2019 GRADUATE Abstracts

Development of Eggshell based Calcium Carbonate Fertilizer

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Purpose: Eggshells are widely considered as waste but contain both inorganic and organic components that can be a potential source for bio-based calcium carbonate ($CaCO_3$). The combination of NPK fertilizers and $CaCO_3$ encapsulated in a biodegradable polymer have the potential to increase crop yields and reduce environmental impact. Methods: In these studies, the synthesis of calcium carbonate was performed using a sonochemical technique to produce high surface area calcium carbonate nanoparticles from eggshells. The eggshell particles were irradiated with a high intensity ultrasonic horn in the presence of hydrogen peroxide. The eggshell $CaCO_3$ and fertilizer was then combined with a biodegradable polymer and collected from the microfluidics machine. Results: The Raman and XRD analyzes confirmed that the calcium carbonate produced from the synthesis was the most stable form, calcite. TEM images confirmed spherical shape with smooth surface. The nanocomposites will be characterized using field-emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), Raman, and X-ray diffraction (XRD). Conclusion: Controlled release fertilizers allow for plants to uptake nutrients as needed. This method of incorporating calcium carbonate for fertilizer applications can be used for the protection of pests and pathogens by adding other agrochemicals.

Investigation of β-cyclodextrin pseudopolyrotaxanes based on poly(ethylene glycol)s for gene and drug delivery

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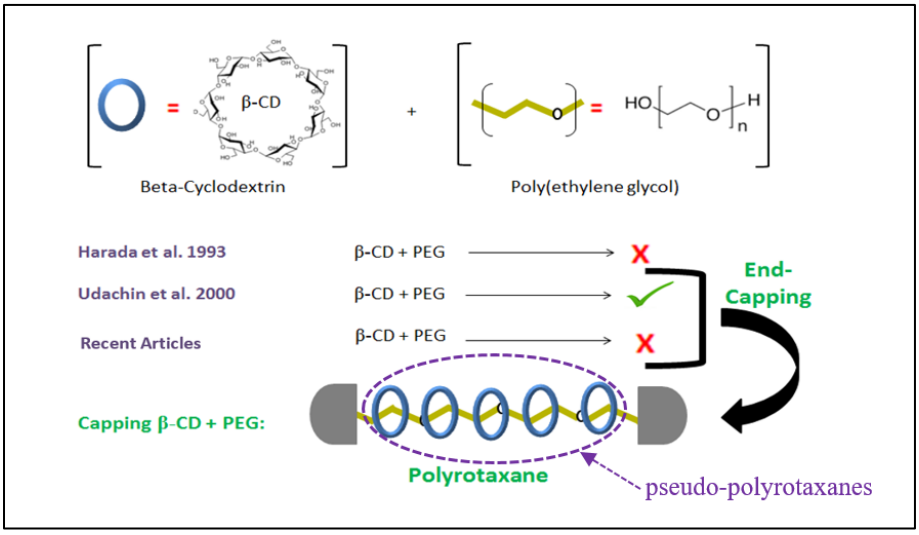
Purpose: β-cyclodextrin (β-CD) plays a vital role as a host in the field of host-guest chemistry that leads to supramolecular structures. In literature, there is a disagreement regarding whether or not β-CD forms inclusion complexes with any molecular weight of polyethylene glycol (PEG). Only one article suggested the formation of inclusion complexes through showing the single crystal structure but no specific PEG molecular weight was mentioned. This research was to investigate the possibility of forming crystalline pseudopolyrotaxanes based on β-CD and two molecular weights of PEG, PEG_{600} and PEG_{1500}.

Methods: β-CD and PEGs were dissolved first separately in appropriate solvents and left on a magnetic starrier for 12 hours. After that, they were combined by adding gradually both PEG having a molecular weight of 600 and β-CD first with PEG having a molecular weight of 1500. Samples stirred up for 24 hours. The sample solutions then were frozen by nitrogen gas for 10 minutes, and solvents were evaporated by lyophilization under vacuum. Solid samples were examined using SEM, FT-IR, WAXS, DSC; while the solution samples were investigated by 1D-NMR (^{1}H, ^{13}C), 2D-NMR (NOESY and ROSY).

Results: 1D and 2D NMR shows that the methylene protons of PEG interact with external and internal protons of β-CDs. WAXS shows the formation of new crystalline structures which are different than β-CD crystalline structure. PEG_{600} is liquid but still forms a crystalline complex with β-CD. SEM shows the formation of large crystals for the β-CD/PEG_{1500}. Furthermore, DSC showed different thermal behaviors and proved the crystallinity of β-CD/PEGs pseudopolyrotaxanes. It should be noted that the solvent plays an important factor which as a result either enhances or prevents the formation of inclusion complexes.

Conclusion: Our approach increases the interaction to farther forming the inclusion complexes which support the one article that illustrated the single crystalline structure of β-CD/PEG even it didn't mention the used molecular weight of PEG. Solid state and solution state examinations suggested the formation of β-CD/PEGs pseudopolyrotaxanes opening the doors for more pharmaceutical application especially for gene and drug delivery. The future work is to cap the PEGs chains to form polyrotaxanes.



Evaluation of Chlamydia Nanovaccine Formulated by Encapsulation of Recombinant MOMP in Poly (Lactic acid) - Poly (Ethylene) Glycol Nanoparticles Administered with Mucosal Adjuvant LT

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Purpose: Chlamydia is a mucosa obligate intracellular pathogen, a leading cause of bacterial sexually transmitted disease worldwide. It has been implicated in infections of extreme concern mostly in females, ranging from systemic mucosal inflammation to acute pelvic inflammatory disease. Despite all efforts and scientific trials to develop a competent vaccine against Chlamydia infections, no prophylactic vaccine capable of inducing complete immune protection in human has been discovered so far. Methods: In the ongoing research study, we are evaluating the immune potentiating effect and protective efficacy of a nano-vaccine by formulating diblock of nanoparticles made up of Poly-lactic acid-Poly-ethylene-glycol nanoparticle (PLA-PEG) polymers. Firstly, we assessed its morphological characteristics using microscopy techniques. Encapsulation of Chlamydia muridarum full length recombinant Major outer membrane protein (MOMP) in PLA-PEG was carried out using a water/oil/water double emulsion evaporation technique. For our in vivo vaccine studies, we employed a mouse infection model. Groups of Balb/c mice received 3 subcutaneous immunizations with either PBS, MOMP, MOMP + adjuvant and encapsulated MOMP + adjuvant. Mice were sacrificed at preschedule intervals. Post immunization cellular and antibody analyses of collected samples were conducted. Subsequently, in this study, mice were genitally challenged with Cm to determine the protective efficacy of the PLA-PEG-MOMP in the presence of mucosal adjuvant (LT). Results: Evaluation of serum (IgG1, IgG2a, IgG2b) and mucosal antibody (IgG1, IgG2a, IgG2b, IGA) isotypes revealed that the encapsulated full MOMP in the presence mucosal adjuvant LT was superior in triggering higher and enhanced mucosal and systemic antibody responses in immunized mice. Conclusion: PLA-PEG encapsulated MOMP in the presence of mucosa adjuvant LT induced enhanced Chlamydia specific antibody responses. This suggests enhanced immunogenicity of encapsulated MOMP when co-administered with mucosal adjuvant against Chlamydia infection. This study might help us find an effective vaccine that could protect against Chlamydia genital diseases.

Cocaine modulates the biogenesis and composition of microglia-derived exosomes

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Exosomes are nanocomposites can mediate oligodendrocytes, astrocytes, neuronal, and glia communication within the central nervous system. Glia cells, such as microglia, are the primary immune cells within the central nervous system that regulate inflammatory processes during infection or cellular damage. Microglia secrete exosomes due to stress and environmental factors stimulated by pathogens and substance abuse (i.e., cocaine). Cocaine is a highly addictive stimulate that is commonly used as a recreational drug in the United States. In this study, we hypothesize that cocaine alters the biogenesis and composition of microglia-derived exosomes. The BV-2 cells were cultured in exosome-free media and were either not treated (control) or treated with 0, 10 nM, 100 nM, 1µM, 10 µM, or 100 µM of cocaine for 24 hrs. Our results demonstrated that cocaine impacted microglia cell morphology, viability, and protein content. Primarily, our studies revealed that biogenesis and composition of microglia-derived exosomes was altered by cocaine treatment.

The Role of Lipopolysaccharide-induced Extracellular Vesicle Biogenesis in Cardiac Cell Death

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Abstract

Exosomes play a crucial role in understanding the progression of infectious diseases. It has been shown that exosome vesicle release and biogenesis can be affected by external factors such as pathogenic infections. To better understand the affect on exosome composition and biogenisis caused specifically by gram-negative bacterial infections, we chose to examine lipopolysaccharide (LPS) as a gram negative model to assess the effect of exosome behavior after LPS treatment of AC16 human cardiomyocytes.

Using a dose dependent gram-negative model (0 µg, 0.1 µg, 1 µg, or 10 µg of LPS), we showed that treatment with LPS substantially altered the composition of AC16-derived exosomes. The relative size and number of exosomes (particles/mL) were decreased significantly with all concentrations of LPS compared to the untreated group. LPS administration decreased the presence of exosomal proteins that are related to exosomal biogenesis. Conversely, we observed an increase in immunomodulators present after LPS administration. *The* evaluation of the impact of lipopolysaccharide on cardiac cell death and exosome biogenesis will yield new insight into the importance of exosomes in a variety of physiological and pathological processes as it relates to disease progression, diagnosis and treatment.

Enhancement of aqueous solubility of hispolon by complexation with sulfobutyl ether β-cyclodextrin

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Abstract:

Keywords: Hispolon, Sulfobutyl ether β-cyclodextrin, Inclusion complexes, Solubility enhancement

Purpose: Hispolon, a structurally related compound to curcumin has been shown to possess anti-inflammatory, anticancer and antioxidant properties. The poor aqueous solubility of hispolon could limit its oral absorption. This study successfully utilized sulfobutyl ether β-cyclodextrin (SBE βCD) to form an inclusion complexes with hispolon to increase the water solubility and dissolution rate for improved permeability across GI tract.

Methods: In this study, the hispolon complexes were formed by using freeze drying technique. The liquid and solid state complexation was confirmed by phase solubility studies, Differential scanning calorimetry (DSC), Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Nuclear Magnetic Resonance (NMR) analyses. Intrinsic dissolution rate of tablets containing anhydrous lactose as diluent was carried out using modified die assembly in dissolution apparatus.

Results: The phase solubility studies showed that the hispolon solubility is enhanced by the presence of SBEβCD with a degree of about 15 folds (0.45 to 6.5 mg/ml). The complexation efficiency was found to be 0.4703 whereas the apparent stability constant as 340.05 M^{-1}. The solid state characterization techniques demonstrated the successful complexation of hispolon in SBEβCD. The DSC thermograms showed disappearance of hispolon peak in the complex. The FTIR spectra of the lyophilized complex lacked the characteristics absorption bands of hispolon. Both of these results indicate the formation of drug complex between drug and cyclodextrin. The Proton NMR has shown deshielding of drug protons indicating the encapsulation of drug inside SBEβCD. The SEM images confirmed the complexation as the surface of the complex showed very less amorphous deposits in comparison to the crystalline cluster of physical mixture. The intrinsic dissolution rate of hispolon was higher (80.43 $\mu gcm^{-2}min^{-1}$) as compared to its physical mixture with SBEβCD (55.76 $\mu gcm^{-2}min^{-1}$).

Conclusion: This study successfully utilized sulfobutyl ether β-cyclodextrin (SBEβCD) to form an inclusion complexes with hispolon to increase the water solubility and dissolution rate for improved permeability across GI tract. The findings provided a new possibility to utilize hispolon for enhanced oral absorption and bioavailability. We plan to utilize the complex formed in various formulation designs and investigate its potential benefits in coming future.

Design of hispolon and doxorubicin nanoparticles for melanoma treatment

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Design of hispolon and doxorubicin nanoparticles for melanoma treatment A hmed Al Saqr1, Ishwor Poudel1, Hamad Alrbyawi1, Manjusha Annaji1, Robert D Arnold1, R. Jayachandra Babu1 1Department ofâ€¯Drug Discovery and Development, Auburn University Purpose: The current chemotherapy drugs are associated with serious toxic effects hence there is a need of novel, safe and effective drugs for the treatment of melanoma. Hispolon is a small molecular weight polyphenol derivativeâ€¯which has antioxidant, anti-inflammatory and anti-proliferative activities. Our recent study has demonstrated hispolon as a potent apoptosis inducer in melanoma cell lines. Doxorubicin is a broad spectrum first-line treatment for various kind of cancers. In this study, an approach for co-delivery of doxorubicin and hispolon using a liposomal system in B16BL6 melanoma cell lines for synergistic cytotoxic effects was investigated. Methods: Liposomes were prepared in certain molar ratio (55:40:5 mol% of DSPC/ cholesterol/PEG2000- DSPE, respectively) using lipid film hydration method and loaded with doxorubicin and hispolon (ratio of 1:10). Liposomes were characterized by measuring size, polydispersity index, release profile and drugs content. In addition, in vitro cytotoxicity, in vitro cell apoptosis, and cellular uptake were evaluated. Results: Liposomes exhibited high drug encapsulation efficiency (>90%) and small size (~ 103 nm). The release profile of doxorubicin and hispolon from liposome was determined by dialysis method. As expected, liposome formulation showed slower release compared to free doxorubicin solution as additional time required for the release of drug from the liposome lipid bilayer. Enhanced cytotoxic effects were noticed between doxorubicin and hispolon in B16BL6 tumor cell lines. Liposome loaded with doxorubicin and hispolon exhibited the highest cytotoxicity against B16BL6 cells. IC50 was 0.05 ÂµM and 0.5 ÂµM for liposome loaded with doxorubicin and hispolon and doxorubicin solution, respectively. On the other hand, liposome loaded with doxorubicin and hispolon also displayed significant increase in cytotoxicity compared to the commercially available liposome formulation (DSPC/Cholesterol/doxorubicin). IC50 was 0.05 ÂµM and 0.1 ÂµM for doxorubicin hispolon liposome and doxorubicin liposome formulation, respectively. Moreover, liposome loaded with doxorubicin and hispolon showed highest cell apoptosis against B16BL6 melanoma cell line. Loading both doxorubicin and hispolon in liposome enhanced cell killing as well as apoptosis more than liposome loaded with doxorubicin alone. Conclusion: Our results showed that such coloading delivery of hispolon and doxorubicin could be a promising therapeutic approach to improve clinical outcomes against melanoma.

3D Tumor Spheroid Models Demonstrate More Realistic Drug Response to Metronomic-like Therapy Compared to Traditional *in vitro* Models

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Purpose: Traditional *in vitro* models growing cells in 2-dimensional (2D) flasks and multi-well plates are a mainstay in drug development due to their ease of use and compatibility with a large array of assay techniques, however, classical *in vitro* studies do not translate adequately to human patients. The development and use of 3D-spheroids better capture the complexity and barrier properties of solid tumors, can be co-cultured with various cell types and can be used for longer drug exposures. Here we show the application of 3D spheroids to determine the activity of anticancer drugs that are administered metronomically, i.e., low-dose frequent drug administration.

Methods: We determined drug potency (IC50) of metronomic *vs* conventional dosing schedules using mitochondrial assays (MTT, Resazurin) in cancer cells using 2D or 3D formats. Human metastatic prostate cancer (PC-3-L-GFP) that are transfected with luciferase (L) and green fluorescent protein (GFP) were grown at 37 C, 5% CO_2 as recommended by manufacture (Perkin Elmer). To simulate conventional "typical" dosing of chemotherapies in human patients, cells were treated after single dose on day one of the study. Metronomic dosing was given daily at a fraction of the conventional dose. Cells were grown using traditional cell culture techniques for the 2D model and a low attachment coating on U bottom plates with Matrigel supplementation to encourage the formation of tumor spheroids was used for the 3D model.

Results: We determined the potency of docetaxel, doxorubicin, vincristine, topotecan, cyclophosphamide, which are all FDA approved anticancer agents. Their activity following metronomic and conventional dosing schedules, comparing 2D to 3D models over multiple time points (3, 7, 14 days).

Conclusion: The shift in drug efficacy demonstrated here using a 3D model may help explain why traditional chemotherapeutic regimens have largely failed to provide consistent and meaningful reductions of tumor volume for human patients. The ability for patients to achieve drug concentrations demonstrated in this study to be necessary for adequate therapeutic response is not possible without extreme toxicity. Additionally, this study suggests that 3D tumor models are more comparable to *in vivo* and clinical data and are more appropriate for the evaluation of *in vitro* drug efficacy and metronomic dosing schedules.

The Effect of *Pseudomonas aeruginosa* on Microglial-derived Exosome Biogenesis and Composition

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Exosomes are comprised of distinct characteristics that exclusively position them as biomarkers. The molecular constituents packaged inside of exosomes allow them to participate in intercellular communication and in the transferring of molecules making them able to help propagate infections in viruses and may be involved in bacterial infections. The goal of our study was to examine the effects of Pseudomonas aeruginosa infection on the biogenesis and composition of exosomes derived from mouse microglia cell line, BV-2. BV-2 cells were cultured in exosome-free media and infected with 0, 1.3 x 10^4, and 2.6 x 10^4 colony forming units per milliliter of Pseudomonas aeruginosa for 24, 48, and 72 hours. This study revealed that BV-2 cell viability significantly decreased after Pseudomonas aeruginosa infection and BV-2-derived exosome concentration decreased significantly in the Pseudomonas aeruginosa-infected group compared to the control group. In addition, Pseudomonas aeruginosa infection increased Let family micro RNAs in BV-2-derived exosomes as compared to the control group. Also, our studies revealed that heat shock protein 70 ($p \leq 0.05$) and heat shock protein 90β ($p \leq 0.001$) levels of expression within exosomes increased after Pseudomonas aeruginosa infection. Overall, our findings suggest that exosome biogenesis and composition was greatly impacted by Pseudomonas aeruginosa infection.

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Formulation of microemulsion containing anti-inflammatory steroidal drug, Difluprednate

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Purpose: Difluprednate has long been used as an anti-inflammatory agent. The aim of this study was to develop a microemulsion formulation for enhanced delivery in ocular or skin disorders.

Methods: Preformulation studies like solubility determination, component selection and pseudo ternary phase diagram construction, were used for optimizing a microemulsion having desirable formulation characteristics. The ternary phase diagrams were constructed with different oil phases (Castor oil, oleic acid), surfactants (Cremophor RH 40 and polysorbate 80), co-surfactant (ethanol) and the ratio of surfactant to co-surfactant (1:1). The formulation was further optimized by using varied water content (20%, 25%. 30%, 32.5%). Different physicochemical properties such as droplet size, pH, and viscosity of the microemulsion systems were measured. A stability indicating HPLC method for difluprednate was established for quantification of difluprednate in the microemulsions. Finally, in vitro drug release of difluprednate-loaded microemulsions was determined and compared with the commercially available product (Durezol®)

Results: The ternary phase diagrams revealed that oleic acid microemulsion has much higher area under curve (AUC) as compared to castor oil (45% versus 34%), which is due to good compatibility of oleic acid with other components in the microemulsion. The water content in the formulation played an important role in the microemulsion stability, droplet size and drug release. The oleic acid based microemulsions with 32.5% water exhibited higher flux than other formulations. The optimized microemulsion was composed of 0.2% difluprednate, 52.3% polysorbate 80 /ethanol (1:1) mixture, 15% of oleic acid and 32.5% of water. This microemulsion has the particle size, 109 nm with narrow size distribution (polydispersity index of 0.263). In vitro release studies revealed a sustained but higher drug release behavior for the microemulsion in comparison to topical difluprednate marketed product.

Conclusion: The sustained drug release pattern observed for the microemulsion as compared to commercially available product, which was probably caused by the different structure of both systems. The results of this study therefore suggest that the microemulsion is a promising delivery system for difluprednate.

Resveratrol Nanogel Formulation for Enhanced Transdermal Delivery across Human Skin

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Purpose: Resveratrol, a natural polyphenol found in grapes and berries, has strong anti-oxidant properties and is believed to be beneficial in treating many disease conditions including skin cancer. This compound is insoluble in many topical vehicles/solvents, leading to problems in developing a topical product. In this study, we presented a nanoparticle based gel (nanogel) formulation and skin permeation data on resveratrol. Methods: The saturation solubility of resveratrol in many topical vehicles such as ethanol, polyethylene glycol 400, LabrfilÂ® 2125, LabrfilÂ® 1944, Lauroglycol FCC, TranscutolÂ®P, LabrasolÂ®, Capryolâ„¢ PGMC, Capryolâ„¢90 was determined. Resveratrol was then solubilized in LabrasolÂ® and added to 1% PluronicÂ®F68 solution under stirring. This resultant suspension was subjected to ultrasonication and high-pressure homogenization to achieve a nanosuspension. The suspension was then gelled using MethocelÂ®K4M (F3). The particle size, drug content, drug release and permeation kinetics of the nanogel formulation were tested. Results: The solubility of resveratrol was the highest in LabrasolÂ®, TranscutolÂ®P, ArlasolveÂ® and polyethylene glycol 400 (>100 mg/ml). However, Labrasol was chosen as a solubilizer for the nanogel formulation because this solvent provided proper viscosity and compatibility with the gel components. The mean diameter of particles in the nanogel formulations F3 120 nm. The F3 formulation showed a much higher amount of drug release (1647.2 Âµg/cm2), which is 1.6 and 1.1 fold higher as compared to F1 and F2 (control formulations), respectively. In the skin permeation study F3 showed a higher flux (1.31Â±0.08 Âµg/cm2/h), which is 12 and 3-fold higher as compared to F1 and F2 respectively. The skin retention of F3 (234Â± 45.9 Âµg/g of skin) was approximately 12 and 5 fold higher as compared to F1 and F2, respectively. Conclusion: An enhancement of resveratrol delivery was observed when nanogel formulation was compared to microparticles incorporated in a gel formulation with the same loaded drug percentage. This enhancement was shown in skin permeation as well as drug retention within skin layers. A high skin permeation in the therapeutically exploitable quantities of resveratrol can be possible when applied the nanogel on a larger surface area of the body.

PLGA encapsulated *Chlamydia* recombinant MOMP nanovaccine subcutaneous prime immunization route enhance immune responses as compared to intramuscular prime immunization route in mice.

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Biodegradable nanoparticles-based vaccines with encapsulated antigen is becoming a popular approach in vaccine development. We have previously developed a PLGA 85/15 (poly (lactic-co-glycolic acid) nanovaccine by encapsulating recombinant MOMP (major outer membrane protein) of *Chlamydia* that induced robust adaptive immune responses and provided enhanced protection against *C. muridarum* (Cm) challenge in subcutaneously immunized mice. In the current study, we compared the immunogenicity and efficacy of priming routes; subcutaneous (sc) or intramuscular (im) followed by two sc-boosts to evaluate serum and mucosal antibodies [Th1 (IgG2a, IgG2b), Th2 (IgG1)] as well as memory and effector T-cells activation after immunization and Cm challenge of mice. We observed significantly high levels of MOMP-specific serum and mucosal antibodies with high avidity especially for Th1 antibodies. A significant increase in Th1 serum and mucosal antibodies with even higher avidity was observed after genital tract Cm challenge in the sc-prime-boost immunized mice. Evaluation of the Th1/Th2 antibody titer ratio revealed that the nanovaccine evoked a higher Th1 and lower Th2 response, which skewed to a dominated Th1 antibody response after bacterial exposure to sc-prime mice. In addition, CD4+ memory ($CD44^{high}$ $CD62L^{high}$) and effector ($CD44^{high}$ $CD62L^{low}$) T-cells proliferation was about two-fold higher in the sc-prime mice. Herein, we reveal that the subcutaneous prime-boost immunization with the nanovaccine was more effective in enhancing the Th1 antibody response and memory and effector T-cell responses against a chlamydial genital tract challenge of mice. Bacterial loads after a genital challenge of immunized mice are being enumerated.

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Influence of Host Circadian Rhythms on Chlamydial Pathogenesis

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Genital Chlamydia infection causes complications such as pelvic inflammatory disease and tubal factor infertility, with some women more susceptible to these conditions than others. However, the effect of circadian rhythms on Chlamydia pathogenesis is unknown. Using a mouse model of chlamydial genital infection, we hypothesize that infections during the day, will cause increased complications compared to infections at night, and that the disruption of circadian rhythms will affect chlamydial pathogenesis. Mice were infected intravaginally with Chlamydia muridarum either at 10:00 am and 10:00 pm. Each group of mice was divided into two subgroups: mice placed under normal 12/12 light and dark cycle, and mice placed under constant light to disrupt the normal circadian rhythm. Infectivity was monitored by periodic vaginal swabbing, and blood/vaginal washes were collected for host immunologic response assessments. The reproductive tracts of the mice were examined for pathological changes, and fertility was determined. Results showed that mice infected during the day shed significantly more Chlamydia and had more pathology than mice infected at night. In addition, mice infected during the day were less fertile compared to mice infected at night. However, for day or night infected mice housed under constant light there was no significant difference in intensity of infection or pathologies. The results suggest that the time of day of infection and circadian rhythm may influence the outcome of chlamydial genital infection and development of pathologies. This represents the first evidence of a possible association between Chlamydia infection complications and the hostâ€™s circadian rhythm.

Analysis of variations within 20S proteasome subunit beta-5 gene in PI-resistant myeloma

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Abstract

Purpose: Multiple myeloma (MM) is the 2nd most common hematological malignancy in USA. Proteasome inhibitors (PI) like Bortezomib (Bz) are often used alone or in combination with other anti-cancer agents to treat both relapsed/refractory and newly diagnosed MM and showed remarkable response. Bz, a boronic dipeptide, specifically inhibits the ATP-independent chymotryptic activity of the 26S proteasome through reversible binding to β5-subunit (PSMB5) of 20S multi-catalytic protease core. This inhibits tumour progression primarily by accelerating unfolded protein response (UPR) in cancer cells, triggering apoptosis. However, despite of an increasing number of approved therapies, MM remains an incurable disease. Bz treatment often achieves only very short-duration responses and drug resistance develops rapidly. Mutations in PSMB5 gene have been proposed as a possible mechanism behind Bz resistance, although the evidences have been conflicting. Bz-resistant human myeloma cell lines (HMCLs) have been shown to harbor PSMB5 mutations, causing conformational changes in the Bz-binding pocket of proteasome that impaired PI binding and decrease in chymotrypsin-like catalytic function resulted into PI resistance. Only recently, clinical studies have reported the increase in mutation frequency in PSMB5 with increasing drug treatment in primary MM. However, there is substantial gap in observation between *in vitro* vs clinical studies pertaining to the discovery of rare variants in PSMB5 vis-à-vis PI resistance.

Methods: Here, we extracted DNA from a large panel of HMCLs (n >60) representing innate and acquired PI resistance to discover PSMB5 mutations associated with drug resistance in MM. All three exons of PSMB5 (Genomic location: chromosome 14:23,016,543-23,035,230 (GRCh38/hg38); size: 18,688 bases; Accession ID: NC_000014) were PCR amplified followed by high throughput bi-directional Sanger DNA Sequencing. Multiple sequence alignment (MSA) of the sequenced data was performed using SeqMan Pro module (DNASTAR Lasergene v16.0) to discover *de novo* and reported variants.

Results: We identified 24 genetic variants within PSMB5 gene. This includes the reported variants rs11543947 (chr14:23034812: G>A), rs769612712 (chr14: 23034874: A>C), rs1470676654 (chr14: 23034944C>T). Rest of the variations are *de novo*.

Conclusions: Currently, we are performing functional genomics analysis on the variants of undermined significance (VUS) to understand the role of these mutations in multiple myeloma drug resistance.

Profiling of immune cell populations in mucosal and systemic tissues of mice following immunization with a VCG-based *Chlamydia* vaccine with and without Flt3L

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Chlamydia trachomatis is a sexually transmitted infection, often asymptomatic and can lead to pelvic inflammatory disease and tubal factor infertility if left untreated. There is currently no commercially available vaccine to prevent *C. trachomatis* infection. Thus, there is need for a vaccine that can protect against infection and prevent *Chlamydia*-associated pathology. We previously showed that the Fms-like tyrosine kinase 3- ligand (Flt3; FL) enhanced the immune responses and protection afforded by a vaccine consisting of *Vibrio cholerae* ghosts (VCG) expressing the chlamydial polymorphic membrane protein D (PmpD) and porin B (PorB) proteins. However, the mechanism(s) by which FL alters the host immune response was not investigated. In this study, we sought to evaluate if differences in the type and number of immune cells infiltrating the mucosal and systemic tissues of mice following rectal immunization could account for the enhanced immune responses observed following co-delivery of vaccine with FL. We hypothesized that immunization with VCG-PmpD/PorB in the presence of Flt3 would result in the induction of higher numbers of specific cell types in the genital tissues of mice. We therefore assessed the differential profile of immune cells infiltrating the spleens, iliac lymph nodes and genital tract tissues of mice following rectal immunization with VCG-PmpD/PorB vaccine in the presence or absence of Flt3 using flow cytometry. The results showed an enhanced number of immune cells present in the spleens, iliac lymph nodes and genital tract tissues of mice following rectal immunization with VCG-PmpD/PorB in the presence of Flt3 compared immunization with the vaccine alone. These results suggest the increased infiltration of immune cells in mucosal and systemic tissues maybe responsible for the enhanced immune responses observed following co-delivery of vaccine with FL.

Utilizing low temperature plasma to morphologically tune carbon black derived from spent espresso grounds

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Purpose: The world consumes about 8.8 million metric tons of coffee annually which produces an exuberant amount of carbon rich biomass. The growing need for carbon nanoparticles, varied in size and shape, encourages the use of techniques to manipulate the chemical activity and structure of said particles. Traditional functionalization requires the use of corrosive chemicals while low temperature plasma is a facile method of etching the surface of materials, therefore activating it. A simplistic method to structurally tune carbon black and biopolymers could resolve processing constraints and lean commercial protocols. The purpose of this work is to investigate the modification of carbon black and biopolymers with O_2 low temperature plasma.
Methods: Spent espresso grounds (SEG) are treated with 5% metal salts of copper, iron, cobalt, and nickel then pyrolyzed at 10°C per minute to 1000°C with an isotherm of three hours. The control SEG produces carbon black while the different metal salts produced various carbon nanoparticles of different degrees of crystallinity. In this study we used 150 RF O_2 plasma at 30cc per minute for varying exposure times to change the surface morphology of the carbon black, poly propylene (PP), and poly methyl methacrylate (PMMA).
Results: The treated samples were analyzed with Raman, SEM, and TGA. Oxygen increases the functionalization of the specimen surface, so when subjected to increased exposure, macropores are filled creating additional secondary bonding which in turn aids in the rigidity of the carbon structure. With increased O_2 exposure, decomposition temperature for PMMA and PP also increased. In the same instance, the increased exposure time is proportional to an increase in structure disorder denoted by the increased Raman intensity of the D' band.
Conclusion: Furthermore, we want to embed the nanocrystalline carbon in PMMA and PP to look for improved mechanical properties. Tunability with respect to carbon black can add to its use as a reinforcement. This tunability in polymers that can lead to increased stability in biopolymers for extended shelf-life or increased degree of degradation for petroleum-based plastics for reduced environmental impact.

Scaffold synthesis for application as vascular grafts

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Abstract

Cardiovascular disease (CVD) is one of the major causes of death in the United States and the leading cause of death globally. This condition can be the result of diseased blood vessels, heart conditions and blood clots etc. Currently bypass surgery is employed as a therapy for the disease. A promising concept is one of a vascular graft, which can be used to replace diseased blood vessels that lead to CVD. Artificial vascular grafts can be synthesized by a range of materials such as polyhydroxyoctanoate(PHO), Polytetrafluoroethylene (PTFE), poly(ε-caprolactone) (PCL) and Poly(glycolic acid) (PGA) etc. These grafts have to be biocompatible, mimic the extracellular matrix and be able to degrade slowly over time. The present studies used Polytetrafluoroethylene (PTFE) scaffolds. These were further treated with Low Temperature Plasma(LTP) to encourage more cell attachment. Human umbilical vein endothelial cells (HUVEC) cells were seeded on PTFE scaffolds. The scaffolds were then visualized using microscopy, we observed growth for up to 10 days. The LTP treated PTFE encouraged more growth than the untreated PTFE. PTFE scaffolds were further treated with fibronectin or collagen to enhance cell attachment. With the addition of fibronectin or collagen, the coverage and growth of HUVEC cells on the surface of the PTFE scaffold increased. We observed more growth with the treatment of fibronectin for up to 15days. We were able to evaluate these results using various types of microscopy, including scanning electron microscope (SEM) and fluorescence microscopy. In the future we look to increase cell attachment and coverage on the PTFE scaffolds, via of protein treatment.

Acknowledgments

This work is supported by the NSF EPSCoR RII-Track-1 Cooperative Agreement OIA-1655280. Funding for this program was made possible through the UAH Graduate Research Assistantship.
Results/Conclusion

Synergistic anticancer effects of 3A.1 and cabazitaxel against prostate cancer

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Purpose: Prostate cancer (PCa) is the second leading cause of non-cutaneous cancer deaths in the United States. The 5-year survival rate for local or regional disease is high, while survival for metastatic disease is 28.7%. Many patients eventually develop the more aggressive form, metastatic castration-resistant prostate cancer (mCRPC). Clinical effectiveness of FDA approved drugs docetaxel (DTX) and cabazitaxel (CBZ) has been hampered by serious adverse effects and emergence of drug resistance. Alternating treatments with first line chemotherapy in combination with new drugs may improve efficacy in PCa. Previously our lab reported andrographolide analogue 19-tert-butyldiphenylsilyl-8,7-epoxy andrographolide (3A.1) effectiveness and proposed mechanism of action in colon cancer. In this study, we investigated the effects of 3A.1, andrographolide alone or in combination with DTX and CBZ against a variety of aggressive human prostate cancer cell lines (PC-3, PC-3M, Du145 and 22Rv1).

Method: Cells were treated with CBZ, DTX, andrographolide and 3A.1 as single agent and in combination for 48 and 72 hr over a broad concentration range; the effect of drug exposure and concomitant therapy was determined used SRB and MTT, classical assays of *in vitro* cytotoxicity. The combination study for both drugs was analyzed by Calcusyn software to determine combination index (CI) and dose reduction index (DRI) to identify synergism and reduce dose for first line chemotherapy drug. Further, we evaluated the effect of treatments on changes in protein expression of important cancer pathway genes by immunoblotting to understand mechanism of action of 3A.1.

Results: 3A.1 alone exhibited dose- and time-dependent antitumor activity in PC3. Co-treatment of 3A.1 with CBZ (at its IC_{50}) 9.5-fold and co-treatment of CBZ with 3A.1 (at its IC_{50}) 18- fold significantly increase activity for 48 hr. All CI values of drug combination were less than one, indicating that co-treatment of 3A.1 and CBZ inhibited cell growth in a synergistic manner. DRI values also indicated that concomitant drug concentration required to achieve estimated potency was reduced ~2-fold for CBZ. Overall, our *in vitro* testing revealed that co-treatment of 3A.1 and CBZ had synergistic growth inhibitory effect against prostate cancer which suggest that the 3A.1 may be useful at increasing anticancer efficacy of CBZ for PCa.

Effects of Plasma Treatment on the Mechanical Properties of 3D Printed FDM Parts

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This study evaluated the use of different plasma treatments on the chemical and physical changes of polymer filaments used to fabricate parts by additive manufacturing. Polycarpolactone (PCL) resomer filaments were treated in a plasma system with various gas sources, powers, and treatment times. The purpose of this project is to investigate the effect of plasma treatments as an effective means to producing stronger 3D printed parts. Fused deposition modelling (FDM) is a popular additive manufacturing technique due to its capability to produce customized parts at a low cost and in a very short time period. Although this is a very useful fabrication method, the FDM printed parts are typically weak in the layer deposition direction (Z-direction) due to insufficient interlayer bonding. The inherent weaknesses limits FDM as a fabrication technique to manufacture 3D printed medical devices in comparison to other processes such as injection moulding. Plasma surface modification is a technique used to functionalize surfaces, improve surface energy and reduce the contact angle by introduction of plasma on the surface. This process can be used as a potential treatment to enhance the interlayer bonding by altering the surface properties and chemical make-up of filament surfaces for 3D printing without changing the bulk properties of the overall material. This study used various sources of plasma treatment to investigate the effects on the mechanical properties of 3D printed FDM parts

One-pot Template-Free Synthesis of Polydopamine Nano-Bowl

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Polydopamine has similar structure to melanin and has excellent biocompatibility. Abundant catechol and aromatic groups endow polydopamine with unique deposition and adhesion properties. There have been many reports of synthesis of polydopamine nanospheres and their application to fields such as biomedical, energy, environmental, etc. But compared with nanospheres, nanobowls with concave structure is expected to give full play to the high specific surface area and high packing density advantages of nanomaterials. Therefore, it is of great interest to study the synthesis of polydopamine nanobowls. However, due to the complexity in fabrication, there are few reports of polydopamine nanobowls or their properties. Currently, the fabrication of asymmetric bowl-like nanoparticles typically utilizes templates such as polystyrene or poly(acrylic acid) nanoparticles, emulsions, etc. The introduction and removal of template complicates the preparation process and can lead to issues in biomedical applications. We report a one-pot template-free method for the rapid synthesis of polydopamine nanobowls. The reactants are simple, dopamine chloride and Tris buffer in an alcohol/water medium, and polydopamine nanobowls are obtained through centrifugation after one hour. Size and morphologies of obtained nanobowls obtained via transmission electron microscopy, scanning electron microscopy, and dynamic light scattering are reported. The effect of reaction conditions on morphology is discussed. An interfacial mechanism for the formation of the bowl is proposed.

Effect of oxygen plasma treatment on Polycaprolactone for tissue engineering applications.

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Purpose: polycaprolactone (PCL) exhibits many advantages like good biodegradability, biocompatibility, excellent processability, and acceptable mechanical strength. Therefore, PCL has many applications particularly in the field of tissue engineering and Nano-medicine. However, low wettability, insufficient biocompatibility and mechanical strength of PCL restrict its application in tissue engineering. The aim of present work is investigate effect of oxygen plasma treatment of PCL pellets on improving the properties during melt extrusion.

Methods: CAPA (PCL) pellets were oxygen plasma treated using a Radio Frequency generated plasma source of 150W for different time periods (5, 10 and 20 minutes). The effect of plasma treatment was characterized using Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and contact angle measurements. The surface treated pellets were then melt mixed using twin screw compounder. The samples were then tested for crystallinity change using Differential scanning calorimetry (DSC) and X-Ray Diffraction (XRD) studies. Thermal degradation behavior was investigated via Thermo gravimetric analysis (TGA).

Results: FTIR results confirmed increase in the presence of oxygen related surface functional groups, XPS results confirmed increase in surface energy due to the oxygen plasma treatment. Contact angle measurements showed increase in wettability of sample with a decrease of about 11 degrees in contact angle with water droplet. XRD results revealed that the crystallinity decreased with plasma treatment. DSC which is a more conventional method of testing crystallinity did not show any significant change in crystallinity of the material. There was very little effect on thermal degradation behavior of melt extruded samples due to plasma treatment which was confirmed by TGA.

Conclusion: Further increased plasma treatment time could result in increased crystallinity. Another approach could be to increase the surface area of the polymer being treated before extrusion. It can be concluded that plasma treatment has potential to increase the desirable properties of polymers particularly in the areas of research where chemical modifications are not desirable.

2019 UNDERGRADUATE Abstracts

Macro and Nano Mechanical Properties of the Graphene Nanoplatelets/ Epoxy Nanocomposites

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Epoxy based nanocomposites are extensible investigated by the researchers because of their high specific properties and extraordinary performance as structural component in the transportation industries. In this work we have investigated the macro and Nano mechanical properties of graphene Nano-platelets (GnPs) reinforced epoxy nanocomposites. SC-15 epoxy resin was used to fabricate the nanocomposites with different percentage of GnPs loading (0.05%, 0.1%, 0.3% and 0.5%). The GnPs was dispersed following multiple dispersion methods. The dispersion and orientation of GnPs in the epoxy resin will be investigated using X-ray diffraction (XRD). 3-point static flexure test will be performed to investigate the macro mechanical properties. To characterize the Nano mechanical properties, Nano-indentation test will be performed on the neat and all the four types of GnPs/epoxy systems. The macro-modulus and strength obtained from the static flexure test and the Nano-modulus and creep compliance obtained from the Nano indentation test will be compared and analyzed. Scanning electron microscopy (SEM) of the flexural fractured samples also will be conducted to investigate the microstructure and fracture mode of the neat and GnPs/epoxy nanocomposites.

Synthesis and characterization of poly(hydroxyethyl methacrylate) hydrogels for drug delivery applications

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Synthesis and characterization of poly(hydroxyethyl methacrylate) hydrogels for drug delivery applications Dell Zimmerman, Armel Boutchuen, Soubantika Palchoudhury* Civil and Chemical Engineering, University of Tennessee at Chattanooga, Chattanooga, Tennessee Abstract Drug delivery system is a major component for the success of nanoparticle based targeted therapy for next-generation medical solutions. The drug delivery agent should be non-toxic, easily up taken by the patient, and capable of controlled release of the drug at the targeted site. Hydrogels are ideal candidates for this purpose as they are composed of crosslinks of hydrophilic biocompatible polymers with the capability to swell in water. In this research, the synthesis of poly(hydroxyethyl methacrylate) (p-HEMA) hydrogels via chemical crosslinking is systematically investigated. The pH-dependent swelling properties of the hydrogels are investigated over a period of 48 h. The surface properties of the series of p-HEMA hydrogels is characterized using scanning electron microscopy, fourier transform infrared spectroscopy, and ultraviolet visible spectroscopy. The capability of the p-HEMA hydrogels to retain and release iron oxide nanoparticles is also investigated in this study using dynamic light scattering and ultraviolet visible spectroscopy. This study will be highly significant for designing new and more efficient drug delivery vehicles.

Thanatomicrobiome in Liver Samples of American and European Cadavers

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Is there any life after death? In many ways, there is. For this reason, there are continuing studies on the microbial lifeforms on and in corpses that may potentially help solve crimes. Human postmortem microbiome research has shown that microbes proliferate in a decaying body and are the primary mediators of decomposition. The source and diversity of microorganisms in regard to geographical factors in which corpses are found have yet to be elucidated after death. Next generation sequencing of 16S rRNA gene amplicons is used in forensic microbiology as molecular biomarkers for the classification of bacteria. Upon death, the immune system in the human body that occur during life stop, and bacteria start to decompose the body. Presumably, geographic location plays a role in postmortem microbial signatures in internal organs, namely the liver). We hypothesized that as a human body decays, microbiome communities within internal body sites will be analytical of the originating country after death. The objectives were to analyze the microbiome of the liver of European and American cadavers. To assess this hypothesis, liver samples were collected from 130 corpses from two countries with postmortem intervals ranging from 3 hours-11 days. PCR and high-throughput DNA sequencing that targeted the V4 region of the 16S rRNA gene using bacterial primers 515F-806R were performed. We used the standard bioinformatics pipelines (QIIME), to identify the most predominant bacteria that correspond to each location. The results revealed that there were statistically significant differences ($p < 0.001$) between locations (Europe versus the United States). Also, this study demonstrated that there was a lack of Clostridium spp. in the European corpses. In conclusion, these results established that the effect of distinct geographic variability in determining the different postmortem microbial signatures provide data that may potentially build forensic models that can predict the origin of bodies recovery for discrete locations.

SCAFFOLD DESIGN USED FOR SKIN TISSUE ENGINEERING

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Abstract

Tissue engineering has been developed as a new system for repairing damaged or diseased tissues to overcome the limitations of current therapies. Aliphatic polyesters, such as polycaprolactone (PCL) and polydioxanone (PDO), have been commonly utilized in biodegradable scaffold. The combination of these polymers, by copolymerization and electrospinning, enables a range of mechanical properties and degradation rates which can be used for biomedical procedures. Likewise, hydrogels are gaining lot of usage in biomedical applications for wound healing, cartilage and bone regeneration due to their biocompatibility and similar properties to natural tissue. In the present study, PDO-PCL scaffolds were synthesized using the electrospinning technique. Likewise 2% and 4% Alginate hydrogels were synthesized by crosslinking with 2% calcium chloride. The polymer scaffolds and hydrogels were individually tested for their mechanical properties along with skin cells: keratinocytes and fibroblast alone and in co-culturing; keratinocytes seeded on top of fibroblast, for compatibility and proliferation. Differential scanning calorimetry (DSC) and dynamic transition analyzer analysis were performed to confirm proper tensile properties suitable for tissue environment. DSC analysis shows PDO with a melting point at 99.80º and glass transition at 76.61 J/g. PCL scaffold with a melting point at 57.56ºand glass transition at 79.15 J/g showing a 3-5º decrease between PDO polymer sample versus when individually sampled. But no significant changes with PCL polymer samples. Cell viability and cell proliferation of each hydrogel scaffold and electrospun scaffold were evaluated over a course of 28 days. MTT cell proliferation assay was done to measure the cell viability on these scaffolds. Neutral red dye uptake and DAPI (4′,6-diamidino-2-phenylindole) staining confirmed the growth of cells on these scaffolds. Results show viability of keratinocytes and fibroblast at 80% up to 28 days and co-cultured cells at 60% up to 28 days. SEM showed confirmation of cell adhesion and growth on scaffolds for up to 28 days. Overall this study aims on identifying various biomaterials which can be used for skin tissue engineering and their several applications in the field.

Selective Expression Patterns of Myosin Light Chain Kinase Isoforms

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Myosin, as well as Actin, are both fibrous proteins that conjointly work together to form contractile filaments of muscle cells. Additionally, both proteins are also interchangeably responsible for the motility in other types of cells. Myosin Light Chain Kinase (MLCK) is a calcium/calmodulin-dependent serine/threonine enzyme that belongs to the immunoglobulin family and phosphorylates the regulatory light chain of myosin, which facilitates myosin binding to actin and aids in contractility. When phosphorylation occurs, this regulates a number of myosin motor activities, which include cell division, muscle contraction, cell motility, and cell adhesion. There are two isoforms of MLCK: long form, also considered the non-muscle form, has a molecular weight of 200-220 kDa. The shorter form, which is found in smooth muscle tissue, has a molecular weight of 100-150 kDa. Though the role of MLCK in contractile tissues is well-established, comparatively less is known about its contractile function in non-muscle cells, including its specific role in inflammatory conditions. Essentially, the overall goal of our research is to determine the molecular and biochemical mechanisms of myosin light chain kinase in the cell-to-cell spread of specific pathogenic bacteria in human cells and in tumor cell migration. The main objective of this investigation is to determine how MLCK is engaged, its utilization, and the consequences of that engagement, in terms of examining inflammatory conditions. Therefore, to address the hypothesis that different pathologic conditions may preferentially express a specific MLCK isoform or both isoforms, we have screened several cancer cell lines from various tissues using western blot and immunoprecipitation to determine which isoform is present. *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).*

Inhibition of *Streptococcus pneumoniae* using metallic nanoparticles

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Streptococcus pneumoniae is a gram-positive, facultative anaerobe most commonly known to cause infection in children under the age of 5 and the immunocompromised. Over the years *S. pneumoniae* has become exceedingly resistant to the common antibiotics used as therapeutics, including penicillin. In our study, we used strain D39, a historically relevant pneumococcal serotype 2. D39 is extremely virulent and frequently utilized in pneumococcal pathogenesis. In recent years, nanotechnology was identified as an emerging field employing nanoparticles as drug delivery systems. Here, we examined a variety of different nanoparticles that can potentially aid in treatment of *S. pneumoniae* infection. The particles used were zinc oxide in water 40nm APS, 20nm silver nanospheres citrate, and 50nm copper oxide nanoparticles. Zinc oxide nanoparticles were found to be more efficacious against *S. pneumoniae*. The minimum bactericidal concentration (MBC) was determined at 0.1µg/ml. The antimicrobial activity of zinc oxide nanoparticles was examined against *S. pneumoniae* in liquid and solid media, as well as against biofilm formation. Zinc oxide nanoparticles are classified as N; R50-53 (ecotoxic) in traces when found in mammals. The human body contains approximately 2-3 g of zinc; therefore, these inorganic metal oxide particles can be safely used as an antimicrobial agent against pneumococcal infections. *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).*

Composite Scaffolds Synthesis for Tissue Engineering

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Tissue engineering has been innovative for repairing damaged tissues. Hydrogels are important in biomedical applications for wound healing due to their biocompatibility and similar properties to natural tissue. Hydrogels mimics the extracellular matrix and high-water content makes them porous which helps in diffusion of nutrients and enhance tissue growth. This research represents a variety of hydrogel scaffolds designed to test fibroblast cell growth for skin tissue engineering. Alginate scaffolds (2%) were synthesized and cross-linked with 2 M calcium chloride ($CaCl_2$). Chitosan scaffolds (1%) were prepared and cross-linked with 5% NaOH. Alginate/Chitosan hydrogels were synthesized by soaking 2% alginate hydrogels in 1% chitosan solution overnight. Alginate/Collagen hydrogels were prepared by soaking 2% alginate scaffolds in 1mg/ml collagen solution overnight. Scaffolds were UV sterilized and soaked in media until ready for cell seeding. Degradation and swelling ratio studies on scaffolds were done to test stability. For swelling ratio, scaffolds were incubated with media for 13 days, after 9 days no weight change was observed. For degradation test, scaffolds were incubated for 13 days with media and collagenase (1mg/ml). After 7 days, no weight change was observed. Degradation and swelling ratio studies confirmed scaffolds stability for cell growth. The viability of cells on the scaffold was assessed by using the MTT assay. In addition, fibroblast cells growth was imaged using microscopy and DAPI staining. Our results show that the alginate-based hydrogel scaffolds provide a good substrate for cell growth. MTT assay results showed the cells viability and proliferation on the scaffolds increased over 14 days. Microscopy studies further confirmed the growth of fibroblast on hydrogel scaffolds. For future studies, the 3D skin model is under progress to study the skin regeneration for co-cultured cells on hydrogel scaffolds. *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).*

2019 Abstracts (Post-Docs, Faculty, etc.)

Toward Forensic Drug Detection using Nanotechnology

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In 2016 NanoBio Summit we have reported our work on forensic drug detection in fingerprint samples using nanotechnology in one of our laboratories in 2014-2016. The previous results showed that combination of nanotechnology and spectroscopy is a promising and informative tool for the detection of illegal substances cocaine, cocaethylene, norcocaine, ecgonine methyl ester, cannobinol, and flunitrazepam. We have used four different types of fingerprint powders and four types of nanoparticles for preparing fingerprint samples. The two procedures for detection of cocaine in the simulated fingerprint samples are effective. The chemical interactions between the cocaine and gold nanoparticle were investigated and summarized in a possible model. The future efforts may use functionalized nanoparticles and investigate the potential improvement in detection of forensic drugs using these nanoparticles. In this work we explored the possible chemical analysis of cocaethylene using DNA nanostructures. The hypothesis of this work is that the infrared spectra of samples containing drugs and nanoparticles should provide the structural information of the drugs on nanoparticles. The DNA nanostructures is two-dimensional crystalline form of DNA (Winfree et al 1998). The samples are 200 uL DNA DX 2D arrays (DNA: 0.5 uM) in TAE/Mg buffer. The volume of 2 uL DNA nanostructures in the absence and presence of cocaethylene are deposited in the diamond window and air-dry. The FTIR spectra were recorded. The FTIR data showed a blue shift of benzene from 720 to 717 cm^{-1}, suggesting that the binding site of cocaethylene is likely phenyl group. In addition, the change of C=O ester IR peak at 1745 cm^{-1} to 1735 cm^{-1} of cocaethylene caused by adding DNA DX 2D array nanostructure was observed. These changes of IR provide insightful information on the interaction between cocaethylene and DNA nanostructures and might be valuable for design of new DNA nanomaterials for detection of forensic drugs. As the purpose of this work is to develop novel methods in forensic drug analysis using nanotechnology, the experimental data might provide potential useful and specific information in assessing whether the fingerprint suspect individual is an illicit drug dealer or even a drug abuser.

Repurposing Clofazimine as a novel drug against PI-resistant stem cell-like subclones in myeloma

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Purpose: Multiple myeloma/MM, the second-most common hematopoietic malignancy in USA, remains a difficult-to-cure disease due to differential drug response/resistance. Cancer stem cell-like sub-populations/MM-CSCs including $CD19^{-}CD138^{-}$ quiescent stem cells, dormant cells, ALDH+ cells and side populations/SP may significantly contribute towards resistance to proteasome inhibitors/PIs, the standard-of-care drug for MM. However, no study so far has attempted to develop drugs specifically targeting drug-resistant MM-CSCs. Further, the gene signature underlying these sub-cellular populations are yet to be revealed. We have previously demonstrated that Clofazimine/CFZ, an anti-leprosy drug, could be successfully repurposed for the treatment of Chronic myeloid leukemia/CML in drug-resistant patients. Here, we hypothesized that CFZ can be used to treat PI-resistant MM by targeting the sub-cellular MM-CSCs.

Method: A panel of >50 human myeloma cell lines/HMCLs representing innate PI-response/resistance and >10 pairs (parental and PI-resistant lines generated using dose escalation over a period of time) of clonally-derived PI-resistant HMCLs representing acquired resistance were treated with CFZ as single agent or in combination with PIs and *in vitro* cytotoxicity was determined using CellTiter-Glo assay. Synergy between CFZ and PIs was analyzed by Calcusyn software based on Chou-Talalay's combination index (CI) theorem. Tumorigenic potential of purified MM-CSCs was determined using colony forming assay, carboxyfluorescein succinimidyl ester (CFSE) assay and analysis of side population.

Results: CFZ alone showed potent inhibition of cell viability, while CFZ + PIs significantly improved the therapeutic index of PI administration to the cells, including P vs VR lines. Next, using flow cytometry, we found that CFZ alone or CFZ + PI significantly reduced the number of quiescent $CD138^{+}$ cells in P vs VR lines and increased CFSE-dim (dividing cell) population. Evaluation of apoptosis in these cells revealed that CFZ alone caused apoptosis in both CFSE-bright and CFSE-dim cells while combining CFZ+PI caused a more robust effect amounting to their near obliteration. Further, %side populations/SP was found higher in PI-resistant cells as compared to parental cells. CFZ alone or CFZ+PI significantly reduced %SP in PI-resistant cell lines.

Conclusion: Based on these results, we propose that CFZ may have strong potential as novel secondary drug against PI-resistant MM-CSC subclones in MM.

Targeting EMT in metastatic breast cancer.

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Delicate balance between cellular plasticity and differentiation is critically maintained during mammary gland development. Disruption of this balance leads to breast cancer initiation and metastatic progression. Recent findings from our lab have revealed that N-Myc and STAT Interactor (NMI) protein is decreased in 70% of primary patient specimens with metastatic breast cancer. Mammary specific Nmi knock out mouse model revealed that conditional Nmi loss disrupts luminal differentiation in the mammary gland affecting alveologenesis and prompted the progression of tumors with aggressive metastatic characteristics. The tumor cells that lack NMI consistently demonstrated elevated attributes of mesenchymal like invasive phenotype confirmed by 2D and 3D morphology, as well as molecular profiling for EMT markers.

Breast cancers cells have wildtype NMI gene; but it fails to express. We undertook a high throughput screen (HTS) of 200K-small molecule compounds, to discover activators of NMI expression. The underlying hypothesis was that by restoring NMI expression, we will be able to successfully curb the invasive, mesenchymal like phenotype of breast cancer cells. In this presentation we will also share the results of the screen as well as validation for biological activity of the leads from the HTS. Our future goal will be to explore effect nano-technologic tools to develop targeted treatment of metastatic breast cancer.

Liposomes for wound healing

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Liposomes are microscopic vesicles that consist of a phospholipid bilayer with an aqueous solution on the inside of the vesicles, varying in size from being a unilamellar (SUV) to multilamellar vesicle (MLV). Research presented here describes the potential of liposomes alone as well as liposome-encapsulated with the therapeutic agent in the process of wound healing. MLVs were produced using conventional thin-film hydration method and transformed into SUVs using probe sonication. The size and surface charge of the empty liposomes was 116.2 ± 2.2 nm and 1.1 ± 0.1 respectively. However, there was slight reduction in size after encapsulation of the therapeutic agent where the size and surface charge of the liposomes was 84.8 ± 0.6 nm and -1.1 ± 0.2 respectively. The percent encapsulation of the therapeutic agent was determined using UV-visible spectrophotometry and was 13.2 ± 1.3 %. MTT assay was used for epithelial cell toxicity in the presence of empty as well as therapeutic agent encapsulated liposomes. Both empty as well as therapeutic agent encapsulated liposomes were found non-toxic to the epithelial cells (fibroblasts and keratinocytes). Fluorescence staining was performed to compare epithelial cell proliferation in the absence and presence of empty liposomes as well as liposomes encapsulated with the therapeutic agent. Confirmation of the cell proliferation results of fluorescence imaging is currently in progress using flow cytometry.

In silico prediction followed by *in vitro* validation identifies YM155 and FK866 as potent secondary drugs for the treatment of docetaxel-resistant prostate cancer

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Abstract

Purpose: Response to anti-cancer drugs is heterogeneous. All patients do not respond equally well to treatment (innate resistance) and those who do often acquire resistance over the course of treatment (emerging resistance). Therefore, identifying secondary drug regimens to circumvent drug resistance is essential for more effective alternative/combination therapy strategies. We have combined a novel greedy algorithm-based set-covering computational optimization method with a regularization technique (secDrug) on sex-hormone related human cancer cell line subtypes (including breast, cervical, endometrial, ovarian, prostate, testicular, vulval cancers) from the Genomics of Drug Sensitivity in Cancer (GDSC) database, the largest public database of drug sensitivity in a vast array of human cancer cell lines, to identify potential secondary drug combinations against cancers resistant to standard-of-care drugs.

In this study, our aim is to validate these *in silico* predictions using *in vitro* chemo-sensitivity assays using our panel of solid-tumor cell lines representing patient diversity in treatment response/resistance.

Methods: To evaluate the potency of predicted secondary drugs in circumventing drug resistance in Prostate Cancer (PCa) cell lines, we treated the PCa cell lines with the top predicted secondary drugs, alone or in combination with the FDA-approved prostate cancer drugs, Docetaxel, Cabazitaxel and Enzalutamide. *In vitro* cytotoxicity assays were then performed to calculate the half maximal inhibitory concentration (IC_{50}) of single-agent treatment and to evaluate drug-drug synergy of combination treatments using Chou-Talalay's combination index (CI) method and the isobologram algorithm.

Results: Our results showed that the *in silico* predicted top secondary drugs, YM155 and FK866, exhibited potency in a panel of PCa cell lines (n=12), alone and in combination with CI values exhibiting high levels of synergistic effect. Currently, we are performing single-cell and bulk gene expression profiling (GEP) analysis using next generation sequencing methods to identify differentially expressed (DE) genes and pathways associated with successful drug combinations.

Conclusions: Combining *in silico* and *in vitro* approaches, we have thus identified novel potent secondary drugs in drug-resistant prostate cancers. Ultimately, we aim to create a universally applicable software application for predicting and functionally validating unique secondary therapies in drug-resistant cancers for any cancer type and any test drug.

Multi-omics analysis of conventional vs metronomic dosing in prostate cancer risk groups

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Abstract

Purpose: Repetitive, low-dose drug administration (metronomic/METRO) has the potential to overcome drug resistance and increase efficacy in many cancers; however, the mechanisms mediating the increased efficacy are not understood fully. We previously showed that METRO dosing of Topotecan/*TOPO* was more potent than conventional/*CONV* dosing in prostate cancer cell lines and xenograft mouse models. Targeted mRNA and microRNA expression studies performed to explore possible mechanisms by which METRO dosing alters tumor growth, and identified potential candidate cancer pathway genes, miRNAs and miRNA-mRNA pairs as treatment-related biomarkers for *TOPO-METRO* therapy.

Methods: Androgen-independent (PC3, PC3-M, DU-145) and androgen-dependent (LNCaP, 22Rv1) human prostate cancer cell lines were treated with *TOPO* following *CONV* and *METRO* dosing schedules. Cancer pathway genes and miRNA expression profiles were assessed at the calculated IC_{50} of *TOPO*. Expression signatures were identified using differential expression analysis and a Spearman's rank-based correlation was used to assess associations between drug response, mRNA and miRNA expression. Gene signatures associated with *TOPO-METRO* therapy were queried against patient profiles in The Cancer Genome Atlas/*TCGA* database. Protein expression of most significant genes was confirmed by immunoblotting.

Result: We identified disparate treatment-related mRNA and miRNA expression signatures and a gene signature (top five genes: SERPINB5, CDKN1A, TNF, FOS, ANGPT1) for TOPO-METRO vs CONV treatment. Further, upregulation of tumor suppressor, anti-proliferation (eg, SERPINB5), activation of the immune system (RPL13A) and down-regulation of genes involved in apoptosis, invasion, metastasis, and inflammation (TNF, FOS, MMP1) are associated with the observed treatment response. MicroRNA expression changes may influenced differential gene expression (miRNA-mRNA) for *TOPO-METRO* dosing. Twenty miRNAs genes (including miR-30c, miR-19a, mir-20a) were associated with *TOPO* cytotoxicity; seven of these bind to 28 mRNAs as mRNA-miRNA pairs (TargetScan) ($p<0.05$). Furthermore, expression of our top genes correlated with patient survival. Interestingly, MMP1, B2M, CXCL8, PDGFA, ERBB2, ITGA1, ITGA3 and JUN was associated with survival in African American ($p<0.05$). Immunoblotting results for top genes corroborated our mRNA expression results.

Overall, these studies address a fundamental gap related to the effects of METRO dosing on key miRNAs, genomic and transcriptomic factors and treatment efficacy. Using a multi-omics approach, we determined gene signatures may be used to individualize patient therapy.

Title: Identification of gene signature for aggressiveness in prostate cancer with Pan-Cancer perspective and influence of metronomic dosing on this GEP signature

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Purpose: Prostate cancer (PCa) is the second leading cause of non-cutaneous cancer related deaths in the United States. The 5-year survival rate for regional disease is high, whereas for distant disease is 28.7%. Many patients develop "metastatic castration-resistant prostate cancer" (mCRPC). Conventional treatment of aggressive PCa with docetaxel or cabazitaxel alone or in combination with other drugs and immunotherapy increases survival rates slightly (median overall survival < 1-year). Current scenario indicates that mCRPC patients in greatest need for new, more effective treatment options that improve survival or delay disease progression. Alternate drug administration schedules such as metronomic dosing (METRO; low-dose continuous exposure of drug) are another approach to increase drug efficacy in many cancers, including prostate. To understand and identify the appropriate target for therapy, we performed mRNA expression studies and identified potential candidate cancer pathway genes as biomarkers for aggressive cancer.

Methods: Expression signatures were identified using differential expression analysis software. Identified Gene signatures were validated in TCGA prostate cancer patient's cohort. Furthermore, we studied significance of top gene in other cancers against patient profiles by systematic Pan-Cancer analysis across The Cancer Genome Atlas (TCGA). Further, protein expression of top significant gene were evaluated by immunoblotting.

Result: Top ten significant gene PLAU, TGFB1, SERPINE1, MET, TIPM1, ITGA3, SERPINB5, PLAUR, CDKN1A, IGF1ware significantly associated with Gleason score and patient survival with PCa patients ($p<0.0001$). PAN-Cancer result showed top genes were significantly associated with other cancers ($p<0.001$) such as PLAU (testicular, skin, lung, Pancreatic, brain cancer), TGBF1 (Bladder, Cervical, testicular cancer), SERPINE1 (Bladder and Cervical cancer), ITGA3 (Endometrial, cervical cancer), TIPM1 with Bladder cancer, SERPINB5 with Endometrial cancer, PLAUR with cervical cancer, CDKN1A with bladder and IGF1 with ovarian cancer. Additionally, Ingenuity Pathway Analysis identified activation of angiogenesis pathway was a one of a key factor for cancer aggressiveness. We also determine the differential gene and protein expression level of these top genes in AAM vs EAM Cell Lines as well as patient cohort. Finally we also exhibited effect of metronomic treatment on top genes, likely played a crucial role in aggressiveness. Using multi-omics approaches we determined gene signatures that play critical roles in diseases aggressiveness and drug sensitivity.

Development of nano bullets programmed with in vivo selected RNA aptamers and capable of delivering high payloads of anticancer drugs to obesity-linked human colorectal cancer tissue in an orthotopic mouse model

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Purpose: Colorectal cancer (CRC) is the third leading cause of cancer mortality worldwide. The epidemiological evidence highlights a strong link between CRC and obesity. Chemotherapy remains the basis of treatment for CRC. Its side effects can be brutal, with some patients succumbing to the adverse effects of the drugs. To minimize these effects, we developed nanoparticles that can deliver high payloads of daunorubicin exclusively to the CRC tissue.

Methods: The key components of our technology are aptamers, small RNA molecules that bind with high specificity to target molecules. Usually they are created *in vitro* via SELEX method. We hypothesized that the aptamers selected *in vivo* will be better equipped to avoid binding to the plethora of non-target cells found in a living organism.

Daunorubicin is a drug used to treat many cancers. It intercalates at the GCA sequences in the double-stranded DNA. We engineered DNA duplexes that are able to bind seven daunorubicin molecules and conjugated them to gold nanoparticles using a combination a sulfur chemistry and polymerization catalyzed by the Klenow fragment of DNA polymerase I.

Results: Using *in vivo* SELEX experiments, we identified three CRC-specific RNA aptamers. Two of them are able to fold into very stable pseudoknots and crosslink, upon UV-irradiation, to a yet unidentified human 58 kDA protein. We functionalized gold nanoparticles with both the daunorubicin-binding DNA duplexes and the CRC-targeting RNA aptamers. Using a chemical approach only, we were able to attach only 62 "preformed" DNA duplexes to 15 nm gold nanoparticles. In contrast, using our enzymatic approach, we were able to attach 149 DNA duplexes to 15 nm. The increased packing density of DNA duplexes on the nanoparticles, which can bind ~800 daunorubicin molecules, we attribute to the 3' exonuclease activity of the Klenow enzyme which yields DNA duplexes with the receded 3' ends.

Conclusions: Our ability to use SELEX for quickly developing novel non-toxic cancer-targeting therapeutics such as RNA aptamers will pave the way for detection and treatment not only for CRC but also for other cancers. When perfected, our technology is expected to have significant impact on personalized medicine.