparticle concentrations (*e.g.,* 5.54×10^{-3} and 27.7 mgL^{-1} Fe) and the control (de-ionized water). In addition, the seedlings are characterized via electron microscopy and Fourier transform infrared spectroscopy to investigate their morphology and chemical composition after interaction with the nanoparticles.
Results: It is found that iron oxide nanoparticles significantly enhanced the growth rate of embryonic roots at low concentrations (5.54×10^{-3} mgL^{-1} Fe).
Conclusion: The combined materials and statistical approach can be used as a facile method to assess both the risk and potential of these nanoparticles as agricultural fertilizers.

GP02 Evaluation of the Inhibition of *Escherichia coli* and *Staphylococcus epidermidis* by Silver-Polyvinyl Pyrrolidone (Ag-PVP), AgNP, and other Functionalized Silver Nanoparticles

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Purpose: Resistance to many commonly used antibiotics has prompted the need for alternative methods of treatment of several Gram-positive and Gram-negative bacteria. Silver nanoparticles have shown promising antibacterial activity but there remains a need to determine the best silver nanoparticle formulation to deliver this activity. The purpose of this study was to test Silver-Polyvinyl Pyrrolidone (Ag-PVP), AgNP, and polymer functionalized Ag nanoparticles for their efficacy against *Escherichia coli* and *Staphylococcus epidermidis*.
Methods: We examined the antimicrobial activity of Ag-PVP, AgNP, and polymer functionalized Ag nanoparticles against *Escherichia coli* and *Staphylococcus epidermidis* using the minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) and bacterial growth curve assays. The interaction of the nanoparticles with bacteria was observed by scanning electron microscopy. On a molecular level we utilized quantitative polymerase real-time chain

reaction (qrt-PCR) to determine the possible mechanism of action by testing a panel of genes essential for viability.
Results: We determined that the MIC for *Escherichia coli* and *Staphylococcus epidermidis* treated with AgPVP and the functionalized Ag nanoparticles ranged from 0.3125 µg/mL to 0.15625 µg/mL with an MBC of 0.6215 µg/mL. However, *S. epidermidis* treated with AgNP had a higher MIC ranging from 1.25 µg/mL and .6215 µg/mL, as well as a higher MBC > 1.25 µg/mL. The bacterial growth curve revealed that *E. coli* and *S.epidermidis* treated with AgPVP showed inhibition at a concentration of 0.15625 µg/mL and 0.3125 µg/mL at both 8 and 16 hour time points, respectively. Both bacteria treated with the functionalized nanoparticles provided stark differences in that E. coli had an inhibitory concentration of 0.15625 µg/mL at both 8 and 16 h time points. Higher inhibitory concentration of 0.6215 µg/mL at 16 and 24 h were required for *S. epidermidis*. SEM analysis showed that the nanoparticles damaged cell membranes and the molecular analysis showed a stress response to the nanoparticle treatment.
Conclusion: AgPVP, AgNP, and functionalized Ag nanoparrticles tested were able to inhibit both *S.epidermidis* and *E.coli*. The polymer coated nanoparticles were effective at lower concentrations.

GP03 Designing Three Dimensional Scaffolds for HEp-2 Cell Culture for *Salmonella Typhimurium* Infection

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Purpose: Salmonellosis, caused by *Salmonella* serovars, is a food borne illness caused by consumption of contaminated food and water. Of relevance, *Salmonella enterica* serovar Typhimurium is a Gram-negative, rod-shaped bacterium, infamously known for causing food poisoning which results in gastroenteritis in humans and other mammals. Worldwide, millions of cases occur every year and the death rate is steadily increasing. Therefore, potential therapeutic agents to control or effectively treat *Salmonella* infections are needed.
Method: In these efforts nanotechnology has emerged as an effective tool as metallic nanoparticles exhibit antibacterial potential. In particular, silver coated Carbon nanotubes (AgCNTs) are well known to have antibacterial activity against several Gram-positive and Gram-negative bacteria. In order to develop 3D models of infection, HEp-2 cells were grown on 3D printed, three dimensional Scaffolds designed at the University of Louisville. Three dimensional cell models were used vs traditional two dimensional cell models to allow in-vivo replication and similar environments. For this experiment, the Hep-2 cells (10^5 cells) were seeded on scaffolds and the cells were infected with the ratios of bacteria to cells as 1:1; 10:1; and 100:1 for 3 h. To determine the MOIs at 3h post infection, the cells were lysed using Triton-X100 and the appropriate dilutions were plated on Luria Burtani (LB) agar plates.
Results: Our results suggested that 1:1 ratio showed 3 log fold increase in terms of MOI compared to the other two ratios. Based on our experiments, our MOI for *Salmonella Typhimurium* is 1 bacteria to 1 cell to establish the infection.
Conclusion: We intend to use this MOI with the 3D cellular model of HEp-2 cells to further investigate the antibacterial effect of silver coated carbon nanotubes against the *Salmonella* Typhimurium infection. The long term goal of this project is to define the antibacterial mechanism of action for these nanoparticles using 3D cellular models.

GP04 Antimicrobial coatings: analyzing the effects of layer-by-layer (LbL) film growth on the loading and release of antibiotics

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Purpose: The transmission of infection is increased after natural disasters due to poor sanitation and other conditions. Exposure to poor sanitation can lead to the risk of individuals being infected by pathogenic agents. Our solution is to use antimicrobial smart coatings to prevent the

transmission of infection. The overall goal is to test and optimize layer-by-layer (LbL) films that will release antimicrobials over long periods of time.
Methods: The chitosan (CHI)/poly-L-glutamic acid (PGA) films were built by the TECAN Freedom EVO 100 deposition robot or manually. The reproducibility of the manually built films was analyzed by using the TECAN Infinite 1000 fluorescent spectrometer. The surface characterization and/or homogeneity of the films were analyzed by using the LSM700 ZEISS confocal microscope. Bocillin, a derivative of penicillin, was the antibiotic that was loaded in the films. The loading and release kinetics of bocillin were measure by using the TECAN Infinite 1000 fluorescent spectrometer.
Results: The TECAN Infinite 1000 fluorescent spectrometer showed the linear growth and slight exponential growth of the manually built films when different pH conditions of the polyelectrolytes were used. The thickness of the manually built films is 1.1 µm or greater. The LSM700 ZEISS confocal microscope showed the homogeneity of the films that were manually built at pH 5. The films that were built in a pH 3 environment were not homogenous. The thickness of the films built by the robot was not measured due to having only one layer of labeled CHI. No significant differences were found between the various loading and release conditions. An abundant amount of bocillin was released within the first hour under various pH conditions.
Conclusion: The CHI/PGA films were built and characterized, the thickness of the films was measured and different conditions of the loading and release of bocillin FL were analyzed. There were no significant changes detected when the various release pH conditions were used for the release of bocillin FL. More conditions of the polyelectrolytes should be tested to determine the conditions that are needed for exponential growth. Future work includes testing different antimicrobial LbL systems (linear versus exponential).

GP05 Magnetic nanoparticle mediated cell death using pulsed electromagnetic waves

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Purpose: Magnetic nanoparticles are multifunctional and can be used for various applications such as hyperthermia, MRI, and drug delivery. In this study, we have developed dipeptide-coated magnetic nanoparticles (MNPs) for enhanced cellular uptake *in vitro* and *in vivo* in lung tumor cells and to test the suitability of the MNPs in inducing localized hyperthermia using a pulsed electromagnetic field (PEMF), which may potentially shrink or destroy tumors.
Methods: The MNPs were synthesized by co-precipitation method and characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS) and vibration sample magnetometer (VSM). The effect of the MNPs on cellular viability was assessed *in vitro* in normal lung cells, HLF-1 and lung cancer cells, A549 and NCI-H460. The MNPs were further tested for heating effects in a pulsed electromagnetic field and its effect on cellular morphology, viability and mRNA levels of heat shock and apoptotic markers was assessed in lung cancer cells.
Results: We determined the shape, size and magnetic properties of the MNPs. The MNPs were found to be non-cytotoxic to normal lung fibroblasts and lung cancer cells. The MNPs showed enhanced cellular uptake in lung cancer cells demonstrated by prussian blue staining and ICP-OES. *In vivo* biodistribution study in nude mice lung tumour xenograft confirmed the presence of the MNPs in the tumors. Viability assays and qPCR studies further revealed that the magnetic nanoparticles mediate cell death in the presence of a pulsed electromagnetic field.
Conclusion: The MNPs in the presence of PEMF induce cell death in lung cancer cells. Thus, the use of targeted-magnetic nanoparticles in an electromagnetic field offers a scope to develop site-specific delivery systems to the site of cancer and overcome the obstacles of toxicity and drug resistance at an affordable cost and provide a disease-free state and better quality of life for the patients. Targeting of magnetic iron oxide nanoparticles in the presence of a pulsed electromagnetic field could offer a promising strategy for the treatment of lung cancers.

GP06 The effect of alcohol on the biogenesis and composition of microglia-derived exosomes

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Purpose: Exosomes are small extracellular vesicles that are formed during the maturation of endosomes. The biogenesis of exosome changes based on external factors, such as alcohol exposure. As a small molecule, alcohol can easily cross membrane barriers and reach different parts of the body very quickly. Alcohol interacts with brain receptors, interfering with communication between nerve cells, and suppressing the excitatory nerve pathway. The purpose of this project was to investigate the effects of alcohol exposure on the biogenesis and composition of exosomes derived from microglia brain cells, BV2.
Methods: The BV2 cell lines was cultured in exosome-free medium and was either not treated (control) or treated with 50mM or 100mM of alcohol for 24, 48, or 72 hours. The cell morphology was examined through light microscopy. The groups of exosomes were isolated using a series of high-speed ultracentrifugation and quantitated using the Lowry dilution method. NanoSight analysis was used to identify and quantify the size of exosomes. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to separate the exosomal proteins based on size and Coomassie blue staining was performed to identify the presence of exosomal proteins each treatment group. Enzyme-linked immunosorbent assay was performed on the exosomes to confirm the presence of exosomal proteins on BV2 cells.
Results: Cell viability decreased after alcohol administration. Cells showed apoptotic behavior after 48-hour and 72-hour treatments after dosing with both concentrations of alcohol. Cells appeared to be shrunken compared to the control untreated cells. The average of three experiments demonstrated through fold change that alcohol treatment at 48 hours at the high dose caused a significant increase of tetraspanin CD63 expression in exosomes. In addition, alcohol treatment after 48 hours caused significant increases of heat shock protein 90 beta compared to the control. At 72 hour treatment with alcohol, we observed a significant increase of cytoskeletal protein actin expression in exosomes when compared to control.
Conclusions: Our studies revealed that exosome biogenesis and composition was affected by alcohol treatment. However, more experiments will be conducted to gain additional knowledge on the impact of alcohol on exosome biogenesis in brain resident cells.

GP07 Excitation scanning hyperspectral imaging as a means of colon cancer detection

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Purpose: Colorectal cancer diagnosis is difficult and time consuming. Tumor detection is often dependent on the skill of the endoscope operator. Additionally, detected polyps must be removed and sent to a pathologist to confirm or refute the pathologic state of the polyp. Recently developed excitation scanning hyperspectral imaging allows detection of spectral shifts associated with disease states which could be used to differentiate healthy and diseased tissue without the need for resection and pathology.
Methods: Diseased human colon tissue and small segments of surrounding healthy tissue were removed from patients by the USAMC surgery department. Samples were classified by the USAMC pathology department and sent to us for spectral imaging. Tissues samples for spectral scanning were sectioned into 2 cm cubes. Spectra of samples were obtained using a custom inverted fluorescence microscope with a Xe arc lamp light source and thin film tunable filter array for excitation wavelength selection. Scans utilized excitation wavelengths from 360 nm to 550 nm in 5 nm increments. The resultant images were compiled into a hyperspectral image cube, which was then analyzed with ENVI and custom Matlab scripts, including linear spectral unmixing and both supervised and unsupervised classification methods.
Results: Initial results show that spectra obtained from healthy sections of colon shared a unique spectral signature across regions of interests, fields

of view, and patient samples. However, all spectra obtained from cancerous regions of colon yielded spectral signatures different in one of several ways from the spectral signature of healthy colon samples.
Conclusion: The results suggest that the spectral signature of the colon doesn't depend on the relative positions of camera and tissue, as changing region of interest, field of view, or even patient samples did not significantly alter the spectral signature. Furthermore, the results suggest that a cancerous area of colon tissue is readily detectable by a shift in the spectral signature of the colon. These results could be used to differentiate healthy and cancerous regions of colon tissue with a cursory scan; a drastic improvement from the multi-step process of operator dependent endoscopy, polyp resection, and pathologist assessment.

GP08 Encapsulation of Interleukin-10 within Poly (lactic acid)-b-Poly (ethylene glycol) Nanoparticles for Therapeutic Control of Inflammation Produced by *Chlamydia* Infections

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Purpose: Inflammation which is induced by the presence of cytokines and chemokines is an integral part of *Chlamydia trachomatis* (CT) infections. Nevertheless, it can be regulated using therapeutics, including anti-inflammatory molecules. We reported that the anti-inflammatory cytokine, IL-10 can effectively regulate CT inflammation by inhibiting its associated-induced inflammatory mediators in mouse J774 macrophages. A major problem with IL-10 is its short biological half-life thus requiring frequent applications at high dosages for biomedical applications. Here, our goal is to encapsulate IL-10 within the biodegradable polymer, PLA-PEG (Poly (lactic acid)-b-Poly (ethylene glycol) nanoparticles in an attempt to prolong its half-life for therapeutic purposes.
Methods: IL-10 was encapsulated in PLA-PEG by the double emulsion method, followed by physiochemical characterizations and functional studies. The anti-inflammatory effect of encapsulated IL-10 was then tested using various concentrations (1-1000 ng/mL) over a 24-72 hour time-point in mouse J774 macrophages exposed to the recombinant major outer membrane protein of CT. We then used specific ELISAs (Enzyme-linked immunosorbent assays) to measure the production of the pro-inflammatory cytokines IL-6 and IL-12 and qRT-PCR to quantify the mRNA gene transcripts of SOCS 1 and SOCS 3.
Results: Data from Ultra Violet (UV) visible and Fourier Transform-Infrared Spectroscopy (FT-IR) revealed the successful encapsulation of IL-10 within PLA-PEG. Encapsulated IL-10 had an average size of ~ 100 to 200 nm, with an encapsulation efficiency > 90 %. Temperature stability of encapsulated IL-10 was up to 89°C as shown by differential scanning calorimetry analysis. Cytokine specific ELISAs showed that encapsulated IL-10 reduced the levels of both IL-6 and IL-12 in macrophages in a time- and concentration-dependent fashion, correlating with its stability and slow release capacity. By Taqman qRT-PCR, we further demonstrated that encapsulated IL-10 induced higher levels of SOCS3 as compared to SOCS1, independent of the encapsulated IL-10 concentration, suggesting their probable role in the encapsulated IL-10 molecular mechanism of inhibition.
Conclusion: Our data shows successful encapsulation of IL-10 and that PLA PEG can prolong the half-life of IL-10. More importantly, encapsulated IL-10 is functional by down-regulating cytokines and inducing SOCS1 and 3 in macrophages exposed to CT at relatively low dosages. Thus, IL-10 may provide therapeutic control of Chlamydia produced inflammation especially in mucosal tissues. *This work was supported by funding from NSF-CREST (HRD-1241701).*

GP09 Construction of Solid State Nanoreactor for the Synthesis and Characterization of Large Scale Metallic Nanoparticles

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Purpose: The current state of nanoparticles fabrication via polymer or solid state means is limited by the instability of the formed nanoparticles against self-aggregation and the ability to produce monodispersed nanoparticles on the macro-scale.
Methods: Hence, motivation for this project is to construct a solid state nanoreactor that uses multifunctional reduction methods (chemical reduction and photoreduction) to produce

technologically relevant monometallic and multimetallic nanoparticles with controllable shapes and sizes and with specific designed cores and shell compositions at the macroscale “gram scale”. In particular, this design will take advantage of polymeric means (Dendrimers and high temperature polymers such as PEI) to control the growth modes of CuNi, CoPt, and CuNiCo particles during formation.
Results: The resulting morphologies and structures of the formed nanoparticles were characterized by UV-vis, TEM, and XRD. Tests were conducted to establish the influence of its effect on biological systems.
Conclusion: Furthermore, TEM characterization of the formed nanoparticles were an average of 5 nanometers. The nanoparticles were proven to be non-toxic based on the results of the biological studies.

GP10 Peptide-Mediated Inhibition of Gram-positive bacteria

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Purpose: Improper use of traditional antibiotics has led to the development and spread of antibiotic resistant bacteria, resulting in difficult to treat infections with increased mortality. The search for alternative novel antimicrobial peptides, to which bacteria have yet to develop resistance, is ongoing. Antimicrobial peptides are naturally occurring oligopeptides of varying length that have antimicrobial activity against a range of microorganisms. In bacterial species, antimicrobial peptides are generally cationic, a-helical, and mainly target gram-positive bacteria. *S. aureus* is a gram-positive bacterial species that is implicated in instances of wound and medical device infections. Antimicrobial peptides are ideal alternatives to antibiotics to slow the development of antimicrobial resistant bacteria and to avoid increase in mortality.
Methods: In this study, six novel antimicrobial peptides, SSU2, SSU3, SSU4, SSU6, SSU7, and GM-1, were screened with minimum inhibitory concentration (MIC) assays to determine if any demonstrated antimicrobial effects against *S. aureus*. SSU (2, 3, 4, 6, and 7) were tested at concentrations of 1 mM, 0.5 mM, and 0.25 mM. GM-1 was tested at concentrations of 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL. Since all peptides were dissolved in DMSO, a control with DMSO was included in the experiment to determine the effect of DMSO on bacterial cell viability. Optical density was measured at 0 and 24 hours to determine the inhibitory effect of the peptides.
Results: SSU7 demonstrated the greatest inhibitory potential against *S. aureus*. DMSO did decrease S. aureus viability in the absence of the antimicrobial peptides.
Conclusion: The structural, inhibitory and/or bactericidal properties of SSU7 should be investigated further since it demonstrated the greatest inhibitory potential against *S. aureus*. While DMSO appeared to decrease the viability of S. aureus, the effect was small, and using less DMSO should reduce the effect on viability. The results of this study will allow us to identify a novel antimicrobial peptide that can be used to treat infections caused by gram-positive bacteria.

GP11 Silver CNTs to prevent *Staphylococcus aureus* adhesion and infection in a 3-D Keratinocytes Model

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Purpose: Although conventional cell culture is useful to track bacterial infections, 3D culture may represent biological infections more accurately. In this study, silver CNTs and peptides will be used to prevent adhesion and infection of keratinocytes with Staphylococcus aureus using 3D culture on scaffolds. This, however, cannot be done without first finding the proper multiplicity of infection or MOI. MOI is important when it comes to finding out which ratio would give enough colony forming units (CFUs) without killing the cells or producing a lawn. The goal of this study was to determine the MOI for use in 3D culture and infection of keratinocytes
Methods: The first step to conduct infection experiments is MOI which stands for Multiplicity of Infection. MOI is defined as the ratio of infectious agents to target cells (bacteria to cells). In this experiment the ratio would be *S. aureus* to Keratinocytes. After growing the Keratinocytes for a week, $2x10^5$ cells were plated in two 12-well plates. While keratinocytes were incubated overnight, *Staphylococcus aureus* was also grown overnight, and cells were infected with $1x10^9$ colony forming units (cfu)/ml. After infection, the plates were incubated for various amounts of time. After the incubation period, 10^3, 10^4, and 10^5cfu/ml were

plated in petri dishes. These plates were incubated overnight and the CFUs were counted the next day to see which ratio gives enough CFUs without completely destroying the cells and creating a lawn.
Results: Incubation for 24 hours resulted in overwhelming bacterial growth with lawns for all dilutions. The three hour incubation produced the optimum amount of CFUs at the 10^3, 10^4 cfu/ml infection rates
Conclusion: After counting all the CFUs from the different ratios and designated time, it was discovered that the optimum MOI ratio was 10:1 (bacteria: cell) for infecting keratinocytes in 3 D culture.
Future studies: These will include SEM and toxicity assays. Eventually silver carbon nanotubes will be added to the keratinocytes to determine their ability to inhibit *S. aureus* from adhering to and infecting the keratinocytes.

GP12 Conversion of Egg and Seashell Waste into a Printable Biomaterial for Dental Tissue Engineering Scaffolds

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Purpose: Hydroxyapatite (HA) based biomaterials have been studied as a promising bioactive material for potential tissue engineering applications due to their favorable biological properties such as bioactivity, biocompatibility, and biodegradability. Every year, about 6 to 8 million tons of sea-shell waste is produced globally along with ~76 billion eggs consumed each year in the United States alone resulting in 1.2 million Kg of eggshell waste per year. In this study, we investigate the conversion of naturally occurring waste material, such as eggshells and seashells, into a valuable nanomaterial that can serve as a bioactive scaffold for bone and tooth regeneration.
Methods: HA was synthesized using an energy efficient microwave-assisted wet chemical precipitation method by utilizing $CaCO_3$ from egg and seashell as a precursor and H_3PO_4 as a source of phosphate ions. The HA was synthesized as follows: In the first step the $CaCO_3$ powder was reacted with a HNO_3 solution to produce a yellowish $Ca(NO_3)_2$ slurry. In the second step, diammonium hydrogen phosphate (DAHP) was synthesized by reacting NH_4OH and H_3PO_4 in stoichiometric ratios. In the third step $Ca(NO_3)_2$ and DAHP were both dissolved separately in an aqueous solution and DAHP solution was added drop-by-drop into the $Ca(NO_3)_2$ solution. Finally the NH_4OH was added to adjust the PH to 11. The reaction mixture was then exposed to microwave irradiation in an isothermal condition of 80^o C for 15 min. The as prepared white precipitate was washed, several times with distilled water followed by ethanol. The final product was dried in a vacuum oven overnight and used for further analysis.
Results: Transmission electron microscopy, Scanning Electron Microscopy, X-ray Diffraction, and energy-dispersive X-ray spectroscopy studies confirmed crystalline HA particles in irregular shapes. The particle sizes measured are within the nanometer range, and displayed a chemical composition that matches very well with that of naturally occurring HA.
Conclusion: Nanoscale hydroxyapatite particles were prepared from natural resources using an energy-efficient microwave-assisted wet chemical precipitation method. This serves as not only an inexpensive but sustainable, and environmentally friendly route to synthesize a valuable nanobiomaterial that can potentially be used in the fabrication of 3D printed dental tissue engineering scaffolds.

GP13 Neuroligin 4X Overexpression in Human Breast Cancer is Associated with Poor Relapse-Free Survival

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Purpose: Neuroligins are neural cell adhesion molecules that have been implicated in heterotopic cell adhesion. Until, recently neuroligins have been shown to be abundantly expressed in blood vessels and also play a role implicated in the growth of glioma cells. Our previous studies using a selection from a combinatorial random peptide library against breast and pancreatic cancer cell lines identified several peptides mimicking neuroligin (NLGN-1, 3 and NLGN4X). In this study, we sought to determine the expression, relevance and functional significance of Neuroligin 4X in human breast cancer.
Methods: NLGN4x expression data for all breast cancer cell lines in the Cancer Cell Line

Encyclopedia were analyzed. Correlation between NLGN4X levels and clinic pathologic parameters were analyzed within Oncomine datasets. Kaplan–Meier curves were generated using a database of public microarray datasets. Immunohistochemistry of breast cancer tissue arrays was performed to analyze expression of NLGN4X. Flow cytometry and immunofluorescence staining were also conducted investigate NLGN4x expression in MDAMB231 and MCF7 cells. To study NLGN4X gene knockdown, MDAMB231 cells were transfected with NLGN4X-specific siRNA. Analysis of NLGN4X Gene Expression was analyzed by RT-PCR and flow cytometry. Post transfection, wound healing and cell viability assays were performed to note the effects of NLGN4X knockdown on migration and proliferation. Caspase detection and Annexin V-FITC apoptosis detection to determine apoptotic activity were analyzed by flow cytometry.
Results: NLGN4X was abundantly expressed in breast cancer tissues. Interestingly, high NLGN4X expression highly correlated with decrease in relapse free-survival in TNBC. Evaluation of bioinfomatic datasets results revealed that NLGN4X expression was higher in triple negative breast cancer cells, particularly the basal subtype. Its level was also observed to be higher in metastatic tissues. RT-PCR, flow cytometry and immunofluorescence study of MDAMB231 and MCF-7 breast cancer cells validated that NLGN4X was increased in MDAMB231. Knockdown of NLGN4X expression by siRNA decreased cell proliferation and migration significantly in MDAM231 breast cancer cells. NLGN4X knockdown in MDAMB231 cells resulted in induction of apoptosis as determined by annexin staining, elevated caspase 3/7 and cleaved PARP by flow cytometry.
Conclusion: NLGN4X might represent novel biomarkers and therapeutic targets for breast cancer. Inhibition of NLGN4X may be a new target for the prevention and treatment of breast cancer.

GP14 Fabrication of scaffolds for tissue engineering using wet-laid technique

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Purpose: Biomaterials that serve as scaffolds for cell proliferation and differentiation are progressively being used in wound repair and regeneration. It is known that some porous structures promote faster healing, so we hypothesized that both the chemical composition and the architecture of the 3-D scaffolds could affect the proliferation and differentiation of new tissue. In this project, the potential regenerative properties of 3-D scaffolds made of biodegradable and biocompatible synthetic polymers Poly-L-lactic acid (PLLA), Polydioxanone (PDO) and Polycaprolactone (PCL) were evaluated.
Methods: The nanofiber scaffolds were generated using wet-laid and electrospinning technique containing high molecular weight chitosan which is ideal for the promotion of wound healing since it possesses multifaceted biological properties. Additionally, purified type I Collagen was used to give additional strength and elasticity to scaffolds and Genipin; a natural biocompatible water soluble cross-linker, to allow the formation of stronger bonds between the scaffolds. Properties of the scaffolds were examined using SEM to view the morphology of the scaffold at the microscopic level, Infrared Spectroscopy was used to determine the functional groups present within the scaffold, Differential Scanning Calorimetry was used to determine the thermal stability and a tensile strength test was carried out using the RSA-G2 solids analyzer to determine the strength of our scaffold.
Results: Scanning Electron Microscopy (SEM) showed the topography and integrity of our nanofiber scaffolds as well as collagen fixation between the fibers. Infrared Spectroscopy showed a significant frequency of collagen accumulation present in our scaffolds. Differential scanning calorimetry analysis showed that for all of our scaffolds they were able to withstand high temperature rates with very high melting points corresponding to each individual polymer, which showed that our scaffolds were very thermally stable. Furthermore, after conducting a tensile strength test for each scaffold, results showed two of our scaffolds had a considerable high stress rate and low strain percentage while others had a low stress rate with a high strain rate.

Conclusion: The initial aim of this work was to describe the fabrication and evaluate the various properties of scaffolds for their ability to influence the proliferation and differentiation of fibroblast and keratinocytes for wound healing. The designing and use of 3-D scaffolds for tissue engineering is

essential and will assist in obtaining further knowledge of 3-D wound healing systems.
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GP15 Porous carbon from Packaging waste to 3D printed energy devices

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Purpose: The growth of portable consumer electronics has led to a tremendous demand for high-performance energy storage devices. Supercapacitors have gained attention for its fast charging time, long cycle-lives, wide range operating temperature, and meeting environmental standards. The objective of this study is to synthesize activated porous carbon from packaging waste material and develop a simple supercapacitor device using 3D printing technique.
Methods: Packaging waste was carbonized at 500°C at autogenic pressure. This carbon was further reacted with Na(OH) at ratios of 2 and 4 and at 700°C to produce activated carbon. The resulting carbon was characterized using BET, Raman, XRD, and SEM. The printable carbon paste/ink was prepared by mixing the activated carbon with PVA/ H_3PO_4 electrolyte at aratio of 1:2. Using predesigned CAD model, the electrodes were printed with VOL-25 printer head commonly used for printing of pastes. After the first electrode is printed, thin electrolyte film was placed on its top and then thesecond electrode is printed. Printed devices were allowed to dry before their removal from the substrate. 3D printed devices were characterized for their charge-discharge performance.
Results: Activated carbon has shown BET surface area of 903 m^2/g and pore volume of 0.5195 cc/g, which is close to commercial activated carbon. XRD and Raman have shown increased graphitization. Printed devices have shown specific capacitance of29 F/g and power density of 750 W/Kg at a current density of 2.5 mA/ cm^2.Two devices connected in series were successfully able to light up an LED for 25 Seconds.
Conclusion: Packaging waste is a promising source of high quality activated carbon.3D printed devices have shown excellent performance, suggesting additive manufacturing is a promising technique for fabrication of versatile energy devices for portable consumer electronics.

GP16 Evaluation of Enzyme-Responsive Liposomes for the Treatment of Triple-Negative Breast Cancer

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Purpose: Secretory phospholipase A2 (sPLA2) cleave phospholipids at sn-2 ester bonds, releasing lysophospholipids and fatty acids, and are over expressed in several pathologies including breast cancer. Herein, we evaluated the therapeutic activity of enzyme responsive liposomes compared to the clinically used, sterically stabilized liposomes (SSL) for *in vitro* response in a triple-negative breast cancer (TNBC) model.
Methods: In these studies, SSL and SPRL formulations were made according to previous studies and resulting in three formulations SSL, SPRL- E and SPRL-G. SPRL were made by the addition of either DSPE (SPRL-E) or DSPG (SPRL-G). Doxorubicin was used as the drug of choice and Dox-loaded liposomes were prepared by remote-loading using an ammonium sulfate gradient. Toxicity studies were performed by the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and uptake studies were performed by fluorescence microscopy and flow cytometry.
Results: Treatment of breast cancer cells with doxorubicin encapsulated in SSL and SPRL resulted in cytotoxicity in the MDA-MB-231 cells line comparable to free drug. Tracking of drug and liposome delivery using fluorescence microscopy and flow cytometry, we demonstrated that drug uptake was liposome-dependent, as encapsulation of doxorubicin in SPRL resulted in greater intracellular drug levels compared to SSL.
Conclusions: These preliminary data show the therapeutic activity of SPRL compared to SSL, and suggest that SPRL may be useful for the treatment of TNBC. Since 2-dimensional models do not fully recapitulate the complexity of barriers to drug delivery, future directions will endeavor to explore the utility of these liposomes in 3-D and microfluidic models.

GP17 A comparison of the visibility and signal migration of non-conjugated quantum dots across two administration routes in an amphibian model

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Purpose: Amphibians are a common model species for a range of research applications, including investigations of physiological pathways following administrations of hormones or pharmaceuticals. The majority of research techniques are invasive and require the sacrifice of the animal model. Quantum dot nanoparticles (QD) offer a unique, non-lethal method to image living systems. While QD visualization has been done in mammals, amphibian models have not been explored. Amphibian skin has a thick dermal layer which contains mucus glands, presenting a unique challenge to in-vivo imaging. The purpose of this study was to determine the visual capabilities and in-vivo fluorescence signal emission of QDs in an amphibian model over time.

Methods: *Anaxyrus fowleri* were administered high and low QD concentrations (5nM and 200nM; Qtracker 655, ThermoFisher Scientific) as intranasal administrations (IN) or intraperitoneal injections (IP). Subjects were imaged using the In-Vivo Imaging System Lumina (IVIS; Perkin-Elmer) to determine visualization, fluorescence emission, and migration of signal at various time intervals (0, 15, 30 and 60 minutes). Subtraction of auto-fluorescence, quantification, and migration of fluorescence emission was performed using the spectral unmixing function and ROI measurements in Living Image 4.5.2 software (Caliper LifeSciences). Excitation and emission filters ranged from 520-620nm/620-670nm, covering the full spectral range of the QDs.

Results: IVIS imaging showed clear fluorescent signal localization at the points of QD administration at both high and low concentrations. The average fluorescence emissions of 5nM and 200nM QDs administered nasally were $1.90x10^9$ and $1.04x10^9$, respectively, while average fluorescence emission was $1.82x10^9$ and $3.07x10^{10}$ following IP. Visible signal migrated an average distance of 1.34cm following IN and 0.96cm following IP over one hour.

Conclusion: At both concentrations and administration routes, QD signal progression can be visualized and quantified in living amphibian systems, although no dose-dependence was observed. The signal progression of non-conjugated QDs provides critical insight into the natural distribution of QDs following two separate administration methods. This is an important step in the use of non-fatal visualization methods of pathways for subsequent research and clinical applications. Work supported by USDA-ARS Biophotonics Initiative #58-6402-3-018.

GP18 Novel anti-microbial nanobiomaterials against *E.coli* infection

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Purpose: *E.coli* can infect many parts of the human body such as intestinal tract, urinary tract and skin (Hilbert 2013). There are a number of treatments that are available for the internal infections that mostly fall in the antibiotic class of drugs; however nanoparticles such as gold and silver are also being studied to determine their affects against *E.coli* (Franková, Pivodová et al. 2016; Balasubramanyam, Altaf et al. 2004). In this study gold nanoparticles (GNPs) and peptide garcinole (GC) are being tested for their efficacy to stop the *E.coli* spread when in contact with human keratinocytes.

Method: Confluent human keratinocytes of when exposed to GNPs and GC to evaluate their toxicity. The MTT assay with varying concentrations of GNPs and GC. Also, the minimum inhibitory concentration (MIC) assay was also performed to determine the optimum concentration for GC.

Results: The MIC assay preformed with GC shows prominent antimicrobial effects against *E.coli* where GC concentration of 0.1 mM shown 40 % bacterial inhibition. Whereas, the MTT assay shows that 0.08 mM GC and 1 nM GNPs are better with over 60 % cell viability.

Conclusion: The initial study was to observe the antimicrobial effects of GC against *E.coli* and it was observed that GC can inhibit *E-coli.* GC found slightly toxic but the cell viability for concentrations below 0.08 mM is over 60 %. GNPs on the other hand found reliable with over 90 % cell viability. Further in this research conjugation of GC and GNPs will be done. It is considered that the conjugation will reduce the toxicity of GC and the conjugation product could show antimicrobial effect.

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GP19 Phantom sensing with infrared sensors to measure temperature rise

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Purpose: Infrared sensors will play a vital role in developing a compact, portable and reliable system, if chosen as the sensors. It has small detection range which is essential for a compact system. It also has small dimensions in the order of millimeters, a wide operational bandwidth, and present no chance of crosstalk when placed at near field distances of each other. Infrared sensor is used to detect the temperature rise in normal and malignant tissues due to the application of microwave signal. The increase in temperature in normal and malignant tissues will be different due to the difference in permittivity and conductivity in these tissues. The knowledge of this difference can be utilized to determine the location and depth of malignant tumors in the breast.
Methods: The ultra-wideband planar ring/slot monopole antenna was designed which is used in the measurement to illuminate the normal and malignant tissues. Basically, the antenna receives 1 Watt of power from the Vector Network Analyzer. The antenna radiates signal to the tissue which generates heat in the normal and malignant tissues. First, the temperature of the normal and malignant tissues was measured in room temperature. Then the tissue was exposed to the antenna for 5 minutes. The antenna signal heat the tissue gently and then removed, the infrared sensor was used to detect the temperature rise. The sensor used is from Microelectronic Integrated Systems (Melexis). Infrared sensors are small in size, low in cost and easy to integrate. It has a wide temperature range from -40^{o} to 380^{o} C. Next, the antenna signal was applied for 10 minutes and the temperature was recorded again. This measurement was verified with the infrared-based FLIR camera (T420) as well which were in good agreement with the infrared sensors.
Results: First, the temperature of the normal tissue was measured in room temperature without applying antenna signal, which was approximately 22.1 Celsius. The temperatures obtained after applying antenna signal for 5 and 10 minutes were 22.4 and 22.5 Celsius respectively. Later, malignant tissue was measured in room temperature without applying the antenna signal, which was approximately 23.6 Celsius. The temperatures obtained after applying antenna signal for 5 and 10 minutes were 24.0 and 24.2 Celsius, respectively. The recorded temperatures using the Flir camera after 5 and 10 minutes were 24.2 and 24.3 Celsius, respectively.
Conclusion: Thermal measurement of normal and malignant tissues were conducted. The temperature rise occurs as the target is exposed to ultra-wideband signals for a short period of time. Depending on the sensitivity of the sensor, the variation of the temperature change in normal and malignant tissues can be captured by infrared sensors. This increase is due to different permittivity and conductivity of tissues. This information can be used to effectively reconstruct the image of the breast to detect early stage breast cancer.

GP20 Synthesis and Cure Kinetics of Epoxidized Soybean Oil

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Purpose: There is an urgent need to replace petroleum based polymers by way of bio-based polymers. Bio-based polymers help in the reduction of fossil fuels usage, and improve economic and environmental concerns. The major sources for bio-based polymers are vegetable oils, which offer advantages such as being renewable, biodegradable, and abundant.
Methods: The current study focuses on synthesizing a high bio content resin via an epoxidation process that uses soybean oil, hydrogen peroxide as an oxygen donor to form the epoxide groups and formic acid as the oxygen carrier. Studies were performed to determine an optimized system by analyzing the effect of reaction time (at 2, 4, and 6 hours), and variation of hydrogen peroxide content (at 1, 1.5, and 2 moles) at 50 ºC. Initial characterization uses ASTM D1652-11 and Fourier transform infrared (FTIR) spectroscopy to determine and analyze the change in functional groups and the formation of epoxy

groups. Cure behavior of the optimum epoxidized soybean oil (ESO) system cured with an anhydride curing agent was investigated using differential scanning calorimetry (DSC) under dynamic and isothermal modes of scans. Results of the scans determined kinetic parameters such as activation energy and reaction rate constants and orders analyzed using Friedman, Kissinger-Akahira-Sunose (KAS), and Kamal models of kinetic analysis.
Results: Activation energy determined from Friedman and KAS were 89 and 107 kJ/mol. Kamal model revealed a diffusion model due to complex reactions in the ESO/anhydride system. An increase in the degree of cure revealed a high rate of conversion at low degree of cures. This phenomenon suggested that the cure temperature be lowered to ensure a controlled curing. However, an increase in the isothermal temperature revealed a glass transition temperature up to 41 °C.
Conclusion: The results obtained from KAS model yielded the best agreement with the experimental data. Comparison of the activation energy from Friedman and KAS models showed a slight decrease at initial conversions followed by an increase as the cross-linking proceeded. This decrease is explained by a diffusion controlled reaction kinetics which is caused by two phenomena, gelation and vitrification, also revealed by Kamal model.

GP21 Functional role of MYB in Ovarian Cancer Pathogenesis

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Objectives: MYB/c-MYB, a cellular progenitor of v-MYB oncogenes, encodes for a transcription factor protein and confers its oncogenic activity through regulation of gene expression. The purpose of our research was to investigate the pathobiological significance of MYB overexpression in ovarian cancer (OC).
Methods: Immunohistochemical and immunoblot analyses were performed to examine the expression of MYB in clinical specimens of OC and established cell lines. MYB levels were altered (stable overexpression or knockdown) in OC cells by genetic engineering to assess its pathological functions in growth, survival and malignant behavior of OC cells.
Results: An intense staining of MYB was reported in all histologic subtypes of OC, while it was detected in normal ovarian tissues. MYB was also expressed at varying levels in all the OC cell lines examined by us. Stable silencing and forced overexpression of MYB was achieved in two high (SKOV3-ip and A2780-cip) and low (SKOV3 and A2780) MYB-expressing OC cell lines, respectively. Stable silencing of MYB led to decrease in growth (37% and 41%) and clonogenic ability (~3.2 and 2.8 folds) in SKOV3-ip and A2780-cip cells, respectively, as compared to their scrambled sequence-transfected (Scr) control cells. MYB-silenced SKOV3-ip and A2780-cip cells also exhibited reduced motility (~4.1 and 3.7 folds, respectively) and invasion (~7 and 6.2, respectively). Accordingly, forced overexpression of MYB in SKOV3 and A2780 cells promoted their growth (44% and 29%), clonogenicity (~2.6 and 3 folds), motility (~3.5 and 3.9 folds) and invasion (~5.1 and 4.3 folds) as compared to vector only-transfected cells. MYB expression in OC cells also correlated with mesenchymal features and reduced sensitivity to cisplatin cytotoxicity.
Conclusions: Our findings establish a direct association of MYB with oncogenic and chemoresistance potential of OC cells, and suggest that it could serve as a novel target for diagnosis, prognosis and therapy.

GP22 Membrane nanoparticles from Hypoxic Pancreatic Cancer Cells enhance survival in Hypoxia

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Pancreatic cancer remains one of the leading causes of cancer-associated deaths in the United States, often diagnosed after metastasis to distant sites or at an advanced late stage. Fibrous stromal tissue encapsulates pancreatic tumors, resulting in insufficient vasculature and reduced oxygen levels within the tumor microenvironment. The pancreatic

tumor is one of the most hypoxic tumors found in humans. The interaction of pancreatic tumor cells with the intratumoral hypoxic microenvironment is a major cause of persistent survival of tumor cells, increased aggressiveness, and therapeutic-resistance. The urgent need to identify underlying molecular mechanisms responsible for adaptation in pancreatic cancer to hypoxia is the focus of our study. In this study, we examined the role of biological nanoparticles, which are vesicles with a lipid bilayer membrane, in imparting survival benefit to pancreatic cancer cells. For this, we first examined the effect of conditioned media from pancreatic cancer cells cultured under hypoxic (0.1% O_2 and 1% O_2) or normoxic (21% O_2) conditions on the growth of fresh pancreatic cancer cells cultured under severe hypoxic (0.1% O_2 and 1% O_2) conditions. We found that hypoxia-conditioned media conferred significant survival benefit to cancer cells under hypoxia as compared to normoxia-conditioned media. Thereafter, we isolated biological nanoparticles from normoxia- or hypoxia-conditioned media and used them to treat pancreatic cancer cells under hypoxic culture conditions. We observed that hypoxia nanoparticles imparted a significantly higher survival advantage to cancer cells under hypoxia as compared to normoxia nanoparticles. Additionally, we characterized differences in nanoparticles released in normoxic (21% O_2) or hypoxic (0.1% O_2 and 1% O_2) conditions. Further studies are underway to identify the molecular effectors in nanoparticles responsible for inducing growth-promoting effects under hypoxia.

GP23 Examining the Viability of Penicillin-Resistant Isolates of *Streptococcus pneumoniae* to Functionalized Nanoparticles

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Streptococcus pneumoniae is a respiratory pathogen which remains a major cause of morbidity and mortality worldwide in spite of available vaccines and antibiotics.The development of a novel antimicrobial drug is needed in order to conquer existing mutating *S.pneumoniae* isolates. Our goal was to investigate the usefulness of penicillin conjugated gold nanoparticles (AuNP-Pen) and encapsulated poly lactic-co-glycolic (PLGA-Pen) nanoparticles as potential multivalent antimicrobials inhibiting the growth of clinically relevant planktonic *S. pneumoniae*.

Methods: Gold nanoparticles (AuNP) were synthesized through the reduction of gold (III) chloride trihydrate ($HAuCl_4$) with sodium borohydride ($NaBH_4$) using the citrate reduction method.The final nanoparticle suspension yields a concentration of 2.5 x 10^{-4}M. Penicillin was conjugated to the surface of the particles via formation of a thioether bond. PLGA-Pen nanoparticles were prepared by emulsification-diffusion method. All planktonic susceptibility studies were done over five hours of incubation in enriched media at 37ºC. Biofilms were incubated at 37ºC for four and twenty-four hours. Following incubation, viable colony counts were performed for all non-adherent bacteria. The *S. pneumoniae* isolates used were isolated from pediatric patients and had varying susceptibility to penicillin.

Results: The AuNP-Pen produced were 5-10nm spherical particles with 1.03 x 10^{-6} mol of penicillin/ng particle. While the encapsulated PLGA –Pen particles were considerable larger at 150-200nm. Following exposure to the nanoparticles, planktonic pneumococci showed reduced viability in the presence of the 0.125ng/µl AuNP-Pen and 0.25ng/µl PLGA-Pen when compared to AuNP and PLGA. However, independent of the level of penicillin susceptibility, there was not a significant difference in the inhibitory ability of free penicillin compared to that of AuNP-Pen and PLGA-Pen. Furthermore, while there was a trend for the isolates to survive less well in AuNP-Pen and PLGA-Pen than AuNP and PLGA, respectively; the difference was not statistically significant. However, AuNP in static biofilms statistically decreased viable non-adherent bacteria at four (P=0.0148) and 24 hours (P<0.05).

Summary: While the tested nanoparticles were not effective against the antibiotic resistant *S. pneumoniae*, they will be examined against other pathogens. Also, because the use of these nanomaterials as therapeutics (against other bacteria) is possible, the genetic response of *S. pneumoniae* to the conjugate and encapsulated nanoparticles will be examined in the future. [*This work was supported by NSF-CREST (HRD-1241701); NSF-PIRE-1545884; and NSF-TPAC HRD-1432991*].

GP24 Intracellular processing and antigen presentation of a *Chlamydia* Nanovaccine in Mouse Primary Dendritic Cells and *In Vivo* Live Animal imaging

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Purpose: Biodegradable polymeric nanoparticle-based antigen delivery systems are an emerging platform for vaccine development. Use of such nanoparticles encapsulated with targeted antigen is an attractive approach because of the provided controlled release of antigen and selective targeting of antigen for presentation. We have formulated a biodegradable PLGA (poly (lactic-co-glycolic acid) nanovaccine by encapsulating recombinant MOMP (major outer membrane protein) of *Chlamydia trachomatis* (PLGA-MOMP). Since dendritic cells (DCs) play a major role in antigen processing and presentation; an essential process for adaptive immune responses, we herein assessed the intracellular distribution and processing of our nanovaccine in mouse bone marrow-derived DCs and distribution in mice.
Methods: MOMP was labeled with fluorescein isothiocyanate and encapsulated within PLGA (85/15) nanoparticles (labeled nanovacine). Labeled nanovaccine and MOMP were incubated with mouse DCs for 4, 8, 24 and 48 hours to assess their co-localization with early endosomes, endoplasmic reticulum and with antigen presenting molecules (MHC class I and II) labeled with their specific fluorescein conjugated primary antibodies. BALB/c mice were injected subcutaneously with labeled nanovaccine or MOMP and their distribution were monitored each day up to three weeks using a live animal imaging system.
Results: Nanoparticles were observed in early endosomes at early time points and an increase in MHC-II expression at 24 hour, indicating antigen processing and presentation. *In vivo* live animal imaging showed that nanovaccine migrated over time from the injection site and localized mainly in lymph nodes, suggesting the initiation or activation of adaptive immune responses.
Conclusion. Our results show the successful visualization of nanovaccine within the subcellular organelles and antigen presentation by primary DCs. *In vivo* live animal imaging show that nanovaccine was slowly released and detected predominantly in lymph nodes within three weeks in addition to the injection site.
This research was supported by the National Institute of Allergy And Infectious Diseases of the National Institutes of Health (NIH) under Award Number R21AI111159, NIH-NIGMS-RISE (1R25GM106995-01) and the National Science Foundation (NSF)-CREST (HRD-1241701) grants

GP25 Structure and Function of Coliphage Qβ Proteins in Evolutionary Bio & Nanotechnology

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Purpose: Evolutionary biotechnology, in contrast to the conventional biotechnology, requires neither natural materials nor organisms for processing. Qβ phage is a small positive stranded RNA suitable for evolutionary systems through its proteins. Qβ's genome has 4220 nucleotides coding for 4 proteins, among which is the A1 protein (read-through) representing the minor coat protein; occurs when the stop codon of the coat protein gene leaks. Thus, A1 is the target and platform for evolutionary nanobiotechnology, with its role and domains being investigated through series of deletion, insertion and substitution.
Methods: The plasmid harboring the cDNA of the Qβ genome pQβ8 and ability to produce phage upon transformation was used for this work. Before genetic manipulation, computer prediction of the recombinant A1 protein was analyzed (MacVector, Pfold & RNA fold). Recombinant plasmids were obtained by insertion, deletion or substitution within the A1 gene and used to produce and characterize phages. The yield of phage production in *E. coli* HB101 and K12, and morphology on the lawn of *E. coli* Q13 were studied. Cryo-EM was used for phage confirmation.
Results: The computer prediction showed a conserved structure with modified C-terminus versus the N-terminus. None of the N-terminus modified A1could give viable phages. The C-terminus could be extend 50 to 250 amino acids, not affected with either Gly-Gly or Gly-Ser linkers. The yield of phage produced was significantly low in comparison to the wild type. The hybrid phage morphology was diversified with predominance of small size plaques with the large inserts and vice-versa, confirming quasispecies nature of Qβ. There was no correlation between the inserted peptide motif with the phage titer but with its size or length. Up to 1/10 of the A1 gene was deleted without affecting the viability but the titer.
Conclusions: We have successfully identified and characterized the portion of A1 for an exogenous gene insertion. Our Study demonstrated, for the first time, a proof of principle of platform for

nanoparticles and/or drug attachment based on an RNA display system. For future research, the nanotags can be used to explore attachment and/or to initiate assembly to nanomaterials.

GP26 Ion-activated in-situ gel for prolonged release and enhanced retention in the porcine cornea

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Purpose: Nepafenac is a nonsteroidal anti-inflammatory drug (NSAID), which has very poor water solubility; therefore, it is available in a suspension form only. Suspensions are undesirable for two reasons: they tend irritate the patient's eye leading to limited residence time and bioavailability of the drug. This decreases the amount of time that the drug has to reach the site of action, the cornea. Therefore, to improve these issues we first solubilized nepafenac using hydroxypropyl-beta-cyclodextrin. Next, we incorporated the complex into an ion-activated in-situ gel formulation using sodium alginate, Protanal PH 1033, to reduce patient repeat administration and increase residence time. This formulation could possibly overcome two problems associated with the commercial product, Nevanac, while still providing the patient with the same dosage concentration.
Methods: A nepafenac in-situ gel formulation (F16) containing 0.3% sodium alginate was produced using a boric acid buffer. Rheological properties of the formulation were determined using a rheometer before and after the addition of STF (Ratio of 25 F16: 7 STF). Release studies were performed using Franz diffusion cell apparatus and porcine corneas, comparing F16 to our solution formulation, our suspension and the commercial product, Nevanac 0.1% (Fig 1).
Results: Rheological studies revealed that the viscosity o F16 doubled when exposed to the STF at 35°. All of the formulations displayed pseudo-plastic flow as exhibited by the shear thinning profile, which is ideal for ophthalmic gel formulations because the viscosity should be low during blinking and high during inter-blinking. The release studies revealed no significant difference in permeation rate across the three in-situ formulations; however, the permeation rate of all three in-situ formulations was approximately 14 times higher than the commercial product ($P<0.0001$) (Figure 2). Additionally, the in situ gel formulations had significantly higher amounts of nepafenac retained in the cornea when compared to the commercial formulation (F15, F17: $P<0.0001$; F16: $P=0.0006$).
Conclusion: An in-situ solution formulation, using Protanal PH 1033, was created for the sustained release of nepafenac on the cornea.

GP27 Zinc nanoparticles may influence microbiota populations and odorant detection

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Purpose: Zinc nanoparticles (ZnNPs) have been isolated within protein nucleating centers present in human and animal blood. A subset of ZnNPs mimicking the endogenous subset ranging in size from 1-2 nm, non-oxidized, non-ionic state, have been studied in their influence on olfaction. At low concentrations, ZnNPs can enhance electroolfactogram (EOG) or whole cell patch-clamp responses to odorants by about 3-fold. Odorants brought to the olfactory epithelium (OE) may traverse the mucous layer interacting with microbiota. The microbiota of the OE is not well established and no available literature evaluates antimicrobial activity of non-oxidized ZnNPs less than <2nm diameter. It is known that Zn^{2+} and zinc oxide nanoparticles have antimicrobial effects. The endogenous presence of ZnNPs in OE has not been described. This study evaluates physiologically analogous ZnNPs in the OE and the effects of ZnNPs on growth of select bacteria.
Methods: ZnNPs were prepared by underwater high-voltage discharge method. Particle size was determined by atomic force microscopy, crystallinity by transmission electron microscopy, and oxidation by X-ray photon spectroscopy. Endogenous nanoparticles were obtained by microsurgical OE collection, homogenization, and filtering (30-kDa/3-kDa). EOG performed on OE evoked by odorant mixture ethyl butyrate, eugenol, and (+/–) carvone. Growth of *Bacillus subtilis* and methicillin resistant *Staphylococcus aureus* (MRSA) strains 1, 2, 5, 13, 26, 34, and 45 cultured with ZnNPs were evaluated. The relative viability of bacteria was determined by spectrophotometric analysis at a 24-hour time point.
Results: The OE nanoparticle filtrate demonstrated analogous EOG enhancement to ZnNPs. Results showed that ZnNPs were generally MRSA suppressive, with approximate 40% maximum reduction in viability ($p<0.05$). Conversely, growth

for *B.subtilis* with no significant effect and a 30% maximum increase in viability.
Conclusion: The presence of ZnNPs within the body may have physiological significance and olfaction enhancing nanoparticles within the OE suggests a role in olfaction. These preliminary studies *in vitro* have demonstrated a differential antimicrobial effect on bacteria of various taxa and suggest their presence may be influencing the microbiota differentially. Further characterization of the filtrate and evaluation to determine the mechanism of action for ZnNPs effects on growth of multiple bacterial taxa are needed.

GP28 The synthesis of polymer scaffolds used for various biological techniques

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Over the past few years' tissue engineering and regenerative applications have yielded many neoteric tissue replacements and implementations. Electrospinning is a valuable technique for the fabrication of 3D fiber scaffolds for advanced wound healing and in this manner reclamation of the skin. Scaffolds are comprised of biocompatible biomaterials, such as collagen, manufactured materials, or in the current study a combination of polycaprolactone (PCL), and polydioxanone (PDO). PCL is a long-degrading polymer and is FDA approved with high porosity and PDO which has been used for biomedical applications, mostly in the form of surgical sutures. These polymers were fabricated into a bioresorbable scaffolds through electrospinning. This newly designed functionally graded composition will be used for skin growth.
Scaffolds were synthesized using PDO and PCL which were dissolved in hexaflouro -2- propanol (HFIP) and stirred 24h. Different polymer solutions of PDO, PCL, PDO+PCL, and PDO+PCL+ Collagen were used for making the scaffolds used. Electrospinning parameters were optimized to get fibers evenly dispersed across metal spiked plate. Scaffolds were then vacuumed dried for 2 days before characterization. Morphology and fiber composition were analyzed with Scanning electron microscopy (SEM). Tensile properties were measured with a dynamic mechanical analyzer (DMA). Scaffolds were sterilized by UV light and afterwards washing with ethanol, sterile distilled water, and finally HBSS. The growth and proliferation of normal human keratinocytes were evaluated in keratinocyte growth serum and our EpiLife medium. Fibroblast was grown in DMEM 10 media until confluent. Scaffolds were then placed into a 48 well plate and seeded with keratinocytes and fibroblast in separate wells for 21 days.
Synthesized scaffolds showed cell growth on scaffolds were 100% viable on days 3 and 5 with a decrease to 90% on days 7 and down to 80% on day 15. Keratinocytes and Fibroblast were viable on all variable scaffolds used.
In conclusion scaffolds were successfully spun for PDO and PCL synthesized polymers. SEM showed ECM mimicking fibrous morphology for each blend that was used. Cell studies confirms cell adhesion and growth on scaffolds used for extended periods. Future studies are to develop procedures for establishing confluent, layers of co-cultured human keratinocytes and fibroblast on the surface of synthesized PDO and PCL scaffolds.

GP29 Effect of Amino functionalized Multi-Wall Carbon Nanotubes(MWCNTs) on mechanical and thermal properties of BMI/CF Nano Composite

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Purpose: To optimize the performance of carbon fiber/bismaleimide composites.
Methods: Bismaleimide (BMI) resin was modified using smaller concentrations of multi-wall carbon nanotubes (MWCNTs) with ultrasound dispersion technique. The carbon fiber (CF) composites were then fabricated by reinforcing modified resin using compression mold method. The CF/BMI composites were finally tested for flexure, ILSS and viscoelastic properties.
Results: CF/BMI composites at 0.2%CNT showed maximum enhancement in mechanical and viscoelastic properties over neat counterpart.

GP30 Fabrication of Polycarbonate-Silica Polymer Nanocomposites Layer by Layer Through 3D Printing

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Purpose: 3D printing is a technique that has vastly grown in the engineering field because of its unique capabilities. Polycarbonate has become a

thermoplastic of interest to 3D print due to excellent mechanical and optical properties. Silica has been used as a filler to improve mechanical properties. Polycarbonate and silica are known for their optical properties here in this study we explored the polymer nanocomposite fabrication layer by layer to improve the optical and mechanical properties. However, preliminary studies on this polycarbonate 3D printing show the change of crystallinity and optical properties.

Methods: The polycarbonate pellets and silica nano powder were heated at 120^{o}C for 6 hours. Afterwards they were blended and extruded at 265-280^{o}C to have the highest transparency. The filaments created were the neat polycarbonate, 0.5% SiO_2, 1% SiO_2, 3% SiO_2 and 5% SiO_2. Then the filaments were 3D printed to create 1 layer and 3 layers of each sample. The materials were then characterized using the UV-Vis for its optical properties, tensile and flexure tests for its mechanical, and the TGA and DSC for its thermal properties.

Results: The transmittance of the material has decreased compared to that of neat polycarbonate. The layer by layer process affects the transmittance due to light traveling the first layer. The second and third layer fails to transmit light as well as the first layer. Increasing the loading of silica into the polycarbonate deteriorates the optical properties. The material becomes translucent. The mechanical properties decreased and the thermal properties received a slight change compared to that of the neat materials.

Conclusion: Polycarbonate-silica has decrease in transmittance due to the layer by layer process from the 3D printer as well as its mechanical properties. However, the thermal properties of the composite received a slight change. With such tests conducted, the application for this material can serve for the automotive industry and for thin film applications.

Postdoctoral Fellows and Faculty Poster Abstracts

Arranged alphabetically by presenting author's last name with names of presenting authors underlined

OP01 Epigallocatechin gallate-Gold Nanoparticles Exhibit Superior Anti-tumor Activity than Conventional Gold Nanoparticles: Potential Synergistic Interactions

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Epigallocatechin gallate (EGCG), the most abundant and active constituents of green tea, is known to have anti-cancer activity. However, the use of EGCG in therapeutics is limited due to its poor bioavailability and limited stability at physiological pH. Gold nanoparticles (GNPs) inhibit angiogenesis and cell-cell crosstalk, and are demonstrated to have some anti-cancer activity as well. Further, GNPs are being used in drug delivery for their ability to protect the drug from degradation and enhance circulation half-life. Here, we investigated if EGCG-formulated GNPs (E-GNPs) would perform better as compared to free EGCG and conventionally-synthesized GNPs due to their synergistic interactions. GNPs and E-GNPs were synthesized by reducing gold(III) chloride with sodium citrate and EGCG respectively. After synthesis, they were characterized with UV–visible absorption spectroscopy, TEM, and DLS. Both the nanoparticles were spherical, ~25 nm in diameter and had uniform size distribution. Cytotoxicity of EGCG, GNPs and E-GNPs was evaluated in cancerous (A375SM, MDA-MB-231, MiaPaCa and PC3) and noncancerous (HaCaT, MCF-10A, HPNE and RWPE1) cell lines by Trypan blue exclusion assay and/or WST assay. Cell viability studies showed enhanced antitumor activity of E-GNPs compared to free EGCG and GNPs. Nanoparticles cellular uptake studies by TEM and AAS revealed that E-GNPs were taken-up quickly and efficiently by cancerous cells compared to noncancerous cells. Further our data show that E-GNPs induce more apoptosis in cancer cells compared to free EGCG and GNPs. Altogether, these findings suggest that E-GNPs exhibit highly selective and significantly more antitumor activity when compared to free EGCG or GNPs alone and can be further exploited for cancer therapy.

OP02 Toward Solar Fuel: Proposed Mechanism for Water Oxidation of a Nanosized Semiconductor/Manganese System

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Photosynthesis is able to achieve solar energy conversion via water splitting in the oxygen-evolving complex of photosystem II in cyanobacteria, algae, and green plants [Suga et al (2017) *Nature*, 543, 131-15]. To mimic photosystem II, we have reported a nanosized semiconductor tungsten oxide/manganese system for efficient photo water splitting at pH 7 [Liu et al (2011) *Angew. Chem. Int. Ed*., 50: 499-502]. In this work we extend our study to the semiconductor iron oxide/manganese nanostructure for water oxidation. Electrochemical behaviors of the manganese compound showed that the manganese materials contain the Mn(III/IV) redox center. The pH dependence of their redox chemistry revealed that the redox chemistry of the manganese materials involves proton-transfer step in the Mn(III) to Mn(IV) transition. In contrast, the Mn(II) to Mn(III) transition seems to be not associated with proton-transfer reaction. The photocurrent at an applied voltage of 0.70 V of the iron oxide/manganese system enhanced by a factor of 5 and 3 than that of the iron oxide. We propose a working model of the semiconductor/manganese systems for photo water splitting, in which the semiconductor serves as the artificial chlorophylls of photosystem II and the manganese compound is the synthetic oxygen-evolving complex, respectively. The semiconductor absorbs photons and generates electrons and holes. The holes are filled by the electrons of manganese (III/IV) center in the manganese complexes. The manganese (III/IV) center is oxidized to form an Mn(V)=O species which is attacked by a water molecule. The formation of the O-O bond is accompanied by the reduction of Mn(V)=O to a Mn(II) species, which seals the water splitting cycle. The Mn(III/IV) center is renewed by the oxidation of Mn(II) species. The structure of the active species in the catalytic cycle of water splitting is unknown and required further investigation.

OP03 Novel nanobio-materials for inhibition of respiratory syncytial virus

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Purpose: Respiratory syncytial virus (RSV) infection is a major concern for the population across all age groups and it is the single serotype with two major antigenic subgroups circulating together but one leads (Borchers, Chang et al. 2013). Viruses of this family fuse their membrane with the plasma membrane of host which results in cell fusion if added to cell in large quantity (Haywood 1978). Several peptides as well as theirs carriers have been reported for the inhibition of RSV (Sun, Pan et al. 2013; Singh, Tiwari et al. 2014). However, possibility of liposomes as carrier of these anti-RSV peptides has been rarely discussed. This works represents inhibition of RSV by liposomes encapsulating anti-RSV fusion peptide (RF-482).

Method: RF-482 is a fusion peptide as well as hydrophilic in nature. This builds a possibility that the RF-482 could get encapsulated as well as fused to the liposome surface and imparts the same or better effect in RSV inhibition. The liposomes were prepared using the conventional thin film hydration method with and without peptide RF-482. The loading of RF-482 was determined using BCA assay and the RSV inhibition was studied using immune-fluorescence imaging, plaque assay and PCR.

Results: Initially, loading of peptide was confirmed by slight increase in particle size. Further it was observed that It was observed that 77.3 % (n=3, ±2.2 %) protein was found non-encapsulated, hence it can be concluded that 22.7 % of peptide RF-482 was loaded. Plaque assay confirmed over 60 % RSV inhibition with just peptide RF-482 and liposomes. However, with peptide loaded liposomes more than 70 % inhibition was observed. The inhibitory effect of liposomes with and without peptide was also confirmed by Immunofluorescence imaging.

Conclusion: From the obtained results and observations it can be concluded that the liposome can become carrier for peptides like RF-482. Moreover, it can also be concluded that the empty liposomes as well as liposomes loaded with RF-482 can inhibit RSV fusion to HEP-2 cells and thereby save HEP-2 cells from infection.

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OP04 Membrane nanoparticle-based delivery of Doxorubicin for improved therapeutic efficacy

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Doxorubicin (DOX) is a conventional anthracycline drug with cytotoxic properties and currently used in cancer therapy. However, the utilization of DOX is limited due to formation of metabolites decreasing availability and increasing organ toxicities. Pegylated liposomal doxorubicin, DOXIL®, was synthesized to overcome these shortcomings. While DOXIL® improves circulation half-life, it is not specific to the tumor and the presence of PEG may reduce its interaction with cancer cell membranes. Exosomes are nanoscale biological vesicles with a lipid bilayer membrane, are secreted by nearly all cell types and mediate cell-cell communication through the transfer of biological molecules. In this study, we investigated if the cells present in a tumor microenvironment; i.e. pancreatic cancer cells (MiaPaCa), pancreatic stellate cells (PSCs) and macrophages (RAW264.7) can package DOX into the secreted exosomes (EXO-DOX) and if these exosomes can be used for the delivery of DOX to the cancer cells to illicit its anticancer activity. For this we treated MiaPaCa, PSCs and RAW264.7 cells with DOX for 48 h and isolated exosomes from media by centrifugation. Size of EXO-DOX was determined by DLS and drug loading in exosomes quantified by HPLC. Out data suggest that MiaPaCa cells are most efficient in loading the DOX in exosome. EXO-DOX from all cell types demonstrated superior anti-tumor activity in comparison to free drug as observed by cell

proliferation assay. Moreover, when cancer cells were treated with equal amount of drug content, EXO-DOX obtained from RAW264.7 demonstrate highest killing in the cancer cells. Together our data suggest that exosomes can be exploited for efficient drug delivery and may potentially be used in pancreatic cancer therapy.

OP05 Proteomic analysis of antimicrobial effects of pegylated silver coated carbon nanotubes in *Salmonella enterica* serovar Typhimurium

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Purpose: Synthesis of silver nano-compounds with enhanced antimicrobial effects is of great interest in the development of new antibacterial agents. Recent studies have revealed the antibacterial ability of pegylated silver-coated carbon nanotubes (pSWCNTs) having less toxicity in human cell lines. The full molecular mechanism underlining the pSWCNTs activity remains unfolded. Here we tested the effectiveness of the pSWCNTs on foodborne pathogenic bacteria and conducted a comparative proteomic analyses of bacteria.
Methods: Major foodborne pathogens including *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *S.* Anatum were cultured in the presence of various concentrations of SWCNTs or pSWCNTs. In experiment-1, bacterial growth kinetics were analyzed by measurement of optical density (OD) and bioluminescence imaging (IVIS) to determine antibacterial activity of pSWCNTs. In experiment-2, fertilized chicken eggs were injected with pSWCNTs to assess toxic effects of pSWCNTs in embryo development. In experiment 3, control and pSWCNTs-exposed *S.* Typhimurium were harvested at the stationary phase and submitted to proteomic analysis using two dimensional electrophoresis (2-DE) combined with matrix-assisted laser desorption/ionization time of flight/ time of flight mass spectrometry (MALDI-TOF/TOF MS) to understand the antibacterial mechanism of pSWCNTs. Data were analyzed by Student's *t*-test with two-tailed nonparametric analysis, with $P < 0.05$ indicating significance.
Results: OD measurements, bioluminescent intensities, and CFU indicated stronger bactericidal activity of pSWCNTs compared to SWCNTs. Chicken embryo administered with pSWCNTs showed similar growth and development to chicken treated with phosphate buffered saline (PBS) solution. The comparative proteomic analysis revealed ten up-regulated and fixe down-regulated by pSWCNTs ($P<0.05$). These proteins were associated with various biological processes including nutrient and energy metabolism, oxygen stress, DNA protection, and starvation.
Conclusion: The present study clearly indicates the pSWCNTs as a promising antibacterial agent acting on dysregulation of proteins synthesis that affect bacterial survival and division. Work supported by USDA-ARS Biophotonics Initiative #58-6402-3-018.

OP06 Prospect of Carbon Coated Ferrite Nanoparticles in Cancer Cell Killing by Microwave Radiation

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Purpose: Temperature rise property of Carbon Coated Ferrite Nanoparticles (CCFN) exposed to electromagnetic wave is investigated to explore its potential in cancer cell killing.
Method: Student evaluates the effectiveness of Carbon coated Ferrite Nanoparticle (CCFN) in heat generation under microwave radiation. This work is conducted by Comsol Multiphysics simulation software and finally verified by an experiment. In the simulation, a solution containing CCFN is modeled as a lossy dielectric material placed in a rectangular waveguide. A fixed frequency electromagnetic signal with various power levels is applied from one end of the waveguide and temperature rise of sample is recorded for 5 minutes of exposure. Next, a fixed power electromagnetic signal with various signal frequencies is applied and temperature rise is recorded for 5 minutes of exposure. Finally, a microwave signal of fixed frequency and power is applied to solutions with different CCFN concentration which is ensured by assuming various loss tangent values for the solution. In all three cases, temperature of the solution is increased exponentially showing effectiveness of CCFN in temperature rise. After simulation an experiment is conducted to verify at least one of the above properties. Two material samples with two different values of loss tangent are taken. One sample is pure water and other one is CCFN

solution which is prepared by mixing 250 milligram of Sodium Cholate, 75 milligram of CFN powder and 250 ml of distilled water. CCFN solution has definitely higher value of loss tangent than that of pure water. 30 ml of CFN solution is poured in 50 ml beaker and put in a microwave oven (1 KW) for 8, 10, 12, 14, 16 and 18 seconds of radiation. In another beaker, 30 ml of distilled water is poured and heated with same microwave oven for the same time intervals as before.
Results: It is noticed that CCFN solution gets heated more as compared to pure water verifying that more loss tangent causes more heat generation.
Conclusion: This observation can be utilized to design radiation frequency or power or duration or CCFN concentration dose to achieve cell apoptosis temperature to kill cancer cells.

OP07 Dithiocarbamates induce cytotoxicity through DNA damage dependent pathways

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Purpose: The mechanism of action of many genotoxic agents, including cancer chemotherapeutics, involves induction of nuclear DNA damage. The single cell gel electrophoresis (SCGE) assay is widely used to measure DNA damage due to its technical simplicity and high sensitivity. We have recently advanced the SCGE assay by facilitating the development of the CometChip®, a 96-well SCGE assay platform.
Methods: Using the CometChip®, we have screened a custom panel of 74 compounds provided by the U.S. National Toxicology Program (NTP). Three of the top five chemicals identified as potent DNA damaging compounds were dithiocarbamate-containing compounds. In particular, Thiram ($C_6H_{12}N_2S_4$), was extremely genotoxic to human lymphocytes at concentrations below 10 μM. Thiram, a potent insecticide, fungicide, pesticide and bactericide has been used in large quantities by farmers for decades however the mechanism of action of the chemical remains contentious. Of particular note, Thiram shares strong structural similarities to the clinical agent Disulfiram ($C_{10}H_{20}N_2S_4$) (aka Antabuse), a drug discovered in the 1920's used to treat alcoholism and more recently used as a chemotherapeutic. As with Thiram, it remains largely unclear how Disulfiram acts as a cancer suppressor.
Results: In the presented research, we show that both Disulfiram and Thiram can induce high levels of DNA damage at clinically relevant concentrations. We also show that human cells (colorectal and kidney) that have impaired DNA repair capacity are more sensitive to the agents. Using the CometChip® technology, we were able to comprehensively assess DNA damage and repair after acute treatment. We can confirm that not only do the compounds produce extremely high levels of DNA damage but also that repair is inhibited long after the agent is removed from the cells.
Conclusion: We propose that the principle mechanism of action of both Thiram and Disulfiram is DNA damage, caused directly and also indirectly, through the induction of mitochondrial dysfunction and ROS production. The ability to characterize the action of these compounds will allow researchers to better evaluate therapeutic potential. *Supported by grants from the National Institute of Health (NIH) [CA148629, ES021116] and the Abraham A. Mitchell Distinguished Investigator Fund to RWS.*

OP08 *Chlamydia* M278 outer membrane peptide encapsulated in poly(lactic acid-poly(ethylene glycol) biodegradable polymeric nanoparticles protects against *Chlamydia muridarum* infection in mice partly by induction of heightened systemic and mucosal antibody titers

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Purpose: *Chlamydia trachomatis* (CT) is the leading cause of bacterial sexually transmitted infections worldwide but, there is no vaccine to control CT infections. Past attempts using subunit vaccines in combination with various adjuvants have failed partly due to inefficient delivery systems. We have developed a *Chlamydia* nanovaccine by encapsulating M278 [a peptide derived from CT major outer membrane protein (MOMP)] in poly (lactic acid-poly (ethylene glycol) (PLA-PEG) nanoparticles that induced enhanced immune responses in immunized mice. In the present study, we first assessed the efficacy of PLA-PEG-M278 in immunized mice challenged with a *C. muridarum* infection as well as the systemic and mucosal antibody responses correlated with its efficacy
Methods: Female BALB/c mice were immunized subcutaneously with 50 μg each of either PLA-

PEG-M278 or bare M278 three times at 2-weeks interval and sacrificed two weeks after the last immunization to measure systemic and mucosal antibody responses. All immunized mice were challenged intravaginally with 10^5 inclusion forming units (IFUs) of *C. muridarum,* three weeks after the final immunization and sacrificed three weeks post-challenged. Blood, cervico-vaginal washes, and swabs were collected from all mice. *C. muridarum* was quantified by growing the swab suspensions on McCoy cell monolayers and IFUs were enumerated by fluorescence microscopy. The serum and wash samples of immunized mice were analyzed for antibody responses by ELISA.

Results: Immunization with PLA-PEG-M278 showed significant ($P < 0.001$) up-regulation of both M278- and MOMP-specific systemic IgG and IgG1 and mucosal IgA antibodies as compared to those of bare M278. Of significance was the PLA-PEG-M278 formulation protected immunized mice, as compared to bare M278, against a *C. muridarium* challenged infection.

Conclusion: PLA-PEG-M278 stimulated significant systemic as well as mucosal antibody responses to protect mice against a *C. muridarium* challenged infection. The enhanced secretion of mucosal IgA suggests they may play a role in protecting mice against vaginal infection. Our data show the potential of PLA-PEG-M278 as a nanovaccine candidate against CT. However, the addition of adjuvants to the formulation may further enhance the protective efficacy in mice.

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OP09 Application of Nanobiotechnology in Veterinary Medicine

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Nanobitechnology is an important research field in veterinary medicine. Application of nano biotechnology has been mainly involved in fields including nano-scale pharmaceutical carrier, nano-scale diagnostic platform, nano-biomaterials, nano-particle feed and feed additives. Antibiotics has been used as growth promoters in animal husbandry for long time, but growing concern over microbial antibiotic resistance, antibiotics free meat is more welcomed in market. Many reports have shown evidence that nanoparticles may be good candidates for animal growth promotion and antimicrobials. The current status and advancements of nanotechnological applications in Veterinary medicine will be the focus of this presentation and the emerging roles of nanoparticles for feed and feed additives, antimicrobial agents, and diagnostic tools in veterinary medicine will be discussed.