Science, Technology and Research Scholars
STARS

Annual STARS II Research Symposium

Yale

Rosenfeld Hall
109 Grove St., Room 101
April 27, 2017

Annual STARS II Research Symposium
Thursday, April 27th, 2017

5:40 p.m. **Opening Remarks and Presentation of Certificates**
Dr. Kenneth Nelson & Sara Katrancha

Featured Presentations

5:45 p.m. **Ultra-small Nanoparticle Formation for Targeted Drug Delivery to Glioblastoma Multiforme**
Cesar Garcia
Department of Neurosurgery-YSM

6:00 p.m. **Sub-diffusive Motion of Tracer Particles in Models for the Cytoplasm of *E. Coli* Cells**
Alizeh Maqbool
Department of Mechanical Engineering & Materials Science-YSEAS

6:15 p.m. **Functional Characterization of a Novel Ferroportin Mutation Identified in Autosomal Dominant Hemochromatosis**
Jihad Womack
Department of Pathology-YSM

6:30 p.m. **Dinner & Poster Presentations**

Ultra-small Nanoparticle Formation for Targeted Drug Delivery to Glioblastoma Multiforme

Cesar A. Garcia[1, 2], Dr. Jiangbing Zhou[3]

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Glioblastoma Multiform (GBM) is the most common and malignant form of brain cancer, where the median survival rate for diagnosed patients receiving optimal care is 14 months. Current treatments for GBM are highly invasive, so it is necessary to develop alternate forms of treatment. One option is to deliver drugs to the brain, but a key problem in targeting brain tumors lies in the difficulty of bypassing the blood brain barrier (BBB), a highly regulated system of tightly packed cells in neural capillaries that control the substances that enter the brain. Approaches using engineered nanoparticles (NP) to deliver drugs have shown promising results in bypassing the BBB. Over the past few years, the Zhou lab has developed several formulations of nanoparticles with diameters ranging from 100-150 nm, and that are surface conjugated with targeting ligands for efficient drug delivery to brain tumors. We hypothesize that nanoparticles with smaller diameters will have enhanced efficiency in bypassing the BBB and targeted drug delivery. To test this hypothesis, we have proposed the development of ultra-small (<60 nm) PLGA-PEG nanoparticles conjugated with the small molecule ligand Gambogic Acid (GA) to act as a drug carrier. GA was selected as the targeting ligand because it has high affinity with Transferrin receptor (TfR), which is upregulated on the BBB and in brain tumors and was found to enhance nanoparticle delivery. NP's were prepared by first forming polymers of PLGA-PEG conjugated to GA with amine-based chemistry (PLGA-PEG-GA), and then performing nanoprecipitation under rotary evaporation with varying conditions. The resulting particles were frozen, lyophilized, collected, and imaged under SEM. Our results show that under most conditions average particle size was larger than 100 nm, but NPs prepared with a mixture of a 5:5 ratio of PLGA-PEG-GA polymer to PLGA-PEG-COOH polymer during nanoprecipitation with rotary evaporation at 30°C, resulted in ultra-small particle formation with a mean diameter of 52.35 nm. These results indicate that ultra-small PLGA-PEG-GA NPs (< 60 nm) can be formed. Further characterization and evaluation of these nanoparticles *in vitro* and *in vivo* are ongoing.

Sub-diffusive Motion of Tracer Particles in Models for the Cytoplasm of *E. coli* Cells

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Tracer particles in the cytoplasm of *E. coli* cells display slow, heterogeneous dynamics similar to those observed in colloidal glasses. The density of the *E. coli* cytoplasm, however, is significantly lower than the onset of glassy behavior in colloidal glasses. The cytoplasmic components are characterized by high polydispersity and a range of polymeric structures. To better understand how entanglements and component shapes contribute towards such heterogeneous behavior, this study utilizes molecular dynamic simulations of closed polymer systems to measure the dynamical properties of the polymer melt; in specific, we will observe the mean squared displacement (MSD) and diffusion coefficients of these polymers. Studies of dilute polymer melts have shown entanglement to suppress diffusion. This study explores the onset of the glass transition in systems of polymers at high densities, as well as the particular effect that entanglement has on dense polymer systems.

Functional Characterization of a Novel Ferroportin Mutation Identified in Autosomal Dominant Hemochromatosis

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Iron homeostasis is important for maintaining proper cellular function and preventing oxidative damage. Ferroportin (Fpn), the only iron exporter protein found in mammals, is a transmembrane protein that transports iron across the cell membrane of many cell types. Mutations in the ferroportin gene result in excessive iron accumulation in the body. We have discovered a novel genetic variant in Fpn (p.Arg88Ile) that cosegregates with disease in a kindred with an autosomal dominant form of hemochromatosis, a clinical iron overload disorder. This project aims to understand the molecular etiology of iron overload in this kindred. Our hypothesis is that the Arg88Ile substitution alters Fpn localization within the cell, thus affecting intracellular iron levels leading to disrupted systemic iron homeostasis. To address our hypothesis, we conducted in-vitro localization experiments by transfecting HEK293T cells with plasmids that encode green fluorescent protein (GFP)-tagged forms of either WT Fpn or mutant Fpn and used confocal microscopy for visualization. Our results show that the p.Arg88Ile mutation impairs trafficking of Fpn to the cell membrane. To assess the effect of the p.Arg88Ile mutation on intracellular iron levels, we used RT-qPCR to measure levels of Transferrin Receptor 1, an mRNA that is stabilized when low intracellular iron levels are reduced. Overexpression of the WT Fpn-GFP increases TfR1 expression levels, and this increase was not observed when the p.Arg88Ile mutant was overexpressed. Because mutant Fpn does not localize to the membrane, overexpression of mutant FPN does not cause lower intracellular iron levels because iron cannot exit these cells. Collectively, these findings suggest that decreased Fpn availability at the cell membrane is the mechanism that results in clinical iron overload in this kindred.

The Role of Endothelial Cell Signaling in Regulating Neutrophil Transmigration through Microvasculature Pericytes

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The first phase of inflammation is neutrophil transmigration, which is required for healing. Despite literature elucidating how neutrophils transmigrate through the endothelial cell monolayer, the mechanism by which neutrophils traverse the pericyte sheath of the microvasculature is not well understood. We investigated endothelial cell (EC) paracrine signals that regulate pericyte (PC) functions to mediate neutrophil transmigration. We found via enzyme linked immunosorbent assay that ECs increase secretion of macrophage migration inhibitory factor (MIF), a proinflammatory cytokine, in response to tumor necrosis factor-alpha (TNFα) stimulation. Results from fluorescence microscopy showed that PC stress fiber formation is disrupted after treatment with TNFα stimulated EC-conditioned media (TNFα-EC-CM), and could be rescued by the addition of either a MIF inhibitor or a MIF receptor specific antagonist, ISO1 or MIF98, respectively. Similarly, transmigration assays across PC monolayers showed that neutrophil transmigration is significantly increased when PC are treated with TNFα-EC-CM, but rescued when treated with ISO1 or MIF98. These results indicate that phenotypical changes in PC actin structures via EC paracrine signaling plays a role in regulating neutrophil transmigration. Dysregulation of inflammatory cascades inhibit the healing process and lead to chronic diseases like rheumatoid arthritis and asthma, and acute conditions like sepsis. This work contributes to a more complete understanding of neutrophil transmigration for the development of better treatments.

Improving CRISPR/Cas9 Efficiency in *Arabidopsis thaliana*

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CRISPR/Cas9 genome editing technology creates specific DNA mutations using guide RNAs (gRNAs) that target a specific region in the genome. The endonuclease Cas9 is directed by gRNAs to create a site-specific double stranded nick, which is subsequently repaired by non-homologous end joining (NHEJ) or homology directed repair (HDR) mechanisms, the first of which results in mutations. Despite its widespread use and advantageousness, there are still improvements to be made to the CRISPR/Cas9 system in plants, mainly increasing the efficiency of creating heritable mutations, which is still very low in Arabidopsis. We hypothesized that increasing DNA accessibility for the gRNA will increase CRISPR/Cas9 efficiency. To test this, we promoted loss of nucleosome density via heat exposure, with the objective of increasing CRISPR/Cas9 efficiency. Our approach involved cycling the Arabidopsis plants between an 37C and 22C during their vegetative growth phase. We targeted the reporter genes HTR5-GFP and MGH3-GFP (found in the germline) with several different gRNAs. To determine whether mutations were created, we relied on flow cytometry for HTR5-GFP mutants and fluorescent microscopy of pollen grain for MGH3-GFP mutants. Our microscopy results indicated that the mutation rate was significantly increased for the T1 plants that received the heat treatment compared to those that did not, with the average mutation rate of pollen grains being 45.06% for heat treated plants, and 1.02% for non-treated plants. Furthermore, the transmission rate to the T2 plants also increased in the heat stressed plants. We conclude that heat stress greatly increases the efficiency of CRISPR/Cas9 in Arabidopsis. The ramifications of this work are far-reaching including the creation of accurate and transmissible mutations in the model plant *Arabidopsis*.

Exploring Limbic Seizure Pathways *in vivo*: Optogenetic and Neuroanatomical Tracing Approaches

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Loss of consciousness during temporal lobe epilepsy, characterized by complex partial seizures originating from limbic structures such as the hippocampus, is not yet well understood. Our current work investigates brain networks including the cerebral cortex interacting with deeper structures to unveil how focal seizures impair consciousness. During temporal lobe seizures, intracranial EEG recordings show sleep-like activity (slow-wave oscillations) in the cortex, accompanied by decreased cerebral blood flow. We have proposed a "network inhibition hypothesis" in which temporal lobe seizures inhibit subcortical arousal systems, causing depressed cortical function. Previous work from our lab has shown reduced cholinergic neurotransmission in both the cortex and thalamus during partial seizures in a rodent model. In addition, focal hippocampal seizures in rats induce increased neuronal firing and cerebral blood flow in the lateral septal area. Electro-stimulation of the lateral septum (LS) in the absence of seizures resulted in cortical slow oscillations resembling deep sleep, suggesting that the LS might contribute to cortical deactivation by impacting subcortical networks. In this study, we explored possible pathways from the LS to the nucleus basalis (NB), a subcortical region that provides the most cholinergic input to the cortex. By using selective Cre-dependent expression of a light-activated channel Channelrhodopsin-2 (ChR2) in NB cholinergic neurons from ChAt-Cre transgenic rats, we found that photo-stimulation of cholinergic neurons in the NB in vivo converted slow-wave oscillations to fast awake-like activity in the cortex, thus reinstating aspects of arousal during induced partial seizures. To identify anatomical circuitry between the LS and the NB, we used a combination of retrograde tracing from the NB and anterograde tracing from the LS. Our histology results revealed no direct anatomical connection between these two regions, suggesting that the LS might innervate cholinergic neurons in the NB via indirect polysynaptic pathways including other subcortical structures. Based on the tracing histology results, the hypothalamus, thalamus, nucleus accumbens and claustrum may be targets for further investigation. Our hope is that by identifying the neural circuitry responsible for impaired consciousness during focal seizures, new treatments can be devised to prevent this alteration of brain function and thus improve the quality of life in patients with temporal lobe epilepsy.

Lithium Abundance in Planet Search Stars

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Stellar lithium abundances are an essential measurement for understanding open questions in stellar and planetary astronomy. Since most lithium in the universe is primordial and is destroyed in stellar interiors, lithium abundance can be used as a probe for various astrophysical processes. Some research has suggested that planet formation may affect lithium abundance in exoplanet host stars (EHS). Additionally, there remains the open question of whether lithium abundance in EHS is measurably affected by the ingestion of planets. However, small and heterogenous samples have made these phenomena unclear. Further study of lithium abundance in EHS is needed to better understand possible physical roles of lithium in planet formation theory. We used a large homogenous sample with accurate, uniformly determined stellar parameters on which we performed both equivalent width analysis and spectral modeling to determine lithium abundances. We report these abundance values and discuss relationships between lithium abundance and fundamental stellar parameters.

Effects of Media Substrate Changes on Engineered Heart Tissues and their Functional Capabilities

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Diagnosis of cardiomyopathy, a genetic heart muscle disease, often occurs in its most advanced stages. The Campbell lab proposes a more effective method of early diagnosis by forming patient specific engineered heart tissue (EHT) and using biomechanical testing to differentiate between healthy and diseased tissues. EHTs are made by seeding decellularized porcine tissue with induced Pluripotent Stem Cells (iPSCs). Current accepted EHT protocols in the Campbell lab and the literature call for high glucose media during culture; this fails to account for the switch in energy source that cardiomyocytes undergo as they mature from glycolysis to fatty acid beta oxidation. Therefore, a critical need exists to explore how different substrates in the media affect tissue growth at physiological ratios. We hypothesized that the addition of physiological supplements would improve cardiac function by increasing biomechanical measurements. To determine the effect of switching to physiological substrates and amounts in neonatal rat cardiomyocytes, cells and tissues were gradually weaned from 25 mM glucose down to a physiological level of 5.5 mM glucose. Four different experimental groups were used to analyze the effects of galactose substitution for glucose along with the fatty acid of interest, oleic acid. Differences between the experimental and control groups were assessed via live/dead staining under confocal microscopy and mechanical twitch force measurements. The experimental media resulted in more prolific neonatal rat cardiomyocytes when they were cultured on coverslips, but resulted in death and significantly weaker force measurements when cultured in tissues, likely due to a lack of controlled oxygen perfusion. Overall, the addition of physiological supplements improves cardiac function in cardiomyocyte cell cultures, but not in tissue culture.

Genome Annotation of the Apicoplast and Mitochondrial Genomes of *Babesia duncani* and Potential Therapeutic Targets

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Babesia duncani is a newly identified causative agent of human babesiosis and responsible for most babesiosis cases reported in Western and Midwestern states United States. Although the parasite causes a much more severe form of the disease compared to other *Babesia* species, not much is known about its biology, pathogenesis, and sensitivity to recommended therapies. Here we report the first complete sequence and annotation of the apicoplast and mitochondrial genomes of *B. duncani*. We found that the apicoplast genome of this parasite consists of a 34kb circular molecule encoding functions that are important for transcription, translation and maturation of the organelle proteins. The linear mitochondrial genome is 5.4kb in size and harbors two inverted repeats at both ends of the molecule. Using the conserved *cob* and *coxI* proteins encoded by the mitochondrial genome, phylogenetic analysis revealed that *B. duncani* defines a new lineage among apicomplexan parasites distinct from *B. microti*, *B. bovis*, *Theileria sp*. and *Plasmodium sp*. Our analysis of the annotated organellar genomes revealed 3 proteins with a primary sequence indicative of sensitivity to FDA approved as well as new experimental drugs. Our studies will set the stage for evaluation of the efficacy of these drugs alone or in combination against *B. duncani* in culture as well as in animal models.

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