STEMcosR 2018 - Abstract List

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#1. Simulating Muscle Contraction With 3D Printed Hydrogels

Gabriel Valdez-Cagua, Dr. Joseph Freeman1, Steve Hermida

3D printing is emerging as one of the most innovative technologies in recent years. Recently, researchers in the scientific community are trying to find ways to incorporate 3D printing into their studies. In Dr. Freemans lab, we are modifying a 3D printer which normally prints plastic objects into a 3D Bioprinter which will be able to print biological materials. Our goal is to conduct research so that one day, scientists will be able to grow muscle implants in a lab for diseased patients.

In the body, muscle contraction occurs when the brain sends a signal to receptor sites in muscle cells. This signal causes the release of calcium ions throughout the muscle. Muscles are made up of microscopic fibers made up of actin and myosin proteins. Myosin binds to actin, pulls on it, and then releases it which closes the gap between adjacent muscle fibers, causing contraction.

Our research aims to simulate muscle contraction by 3D printing hydrogels (water absorbing polymers) in the same pattern as the microscopic fibers that our muscles are made out of. Hydrogels have previously shown the ability to bend in the presence of an electric field. In addition, stem cells seeded in hydrogels are able to thrive and change into muscle cells. Ideally, the electric field will cause our hydrogel "fibers" to bend and close the gap between adjacent fibers, simulating muscle contraction.

#2. The Effects of KPT-9274 Drug Treatment on Gene Expression in SUM159 Breast Cancer Cells

Emma Cordover, Rebecca Della Croce, Alexa Podolsky, Audrey Minden

The p-21 activated kinase 4 (PAK4) is a critical component in many signal transduction pathways as it mediates cytoskeletal and cell shape changes. This kinase is overexpressed in many human cancers, including triple negative breast cancer (TNBC). Because TNBC lacks certain hormone receptors and druggable targets, it is difficult to treat. However, previous studies show that KPT-9274, a compound synthesized to inhibit PAK4 expression and function, is promising for the treatment of TNBC as it triggers cell death in vitro. The drug has additionally been deemed effective in treating TNBC in mouse models. Previous data from Next Generation RNA Sequencing and Ingenuity Pathway Analysis (IPA) indicates that various cancer-related genes are likely affected by the decreased level of PAK4 caused by KPT-9274. The overall purpose of this study is to confirm whether the changes in protein levels of certain target genes are consistent with what was observed in the RNA sequencing. In order to analyze the extent to which KPT-9274 affects PAK4's role in the expression of other genes, SUM159 cells were treated with different doses of the drug. Western blot data was analyzed to determine the differences in the presence of cancer-related genes GADD45A, MAT2A, and ENO1 in the cells treated with the drug versus untreated. When treated with KPT-9274, the expression of GADD45A and ENO1 increased while MAT2A decreased. This study also conveys that treating SUM159 cells with the drug leads to cell death. Further experimentation is necessary to analyze potential pathways for PAK4 and the associated genes of the study.

#3. The Effect of Electroporation Buffer Composition on Cell Viability and Transfection Efficiency

Joseph Sherba, Stephen Hogquist, Jerry W. Shan, Hao Lin, David I. Shreiber, Jeffrey D. Zahn

Electroporation (EP), is a technique used to introduce foreign molecules, such as plasmid DNA vectors, into the intracellular space of biological cells using an external electric field. Although this technique has been in use for a few decades, there is still an incomplete understanding of the effects that different molecular solutes in the cell suspension buffers has on the overall transfection outcomes. With gene therapy beginning to be adopted in clinical settings and EP as one of the techniques used for DNA transfection, a better understanding of how different buffer compositions effect EP outcomes will aid in the rational development and optimization of DNA transfection protocols. Thus, the purpose of this study is to investigate how EP buffer composition affects cell viability and transfection efficiency (TE) following EP.

Sterile EP buffers were prepared with matching physiological pH (7.4) and osmolality (~300 mOsm). Either sucrose or trehalose was used to balance osmolality. Final buffer conductivity was adjusted to 100 μS/cm, 500 μS/cm or 2000 μS/cm, using $MgCl_2$, KCl, $MgSO_4$, or a mixture of $MgCl_2$ and KCl. 3T3 cells were harvested and washed in EP test buffer prior to resuspension at a concentration of 3x106 cells/mL in a 2 mm gap EP cuvette. This resuspension contained the Lonza pMAX GFP vector at a final concentration of 20 μg/mL. A single square wave pulse was applied to each cell population. The prescribed electric field and duration ranged from 1.2 kV/cm to 4.8 kV/cm and 0.063 ms to 1.0 ms, respectfully. Electric field / pulse duration combinations were chosen to either keep the total energy dissipation (σE2t) or charge movement (σEt) constant (σ—conductivity, E—electric field, t—pulse duration) during the pulse. Following EP, cells were re-plated and incubated for 24 hours then imaged under phase contrast and fluorescence microscopy to determine the resulting cell viability and TE, respectfully. Cell viability was determined as the total number of cells imaged in the experimental condition normalized to the total number of cells imaged under a 'no-pulse' control. TE was defined as the ratio of total number of GFP (+) cells to total number of cells per condition.

To quantify the results gathered from this study an electroporation scoring metric was created. This metric is the product of cell viability and efficiency percentages for each condition. The results show three distinct clusters. The first cluster resulted in good cell viability, but poor TE. This was observed primarily in conditions with lower energy pulse applications. The second cluster displayed the opposite: high efficiency with low cell viability. This cluster is primarily of EP buffers that lack Mg^{2+} as the cation used to balance conductivity. The third cluster resulted in a balance of both good cell viability and TE. This cluster is composed of EP buffers that had the lowest concentrations of Mg^{2+} tested. From these results it appears that an optimal concentration of Mg^{2+} is required to preserve cell viability while still allowing for high TE. Mg^{2+} is known to be a cofactor for many biological enzymes, in particular DNases and ATPase membrane ion channels. High concentrations of Mg^{2+} may result in lower TE due to higher activity of DNase enzymes. However, it is believed that removing Mg^{2+} altogether does not allow the cell to re-establish physiological equilibrium, resulting in higher amounts of cell death. The EP buffer with both K^{+} and Mg^{2+} is found under all pulse conditions tested to result in both high viability and TE. We believe that this is because the lower overall Mg^{2+} concentration allows cell recovery while limiting DNase activity.

The composition of EP buffer appears to play a major role in terms of cell viability and TE following EP. Mg^{2+} is crucial for preserving cell viability, but at the same time detrimental for transfection success. Future will investigate the molecular mechanisms by which Mg^{2+} plays a role in the cell recovery and transfection process, with hopes of further enhancing overall EP outcome scores.

#4. Tissue Specific Distribution of Thyroid Receptor Isoforms

Sanya Bansal, Shaan Tadepalli, Michael Brotherton, Rucha Janodia Svetlana Minakhina, Fredric Wondisford

The thyroid hormones (T3 and T4) and the thyroid hormone receptors are responsible for regulating the body's metabolism along with controlling the function of various tissues like brain, liver, lung, kidneys. Major receptor isoforms, Thrα1, THRα2, THRβ1, and THRβ2 are encoded in two genes *Thra* and *Thrb*.

Currently, there is not a lot of data portraying relative expression of THR protein isoforms since there are no reliable isoform-specific antibodies. We created mouse models by adding a 2X hemagglutinin (HA) tags via CRISPR into endogenous *Thra* and *Thrb* genes. Homozygous mice are viable and have no overt phenotypes, and individual THR isoforms now can be detected within the tissues using anti-HA antibody. Understanding relative expression of thyroid hormone receptor isoforms will help us understand the molecular basis for the THR isoforms function. It will also pave the way forward to developing targeted therapy for various thyroid hormone related diseases.

#5. Optimizing PLGA Nanoparticle Encapsulation for Bupivacaine Delivery

Mayur Patel; Mollie Davis; Rene Schloss, Ph.D.; Martin Yarmush, M.D. Ph.D.

Osteoarthritis (OA) is the most common chronic condition of the joints characterized by pain, stiffness, and swelling. OA is caused by the progressive degradation of the cartilage, or cushion, between the joint [1]. Currently, OA does not have a cure, but we believe the first step to create a cure is to develop a therapy to relieve pain with minimal side effects.

Local anesthetics (LA) are used to reversibly block pain sensation in the target area. Compared to other OA pain treatments, such as opioids and NSAIDs, LA are non-toxic in small doses and less addictive. However, local anesthetics are not as potent and long lasting as opioids and NSAIDs.

Poly(lactic-co-glycolic acid) (PLGA) is one of the most widely used biodegradable polymers in controlled drug delivery. LA encapsulated in PLGA nanospheres have been known to prolong diffusion for up to 1 month. Compared to other means of drug delivery, such as liposomes, PLGA nanoparticles are more durable, affordable, and practical to create.

Our research works to develop a multimodal therapy that targets pain relief, inflammation, and regeneration in OA. Currently, we are aiming to optimize a therapy that only targets pain relief. In particularly we are encapsulating bupivacaine, our LA of choice, in PLGA microparticles and liposome nanoparticles in order to create a product that can provide pain relief for a long duration and allows for high cell viability.

A major goal when creating this therapy is to optimize a protocol that is economical. To do so we measure the encapsulation efficiency, the percent of drug that successfully got encapsulated, and the loading capacity, the percent of mass of the nanoparticles that is drug. Using tools such as an HPLC, we performed chromatogram to measure the amount of bupivacaine encapsulated. In this way, we hope to optimize a protocol that is efficiently loading bupivacaine so we can get a practical mean of relieving pain in OA patients.

#6. A Hand-held, Portable, and Low-cost Robotic Device for Autonomous Blood Drawing

Josh Leipheimer

Venipuncture, the process of obtaining intravenous access for blood sampling or fluid delivery, is one of the most common clinical procedure performed worldwide, and also one of the most problematic. Because of accidental needle stick injuries and unnecessary long procedure times, difficult venipuncture costs the U.S. healthcare system on average 4.7 billion dollars every year. Previously in our lab, a benchtop robotic device was created to automate the venipuncture process. This device utilized 9 individual motors, a near-infrared (NIR) imaging system, and an ultrasound imaging probe to automatically identify a vein for cannulation and perform the needle insertion. However, its large size, lack of mobility, and high costs makes it impractical for clinical translation, as most venipuncture procedures will be done on patients that are either bed ridden or incapable of accessing the device.

To solve the major limitations with the previous device and difficult venipuncture, I am working to develop a hand-held, cost-effective, portable robotic venipuncture device, capable of both identifying a suitable vein for insertion, while also quickly, safely, and efficiently cannulating said target vein for difficult venous access patients. The device will utilize out-of-plane ultrasound imaging to segment suitable veins for insertion and utilize a custom force sensor feedback system for vein puncture detection. The device will be intended for easy clinical translation, featuring a simple to use interface that requires little to no human intervention or training required. The device will be designed for compact, hand-held use so that clinicians can use it easily in any clinical environment. By developing a hand-held, miniature device to quickly and safely automate venipuncture procedures, we plan to reduce injuries and problems associated with difficult venous access, while also reducing procedure times for healthcare clinicians, thereby reducing healthcare related costs.

#7. Encapsulation and Loading Efficiency of Bupivacaine in PLGA-based Nanoparticles

Kyle Lee, Mayur Patel, Mollie Davis, Rene Schloss, Ph.D., Martin Yarmush, M.D., Ph.D.

Osteoarthritis (OA) is a degenerative joint disease that involves the wearing and tearing of articular cartilage[1]. Known for being a disease that is associated with pain and disability, OA affects around 630 million people worldwide[2]. There are no current cures for the disease but treatments available for combating pain include steroids, NSAIDs and opioids. However, many of these treatments come with unwanted side effects including addiction. As an alternative to these options, we are using local anesthetics (LA) as a treatment option for pain relief because they are localized and are non-toxic in small doses. LAs reduce pain in a local area by inhibiting nerve transmission, which produces a reversible loss of sensation in the targeted area of the body[3]. Current LA application protocols do not deliver sustained doses for effective treatment; however, the benefit of using them is that they do not have the unwanted side effects. In order to utilize this drug safely at localized areas in the body for longer periods of time, PLGA-based nanoparticles may provide a drug delivery alternative for sustained drug release. Having the drug delivered using this approach may ensure that the drug is not released all at once, and may simultaneously protect neighboring cells from losing viability or function.

Drug loading efficiency and encapsulation efficiency are two important factors to consider in making LA encapsulated PLGA-based nanoparticles. Our research works to develop consistency in these two aspects by experimentally devising a protocol that maximizes overall efficiency. Using high performance liquid chromatography (HPLC), our goal is to measure the concentration of bupivacaine in nanoparticle samples to determine the percentage that is encapsulated in a given sample based upon the original amount of loaded bupivacaine. It is imperative to know these two characteristics because they help determine overall diffusion profiles and cell apparent dosages, which are both important in the perspective of evaluating an overall therapy. By understanding methods of maximizing encapsulation efficiency and better understanding diffusion profiles, we will eventually be able to test this concept on chondrocytes cells that are found in cartilage. Analyzing how this construct affects chondrocyte cells helps us closer to reaching our goal of discovering the most beneficial pain relief treatment for OA.

#8 High Throughput Identification of Polymers for Protein-Like

Rahul Upadhya, Shashank Kosuri, Matthew Tamasi, Adam J. Gormley

As tiny microscopic factories, cells manufacture proteins using 20 types of amino acid building blocks. These amino acids can be classified as either hydrophilic (water-loving) or hydrophobic (water-repelling). The sequence of hydrophilic and hydrophobic amino acids determines the protein structure, as hydrophilic building blocks tend to interact with the aqueous environment hydrophobic ones interact with each other. Protein structure determines function as unique structures enable particular interactions with cells and receptors.

The manufacture of biologic protein-based drugs is becoming more common in the pharmaceutical industry, particularly for cancer therapies. Despite the advantages of biologics, proteins are costly to produce, can set off an immune response in the body, and can be rendered unstable with time. We aim to improve upon these flaws by making protein-like structures from synthetic polymers.

In general, a polymer is any structure that is made up of smaller building blocks that are called monomers. For example, a protein is a polymer that is made up of amino acid monomers. Our lab works with synthetic polymers, which are human-made and can be formed using hydrophilic and hydrophobic monomers. Polymers are less expensive to manufacture than proteins and can be designed to be remarkably stable in aqueous solution and biocompatible (not trigger an immune response).

The major question of our research is: can we create synthetic polymers from both hydrophobic and hydrophilic monomers that have protein-like structures?

While our ability to control the exact sequence of monomers is not yet at the level of precision of nature, we can control polymer size and the combination of various monomer types. Additionally, we can synthesize several combinations of polymers in a short period of time. To gain clues about the structure of all the many polymers we create, we take advantage of rapid analytical techniques. These methods provide molecular weight distributions, size, and folding information.

We can further incorporate peptides on the surface of these polymers to bind to specific cell receptors and activate cellular signaling pathways. Overall, by understanding the structure of these synthetic polymers and polymer-peptides, we aim to engineer materials that have similar structure and function as therapeutic proteins with improved characteristics.

#9. Neuronal Vinculin Protein Distribution as a Function of Growth and Maturation In Vitro

Shivani Vyas, Dr. Nada Boustany

Our goal is to investigate how vinculin protein distribution in neurons alters over time. There is a significant and noticeable difference between the appearance and distribution of vinculin in the cell. At earlier days-in-vitro (DIV), vinculin is more continuous throughout the neuron. At later DIV, the protein is more punctate and gathers at specific spots throughout the cell, especially at the edges of cellular processes.

Immunofluorescence was used to illustrate the distribution of native vinculin in cultured neurons. By immunostaining for vinculin, establishing an improved incubation protocol, and confirming the visibility of the protein by comparing to a microtubule-associated protein (MAP2), it is clear that vinculin in neurons gathers at regions throughout the cell. When imaged with fluorescent microscopy, vinculin is seen to adopt a spotted appearance in the cell at later DIV.

A MATLAB image analysis regarding neuron image intensities based on varied incubation periods was conducted, demonstrating that the longer incubation periods previously stated were producing significantly brighter images. Quantitatively, with MATLAB image analysis, neurons randomly sampled from the earlier DIV contained less circles and dots representing the vinculin protein in comparison to the later DIV images. Additionally, the density of vinculin distribution was greater in the later DIV than the earlier DIV. However, as compared to epithelial cells, neurons seem to have less vinculin, yielding a much dimmer image.

In the future, reasons for why vinculin appearance within neurons is changing over time need to be determined. Although the neuronal vinculin immunostaining procedure was improved upon, it still appears dimmer in neurons than other types of cells. Therefore, the immunostaining methods still need to be developed further to yield optimal results when imaging.

#10. Improving Cancer Screening with an Optimized Blood Test

Zachary Fritz, Lawrence Williams, Anil Shrirao, Rene Schloss, Martin Yarmush

Detecting cancer early in its progression is critical for administering safer, more effective treatments. Recently research has shifted toward developing "liquid biopsy" techniques that test blood and other bodily fluids for the presence of certain disease-associated molecules, known as "biomarkers"; these tests may detect cancer earlier and would be less invasive than established screening methods, such as colonoscopy. Autoantibodies are one class of biomarker that appear very early and persist in many types of cancer, but tests to detect them are not widely used due to a low reported prevalence of individual autoantibodies in cancer patients. However, we believe that this low prevalence is due to the flawed molecular design these tests use to capture (and thereby detect) autoantibodies from a blood sample. By using a new computational molecular model, we hypothesize that we are able to design a superior, optimized blood test that will capture and detect a larger proportion of the autoantibodies in a cancer patient's blood, significantly improving the efficacy of this screening test. Additionally, we plan to develop an integrated device that will be able to perform the test quickly, with minimal user expertise/input, and that can be used in clinical settings. Our improved test will be applicable to a wide range of cancers, and may be useful for purposes other than screening, such as monitoring for disease recurrence or determining which course of treatment to take.

#11. Alginate Encapsulated Mesenchymal Stromal Cell Therapies for Osteoarthritic Treatment

Rishabh Hirday, Ileana Marrero-Berrios, Rene Schloss, Martin Yarmush

Osteoarthritis (OA) is a chronic age-related disease characterized by the progressive destruction of articular cartilage (the smooth tissue that covers bone ends in a joint). OA is considered the most relevant joint disease in adults, affecting more than 12.4 million adults over the age of 65 in the U.S. and amounting more than $185.5 billion per year in healthcare costs. While previously described as a non-inflammatory wear and tear disease, there are known pro-inflammatory factors that play a key role as mediators in the disease. There is no cure for OA; current treatments can only relieve symptoms initially and for a limited period of time, as disease progression eventually proceeds.

Mesenchymal stem cells (MSCs) have been shown to secrete anti-inflammatory and regenerative factors that may help change the inflammatory state in OA. However, simply injecting cells into an afflicted join it is not reasonable since millions of cells are required, and many die after injection or migrate from the insertion site. Hence, in order to create a long term treatment, modification of the delivery of the cells is needed; which our group has achieved by encapsulation in alginate, a polysaccharide found in brown seaweed. Alginates are widely used in the food industry as thickeners and interestingly, encapsulating MSCs in alginate has shown to protect cell health and promote anti-inflammatory factor production.

Our research aims to develop new long lasting stem cell based therapies with the potential to stop disease progression and promote healing in OA afflicted joints. Therefore, we are using alginate encapsulated MSC as a treatment for OA. To determine the treatment efficacy, we used simplified models that mimic the diseased state in the joint by culturing chondrocytes (the only cell component in articular cartilage) and macrophages (immune cells present in the joint) in inflammatory conditions and then adding encapsulated MSCs as a treatment. To measure the MSCs' effect, the gene expression and protein secretion changes in chondrocytes were analyzed using molecular biology techniques, such as quantitative reverse transcription polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively.

Generally, our results indicate that under inflammatory conditions, chondrocytes presented a characteristic OA response (high inflammation levels); however, in the presence of MSCs this response was worsened. A potential explanation for this phenomenon is the lack of target cell for MSC function in our model. Further studies will investigate the effects of encapsulated MSCs in an environment containing both macrophages and chondrocytes, as macrophages are known to respond in a favorable manner to MSC presence and can also affect the chondrocyte inflammatory state. This will paint a more realistic picture of the effectiveness of encapsulated MSCs as a treatment for OA.

#12. Title: Chemical Vapor Deposition of Twisted Bilayer Graphene on Copper Foil Substrates

Lucas Hanson, Nikhil Tilak, Micheal Altvater, Eva Y. Andrei

Graphene, a one atom thick sheet of carbon atoms, has been a material of great interest to the condensed matter physics research community since its discovery in 2004. It is the strongest material ever discovered (200 times as strong as steel), has remarkably high electrical and thermal conductivity for a semimetal, and can be synthesised simply by peeling a piece of scotch tape off of the surface of a block of graphite. Of increased research interest is bilayer graphene, a graphene superlattice where two sheets of single layer graphene are stacked on top of each other. Earlier this year it was announced at the March meeting of the American Physical Society that bilayer graphene with a small twist angle between the layers exhibits unconventional superconductivity, an exotic phenomenon that is of tremendous theoretical and experimental significance. However, the applications of this discovery to new technologies are limited, as the scotch tape synthesis method can not produce a consistent number of layers or twist angle, and can only produce small amounts of graphene at a time. One promising method for large scale twisted bilayer graphene synthesis is chemical vapor deposition (CVD). In CVD, graphene is grown on a catalysing transition metal surface (typically copper or nickel foil) through the deposition of carbon atoms from a gaseous carbon based feedstock (in our case methane). By flowing a mixture of argon, hydrogen, and methane gas over the foil while it is heated to more than 1000^{o}C in a tube furnace, methane molecules adsorb to the surface, the copper separates the hydrogen atoms from the lone carbon, the hydrogen gas is desorbed from the foil, and the carbon remains on the surface. As the temperature in the furnace is decreased the carbon atoms bond with each other to form single, bilayer, and multi-layered wafers of graphene on the surface of the foil. Preliminary results suggest that increased bilayer grain sizes can be achieved by flowing higher methane concentrations for longer time periods during the growth stage, and optical microscopy of single and bilayer grain alignment suggest that small twist angles can be achieved using our CVD method.

#13. A Spectral Exploration of Obscured and Unobscured AGN

Antoine Washington

There is an ongoing debate as to whether the differences we see in certain active galactic nuclei (AGN) is due simply to their orientation relative to their observer or there exists some real, physical difference in these objects that drive these differences. The unification model stresses the former, that these objects are simply look different at different orientations, and otherwise, these objects are all essentially the same. Andy Goulding at Princeton University devised a method of differentiating between obscured and unobscured AGN using WISE and HSC data. However, this method cannot function on objects at lower redshifts. I look into the spectral data of these objects to determine how well Andy's classification functions, and I ultimately find that this method works better with more luminous, higher redshift objects.

#14. A Thermoreversible and Photoactive Collagen-Based Scaffold to Treat Deep-Skin Wounds

Yoliem S. Miranda Alarcón, Dorota Jazwinska, and David I. Shreiber

Current treatments for regenerating tissue like skin, muscle, ligaments, and cornea largely rely on grafts patients, human donors, or animals. There are many drawbacks to these grafts, including the creation of a wound on the patient's healthy tissue, lack of biocompatibility, lack of availability, or undesired immune response from donor tissue. One goal in tissue engineering is to develop implantable scaffolds for soft tissues that have been damaged through injury or disease. As the most abundant protein in mammals, collagen can be found in a range of tissues from skin and bone to ligament and cornea. Collagen, therefore, can be employed to develop scaffolds as an alternative to grafts. It provides mechanical structure to our tissues by not only providing strength and stability, but also through its versatility, as collagen can be modified by various chemical, biological, and thermal processes. Further, this protein promotes cell growth and proliferation at physiological pH and temperature. But in order to have the stiffness and strength needed to mimic tissue, collagen needs to be crosslinked. To this end, we are modifying Collagen type-I to make it (1) thermoreversible and (2) photocrosslinkable. Our collagen type-I modification involves reacting collagen with methacrylic acid to synthesize Collagen Methacrylamide (CMA). (1) As a thermoreversible material, CMA behaves as a gel at physiological conditions—pH of 7 and 37◦C—and CMA can go back to a liquid suspension at 4◦C. (2) As a photocrosslinkable material, when CMA is exposed to UV light, the gel stiffens to up to three times its mechanical strength and becomes thermally irreversible. These properties make CMA a promising material for tissue engineering, specifically 3D printing and cell entrapment.

In this work, we have studied the optimal conditions for CMA crosslinking to entrap human Mesenchymal Stem Cells (hMSCs) in the hydrogel to induce further differentiation. hMSCs can differentiate into a variety of cell types, such as adipose cells or cartilage cells, when exposed to surfaces with a stiffnesses that reassembles native tissue. One application we are investigating is to tune the stiffness of CMA to use it as a matrix to grow and direct hMSCs differentiation through cell entrapment. We aim to differentiate hMSCs in CMA hydrogels into different cell types to develop tissue-specific connective tissue scaffolds.

#15. Stimulus-responsive Self-Assembly of Protein-Based Fractals by Computational Design

Nancy Hernandez, Sagar Khare

Fractal topologies, which are statistically self-similar over multiple length scales, are pervasive in nature. The recurrence of patterns at increasing length scales in fractal-shaped branched objects, e.g., trees, lungs, and sponges, results in high effective surface areas, and provides key functional advantages, e.g., for molecular trapping and exchange. Mimicking these topologies in designed protein-based assemblies will provide access to novel classes of functional biomaterials for wide ranging applications. Here we describe a modular, multi-scale computational design method for the reversible self-assembly of proteins into tunable supramolecular fractal-like topologies in response to phosphorylation. Computationally-guided atomic-resolution modeling of fusions of symmetric, oligomeric proteins with Src homology 2 (SH2) binding domain and its phosphorylatable ligand peptide was used to design iterative branching leading to assembly formation by two enzymes of the atrazine degradation pathway. Structural characterization using various microscopy techniques and Cryo-electron tomography revealed a variety of dendritic, hyperbranched, and sponge-like topologies which are self-similar over three decades (~10nm-10□m) of length scale, in agreement with models from multi-scale computational simulations. Control over assembly topology and formation dynamics is demonstrated. Owing to their sponge-like structure on the nanoscale, fractal assemblies are capable of efficient and phosphorylation-dependent reversible macromolecular capture. The described design method should enable the construction of a variety of novel, spatiotemporally responsive biomaterials featuring fractal topologies.

#16. Nkx6.1 Induces in Neurogenesis after Spinal Cord Injury

Misaal Patel, Jeremy Anderson, Shunyao Lei, Rebecca Risman, Li Cai

Spinal cord injury (SCI) results in permanent loss of function leading to paralysis. Unfortunately, there are no therapeutics that promote complete repair and regeneration after SCI. Identification of neural stem cells (NSCs) and their role in injured spinal cords have provided promising opportunities for spinal cord regeneration. However, adult NSCs mostly generate astrocytes and oligodendrocytes and the amount of neurogenesis is not sufficient to replenish all the neurons lost due to the injury. Our previous studies have established a novel role of Nkx6.1, a transcription factor, in regulating Notch1 signaling during spinal cord development. Nevertheless, the role of Nkx6.1 in the adult spinal cord and after injury has not been studied. Our goal is to determine the cellular role of Nkx6.1 on neurogenesis and specific interneuron differentiation after SCI.

In this study, we used a lentivirus-mediated gene expression system to transduce Nkx6.1, with RFP reporter, into the spinal cord of the young adult (8-12 weeks old) mice immediately after hemisection SCI. Lentivirus carrying only RFP gene is used as a control. Injured animals are harvested at 3, 7, 14, 35, and 56 days post-injury (DPI) and immunohistochemistry is performed with various markers to determine cellular changes around the injection site.
We identified that at 3 DPI Nkx6.1 induces proliferation and NSCs activation and reduces inflammation and cell death. Nkx6.1 induced NSCs differentiates into neural progenitors at 14 DPI and into mature neurons, specifically into cholinergic interneurons, by 56 DPI. Collectively, our findings suggest that Nkx6.1 is a potential therapeutic target for neuroregeneration after SCI. Successful completion of the proposed study will provide new insights into the cellular and molecular mechanisms underlying neurogenesis after SCI, which will accelerate the development of regenerative medicine for SCI.

#17. Response After Lentivirus Injection on Traumatic Brain Injury

Rebecca Risman, Jeremy Anderson, Dr. Li Cai

Traumatic brain injury (TBI) causes cell death and temporary or permanent loss of cognitive and/or motor function. Approximately fifty thousand people in the United States die annually from TBI due to falls, motor vehicle accidents, and assault. There is currently no cure for TBI. It is known that injury activates neural stem cells (NSCs) to generate mostly astrocytes and oligodendrocytes in the damaged brain. Notch signaling plays an important role in neural development and regeneration after injury. The goal of this research is to improve TBI recovery by motivating NSCs to generate neurons that will help to restore damaged neural circuitry. Using a Notch-GFP animal model where neural stem cells are tagged with green fluorescent protein (GFP), we target Notch signaling in mice after TBI. A closed head injury on mouse was induced by weigh-dropping. Lentiviruses carrying Notch pathway genes were also injected into the mouse brain at the injured site to further study the affect of the Notch pathway on stem cell response to brain injury. The brain tissues were analyzed at various time points after injury (i.e. 7 days and 21 days). Immunohistochemistry was performed to study the role of Notch signaling on neuronal differentiation using markers for neurons (Tuj1, DCX, and NeuN), oligodrocytes (PDGFRa and Olig2) and astrocytes (S100b and GFAP). Results with help determine the potential therapeutic effects in patients with a traumatic brain injury.

#18. Alginate Encapsulation for Bupivacaine Delivery and MSC Co-therapy

Mollie S. Davis MSE, Xiomara I. Perez BS, Ileana Marrero-Berrios M.Eng, Timothy Maguire PhD, Charles P. Rabolli, Rene S. Schloss PhD, Joel Yarmush MD, and Martin L. Yarmush MD PhD

Local anesthetics (LA), which reversibly blocks nerve transmittance, are commonly used to minimize patient discomfort and can also be co-administered with cellular therapies to improve regeneration and inflammation. However, LAs have been shown to have negative effects on mesenchymal stromal cells (MSC) in a potency-and-time-dependent manner [1]. To help mitigate these effects, we have developed a sustained release bupivacaine alginate-liposomal construct which enables four days of bupivacaine release. However, a co-therapy between MSC and LA would require positional control of the cells. Our lab utilizes alginate encapsulated MSC (eMSC), and the effect of LA on these cells is assessed. Unilamellar liposomes containing bupivacaine were encapsulated in a 2.2% ultrapure alginate hydrogel. MSC were freely cultured or encapsulated in alginate microspheres. The cells were left untreated or dosed with bolus, liposomal, or construct bupivacaine and were untreated or activated with TNF-α and IFN-γ. Matlab was used to model the diffusion of bupivacaine from the construct to the eMSC. It was found that eMSC secrete similar levels of IL-6, PGE2, and TGF-β regardless of LA modality. The model showed that eMSC are in the presence of 18% of the bolus dose for the first 8 hours, followed by negligible amounts of LA. Using the construct, the eMSC were in the presence of around 1% of the initial dose. Therefore, eMSC are protected from LA, regardless of LA modality and the model indicates that a higher LA concentration can be used within the construct while still allowing cellular functionality and viability.

#19. Dual Encapsulation of Neurotrophic Factors for Targeted Drug Delivery

Elisheva Strauss, Xiomara I. Perez, Hedaya Walter, George Durrant, Mollie Davis, Rene Schloss, Martin L. Yarmush

Traumatic brain injury (TBI) leads to a cascade of neurodegenerative events. As a result of primary injury, neuronal axons are physically damaged, leading to secondary injury events characterized by ischemia and inflammation. To aid in functional recovery of neurons, a multimodal therapy which can promote neuro-regeneration is required. Although current TBI therapies aim to treat the associated symptoms, the addition of positional control of therapeutics will allow for directed and sustained delivery to the injury site. Therefore, our group is encapsulating neurotrophic factors (NFs) in poly-lactic-co-glycolic acid nanoparticles (PLGA NPs) and a hydrogel. Encapsulating NFs, which aid in survival and regeneration of neurons, in PLGA NPs will assist in drug delivery; while further encapsulating the biomolecule-loaded NPs in alginate will enable localization and sustained release. The purpose of this study was to optimize the parameters for developing uniform NPs and to encapsulate them in alginate-microbeads. The NPs were synthesized using a double emulsion method followed by 2 hours on a rotary evaporator and lyophilization. Once optimized for size, Rhodamine B, a fluorescent dye, was encapsulated within the NPs to ensure that a molecule could be encapsulated. For the hydrogel encapsulation, Rhodamine B-loaded NPs were uniformly distributed in 2.2% alginate followed by microsphere formation using an electrostatically assisted encapsulator. The resulting nanoparticle sizes ranged from 270 to 540 nm and images taken with confocal microscope indicated that the subsequent hydrogel encapsulation was successful. Future work includes an *in vitro* controlled release study of the dual-encapsulated biomolecule from the PLGA-alginate drug delivery vehicle.

#20. Therapeutic Affect of Phosphatidylglycerol (PG) on Neimann Pick Type-C Disease

Miriam Waghalter, Olga Ilnytska PhD., Ran Li, Judith Storch, PhD.

Neimann Pick Type C (NPC) Disease is a rare lysosomal storage disorder in which one of the genes that code for NPC proteins (1 or 2) is mutated, resulting in absent or nonfunctional protein. Without either of these proteins, cells lose their ability to transport cholesterol through the endo-lysosomal compartment. The subsequent accumulation of cholesterol and secondary accumulation of other lipids in cells cause detrimental motor and neurological symptoms and ultimately death. Chevallier *et al (J.Biol.Chem 2008)* discovered that exogenous treatment of NPC1 cells with lysobisphosphatidic acid (LBPA) results in cholesterol clearance from late endosomes. We have recently shown that LBPA enrichment can occur by incubating cells with phosphatidylglycerol (PG), the precursor of LBPA. In this work, we induced the phenotype of NPC disease on apparently healthy human fibroblast cells using drug U18666A, which inhibits NPC1 from exporting cholesterol. We examined whether treating these cells with PG has an effect on clearance of cholesterol. We used three different concentrations of U18666A (0.5 μM, 1 μM, and 5μM) and treated the cells with PG over four different time points (48h, 40h, 34h, and 16h). Using flow cytometry and microscopy, we measured the cholesterol content in the cells after treatment and found that PG does reduce the amount of cholesterol within the U18666A-treated cells. These results on this isogenic model confirm our lab's previously obtained data on the therapeutic potential of exogenous PG/LBPA enrichment in cells with defects in NPC1.

#21. Imaging Rare-Earth-Doped Nanocomposites Using Line-Scanning Confocal Microscopy

Carolina Bobadilla-Mendez, Harini Kantamneni, Vidya Ganapathy, Mei Chee Tan,Prabhas V. Moghe, and Mark C. Pierce

Currently, the standard method to diagnose cancer is through qualitative assessment of histopathology slides prepared from a biopsy specimen. However, this process is subjective, and the result can vary depending on the expertise of the pathologist. There is a clinical need to develop new methods that can provide an objective assessment of tissue state and to quantify the different cell types present.

We are exploring the use of rare-earth (RE) doped nanoparticles to target specific cancer biomarkers for optical imaging. After being excited with light at a single Near-Infrared (NIR) wavelength, RE doped nanoparticles can emit light at both visible and Short-Wave Infrared (SWIR) wavelengths. In addition, different RE dopants such as Thulium and Erbium exhibit spectrally distinct emissions, potentially enabling imaging of different targeted biomarkers in a single sample.

A line-scanning confocal microscope was developed to image RE emissions and to isolate specific RE spectra in the visible and SWIR wavelength ranges. We demonstrate being able to isolate each RE emission type *in vitro* by selecting the appropriate optical filters. We are currently analyzing the system's ability to measure multiple RE emissions from cells labeled *in vitro* and relate them to the targeted cancer cell type.

When these different REs are tailored to target specific cancer biomarkers, they could be used as contrast agents to aid in cancer diagnosis and the study of tumor heterogeneity.

#22. An Improved CSI Based Device Free Indoor Localization Using Machine Learning Based Classification Approach

Tahsina Farah Sanam, Hana Godrich

Indoor positioning system (IPS) has shown great potentials with the growth of context-aware computing. Typical IPS requires the tracked subject to carry a physical device. In this study, we present MaLDIP, a novel, machine learning based, device free technique for indoor positioning. To design the device free setting, we exploited the Channel State Information (CSI) obtained from Multiple Input Multiple Output Orthogonal Frequency-Division Multiplexing (MIMO-OFDM). The system works by utilizing frequency diversity and spatial diversity properties of CSI at target location by correlating the impact of human presence to certain changes on the received signal features. However, accurate modeling of the effect of a subject on fine grained CSI is challenging due to the presence of reflections, fading and scattering. We propose a novel subcarrier selection method to remove the multipath affected subcarriers to improve the performance of localization. We select the most location-dependent features from channel response based upon the wireless propagation model and propose to apply a machine learning based approach for location estimation, where the localization problem is shifted to a cell identification problem using the Support Vector Machine (SVM) based classifier. Experimental results show that MaLDIP can estimate location in a passive device free setting with a high accuracy using MIMO-OFDM system.

#23. Chemogenetic activation of the PRAM pathway prevents light deprivation-induced augmentation of orexin/hypocretin expressing neurons and depressive-like behavior

Shayna L, O'Connor, Hannah E. Bowrey, Morgan H. James & Gary Aston-Jones

Introduction: The 24 h light-dark (LD) cycle has a robust influence on behavior and mood. Deviations from this light cycle induce depression and depressive-like behavior, which can be ameliorated by artificially restoring normal light pattern information via phototherapy. We have recently demonstrated that the activation of a trisynaptic (retina->supra chiasmatic nucleus (SCN)->dorsomedial hypothalamus (DMH)->locus coeruleus (LC)) pathway, termed the PRAM (photic regulation of arousal and mood) pathway, prevents light deprivation-induced depression-like behavior. Previous research indicates that an orexin-dependent mechanism may underlie this depression-like behavior. As the DMH is a critical node in the PRAM pathway, we analyzed the expression of orexin in the DMH and the surrounding perifornical area (PF) and lateral hypothalamic (LH) orexin cell fields in animals that received PRAM stimulation during chronic light deprivation.

Methods: Male Sprague Dawley rats received intraocular injections of an AAV encoding a Gq-linked designer receptor exclusively activated by designer drugs (DREADD: AAV2-hSyn-hM3D(Gq)-mCherry; n=12) or control virus (AAV2-hSyn-EGFP; n=10) . Rats were placed in continuous darkness for 8 wk, and those that received virus were concurrently subjected to daily i.p. injections of the DREADD agonist clozapine-N-oxide (CNO). A control group (n=10) received no virus and was maintained on a regular 12:12 light/dark cycle. Rats were then subjected to assays of mood (saccharin preference test, elevated plus maze and forced swim test) and vision (electroretinagram: ERG). Rats were perfused using 4% paraformaldehyde, and brains were cut into 40 μm thick cryosections. Hypothalamic tissue was immunohistochemically stained for orexin-A and c-Fos.

Results: PRAM pathway stimulation enhanced retinal ERG signals and activated key PRAM pathway structures, including orexin neurons. Constant light deprivation induced a depression-like phenotype in control animals, which was augmented in DREADD animals given daily CNO. The abundance of orexin-A-immunoreactivity (IR) in DMH, LH and PF was affected by chronic light deprivation. Activation of the PRAM pathway prevented the light deprivation-induced reduction of orexin-A-IR in DMH, LH and PF.

Conclusions: Dysfunctional orexinergic signaling may underlie light deprivation-induced depression-like behavior. PRAM pathway stimulation may prevent the augmentation of orexin-A-IR that occurs as a result of light deprivation. The PRAM pathway presents a novel circuit for the regulation of mood, and thus a possible new direction for the treatment of depression in humans.

#24. Autophagy Modulates Lipid Metabolism to Support Liver Kinase B1 (LKB1)-Deficient Lung Tumor Growth

Vrushank Dharmesh Bhatt, Zhixian Hu, Xiaoyang Su, Jessie Yanxiang Guo

Autophagy degrades and recycles macromolecules for cells to survive starvation. In genetically engineered mouse models (GEMMs) for human non-small cell lung cancer (NSCLC), autophagy supports Kras-driven lung tumor growth with or without Trp53. Tumor suppressor liver kinase B1 (LKB1) activates 5'-adenosine monophosphate protein kinase (AMPK) to maintain energy homeostasis. LKB1 mutations are detected in 20-30% of NSCLC, causing aggressive tumor growth and resistance to chemotherapy. Identifying novel target to improve LKB1-deficient tumor treatment is urgently needed. Using GEMMs for NSCLC with oncogenic Kras and LKB1 loss (**KL**), we found that autophagy deficiency increased the survival of the mice bearing $Atg7^{-/-}$ tumors compared to mice bearing wild-type (WT) tumors. To determine the mechanism of autophagy in supporting LKB1-deficient Kras-driven lung tumor growth, tumor derived cell lines (TDCLs) were generated from the lung tumors of these mice. We found that *Atg7* null TDCLs were more sensitive to glucose and glutamine deprivation-induced cell death compared to *Atg7* WT TDCLs. *Atg7* null TDCLs were also more sensitive to starvation-induced cell death than *Atg7* WT TDCLs, which can be rescued by glucose, glutamine, pyruvate, lactate and nucleotide supplementation. Thus, autophagy is required for KL TDCLs to tolerate metabolic stress. Furthermore, we observed that palmitate supplementation successfully rescued starvation-induced *Atg7* null TDCLs death, indicating that autophagy is required to maintain free fatty acid level for cells to survive starvation. We further performed metabolomics in TDCLs in normal and starvation conditions and found that level of amino acids and intermediates for glycolysis and TCA cycle metabolism were significantly lower in Atg7 null TDCLs compared to Atg7 WT TDCLs during HBSS starvation. Surprisingly, we observed a significant increase in the levels of biotin, a precursor for fatty acid synthesis, in Atg7 null TDCLs than that in WT TDCLs, indicating that accumulation of biotin in autophagy deficient cells might be due to defective fatty acid synthesis. In support of this, we found that the lipid droplets accumulation is significantly lower in *Atg7* null KL TDCLs than that in *Atg7* WT cells. To further evaluate the functional consequences of autophagy-mediated lipid metabolism in supporting KL tumor growth, we treated *Atg7* null and WT cells with etomoxir, an irreversible inhibitor of carnitine palmitoyltransferase-1 (CPT-1) to inhibit fatty acid oxidation, in normal and starvation conditions. We found that *Atg7* null TDCLs are much more sensitive to etomoxir treatment than WT cells. Taken together, autophagy plays a critical role in supporting lipid metabolism for cells to survive metabolic stress. Thus, a combination of autophagy inhibition with interruption of lipid metabolism could be a novel therapeutic strategy to treat LKB1-deficient lung tumor.

#25. An Attempt to Investigate the Metal Active Properties of Bacteria Found in Chitradurga Copper Mines, Karnataka

Aishwarya Santosh Deshpande, Rajni Kumari, Anand Prem Rajan

The diversity of microorganisms found in mining areas is immense and the exploration of diversity holds tremendous potential for the discovery of unique and useful strains. Microbes have the ability of interaction with minerals and metals in synthetic as well as natural environments which bring about changes in their chemical and physical states. In turn, the growth, survival and activity of microbes are also affected by the minerals and metals. Due to this ability of microorganisms, they find application in the elimination of toxic heavy metals from the environment through the process of bioremediation and other biological approaches such as bioaugmentation, bioleaching, biostimulation, composting and bioventing. These methods are inexpensive, eco-friendly (utilize solar energy) and natural processes. Among extremophiles, Chemolithotrophic bacteria play a very important role in nature. Their metal solubilizing ability is mainly used in bioleaching process and holds applications in electronic waste management, metal pollution reduction, bioremediation etc.

In this study, we have isolated and characterised a strain from Chitradurga copper mines, Karnataka. The results of gram staining, biochemical tests and scanning electron microscopy revealed that the isolate was gram positive, rod shaped, used sodium citrate as its only carbon source, could perform mixed acid fermentation upon glucose supply and had the ability to produce acetoin as well as the enzyme cytochrome oxidase. Through 16S rRNA sequencing, it was observed that the bacterial isolate showed maximum sequence similarity with *Bacillus humi*. The isolate was tolerant to high concentrations of cobalt, zinc and nickel and could accumulate cobalt, cadmium and to a lesser extent, copper. The new strain has been deposited in NCBI database. This study has helped in the discovery of yet another useful bacterial strain from mining areas.
Since the strain displays tolerance for high concentrations of cobalt, nickel and zinc and can accumulate significant amount of cobalt and minimal amount of copper and cadmium, it can be concluded that this strain possesses metal active properties which can have potential applications in various biological processes for elimination of toxic metals.

#26. Macrophage Response to Hemoglobin-based Treatments for Chronic Wounds

Paulina Krzyszczyk, Kishan Patel, Kristopher Richardson, Rene Schloss, Martin Yarmush, Andre Palmer, Francois Berthiaume

Chronic wounds affect over 6 million Americans, including those that are bed-ridden, have blood flow problems and suffer from diabetes. Unlike small cuts and scrapes, chronic wounds resist typical treatments and do not heal. The skin remains open, painful and susceptible to infection. Oftentimes, amputation of the affected limb is necessary to prevent further spread of damage. Chronic wounds can develop as a result of low levels of oxygen as well as the presence of a high number of cells that promote inflammation and tissue damage, called M1 macrophages. Wounds will heal when there are higher numbers of a different type of macrophages, called M2 macrophages, which promote tissue growth. Chronic wounds unfortunately lack M2 macrophages, and therefore, do not heal.

This work aims to develop a hemoglobin-based therapy to overcome the problems of low oxygenation and overabundance of M1 macrophages in chronic wounds. Hemoglobin is the protein in our blood that binds and delivers oxygen. In some circumstances, it can also act on macrophages by changing them from M1 to M2, thereby reducing damaging effects and promoting healing. Some characteristics of M1 macrophages are that they have higher levels of damaging reactive oxygen species (ROS) and they are more rounded in shape compared to M2 macrophages.

We have modified hemoglobin to form PolyHbs, which consist of several hemoglobin molecules bound together. The benefit of PolyHb is that more oxygen can be delivered depending on the structure of the Hb molecules contained within them, which could have a beneficial effect on wound healing. In addition, PolyHbs may be tolerated better by cells compared to regular Hb, as PolyHbs contain bonds that make it more difficult for the heme group of Hb to be released. Extracellular heme can create more ROS and result in cellular damage and death, which must be avoided to promote tissue growth and wound healing.

In this study, we test the effects of Hb and PolyHbs on macrophages from three different human donors. We measure the levels of intracellular ROS and degree of cellular elongation in response to the treatments. We aim to understand the direct effects of Hb/PolyHbs on macrophages in cell-culture dishes, with the overarching goal of creating an anti-inflammatory treatment to promote healing of chronic wounds.

#27. A novel treatment for diabetic nephropathy using the targeted administration of an extracellular matrix inhibitor

Oren Merhav, Alexandra Pastino, Mariana Nogueira de Lima, Joachim Kohn

Approximately 40% of all type 1 and type 2 diabetic patients are affected by diabetic nephropathy (DN), which is characterized by excess production of extracellular matrix (ECM) proteins such as fibronectin and collagen IV by mesangial cells in the glomeruli of the kidney. This causes the obstruction of the blood vessels that compose it, the impediment of the total functionality of the glomerulus, and ultimately kidney failure in severe cases. The body largely cannot degrade ECM proteins on its own, and so it is up to external medication to artificially degrade excess ECM or to inhibit its production. Today, the only reliably successful treatment for DN is ACE inhibitors, which delay the need for a kidney transplant, but do not eliminate it altogether.

The use of ECM inhibitors has very limited use in clinical practice due to their harmful effects on healthy tissue. This creates the need for an accurate targeted drug delivery system that will unload a drug within the kidney and nowhere else in the body.

Our research aims to develop a drug delivery system made of biodegradable materials that will deposit an antifibrotic drug in the kidney. This study focuses on the delivery of the peptide sequence SDKP, which has been shown to reduce the transcription of genes coding for fibronectin expression. We conducted tests for cytotoxicity and reduced fibronectin assembly in cells exposed to SDKP *in vitro*.

The peptide will be housed within self-assembling tyrosine based nanospheres, or TyroSpheres, which fully biodegrade after around six months. Mounted to the outside of the Tyrospheres will be peptide chains of the sequence CLPVASC, which has been shown to have significant preferential binding to kidney cells. The CLPVASC chains will anchor the TyroSpheres to kidney cells until they degrade and release the SDKP sequences into the cells.

#28. Polymerized Hemoglobin for Enhanced Oxygen Transport in Liver Bioreactor

Andrew Pskowski and Jeffrey Zahn

Introduction: Blood flow in the microvasculature plays a large role in the transportation of nutrients and oxygen to the surrounding tissues. With vessels as small as 4 μm in diameter, transportation through the microvasculature is contingent upon deformability of the red blood cells (RBCs). This leads to interesting dynamics occurring. Recently *in silico* modeling demonstrated RBCs "lingering" at bifurcations in the microvasculature[1], causing changes in vessel resistance and hematocrit distribution. In order to further understand this phenomena and others, we present the use of microfluidics to study RBC dynamics in the microvasculature.

Materials and Methods: *Sample preparation*: Blood was collected through finger prick from healthy consenting volunteers into a heparinized tube (Ram Scientific, Nashville, TN) and washed 3 times in phosphate-buffered solution (PBS). The sample was then diluted in PBS to reduce the hematocrit to approximately 25% and 5% hematocrit. *Experimental Setup:* A Y shaped bifurcating microchannel (figure 1A) was fabricated using traditional soft lithography techniques. The channel dimensions are as follows: the feeder channel width was 8 μm wide, both daughter channels were 6 μm wide. 1mm holes are punched in the feeder and daughter channels. The height throughout the device is 6.5 μm. Prior to perfusion the device was incubated in a 1% bovine serum albumin for 1 hour to prevent non-specific brining. Electronic pressure regulators control both the input pressure (P_{in}) and a back pressure (P_{back}) on one outlet in order to control flow rate in the daughter channels. The other daughter channel outlet is left open to the atmosphere. Images were captured at 40X magnification on a high speed camera and were analyzed via custom MATLAB software.

Results and Discussion: We were successful in perfusingg RBCs through the device as well as recording and quantifying the flow. We were able to observe evidence of both the Zweifach-Fung effect and RB C ligner.

Conclusions: The study shows that this cell lingering does occur in our bifurcation vessel model as a function of HCT. Further studies will involve both changing the angle of the bifurcation, channel size, modulating the rigidity/deformability of RBCs, as well as the use of an artificial microvasculature network with physiological levels of hematocrit in order to study how the increase in channel resistance effects cell partitioning both upstream and downstream of the bifurcation.

#29. Developing a Solid-State Nuclear Magnetic Resonance (ss-NMR) Methodology to Characterize Interactions between pH domains and PIPs in the Cell Membrane

Nuozhou Chen, Donald Andrew Belcher, Sunshine Littlecreek, Jeffery D. Zahn, Andre Palmer, Francois Berthiaume

Liver is the second largest organ inside human body located in the right upper abdomen under the rib cage. It burdens many synthetic, metabolic, biotransformatic functions and detoxification. All liver injuries and diseases progress in similar ways towards end-stage liver disease (ELSD) which means a life-threatening loss of liver function. Unfortunately, the only clinically efficient treatment for end-stage liver disease is liver transplantation, but many patients die before a suitable donor is found. Thus, there is a need for a liver-supporting therapy that could serve as bridge to transplantation. This could be accomplished by a bioartificial liver device consisting of a bioreactor containing liver cells that mimic native liver function.

Oxygen transfer is a great challenge in bioartificial livers since liver consumes up to 30% of body's basal oxygen supply. The liver exhibits a metabolic zonation along with an oxygen gradient between the periportal inlet and the perivenous outlet. We hypothesize that by recreating an *in vivo*-like oxygen gradient in a hollow fiber-based bioreactor, this metabolic zonation will be recapitulated with an improved device performance. To test this, we perfuse a bioreactor loaded with hepatoma cells (HepG2/C3A) with polymerized hemoglobin (PolyHb) as oxygen carrier through hollow fibers. PolyHb is a cross-linked form of hemoglobin (Hb) with decreased cytotoxicity available in different oxygen binding affinities.

A tolerable PolyHb dose was determined by exposing HepG2/C3A cells to different concentrations of PolyHb and uncrosslinked Hb for up to 6 days. The data suggest that HepG2/C3A cells tolerate up to 10mg/ml of PolyHb with no significant decrease in total cell metabolic activity compared to vehicle controls. In general, viable cell number was higher in the PolyHb groups compared to the Hb group, highlighting the advantage of using PolyHb. We also evaluated the impact of 10mg/ml PolyHbs on common liver cell functionalities such as albumin production, ammonia removal, and xenobiotic metabolic activities. These data will inform the design of a small-scale bioreactor system where the effect of a physiological relevant oxygen gradient will be evaluated. Using microfabrication techniques we will be able to explore operational conditions of a full-sized bioartificial in a much smaller scale, which allows a time and cost efficient screening of different conditions.

#30. Novel Impact in Actinide Chemistry: Thorium (IV) Chalcogenido Cubane Clusters

Stefany Lazieh, Jacqueline Perodeau, Ashley Bernstein, Andrew Nieuwkoop

The human body is constantly perceiving external signals and reacting to them. Similarly, all of our cells are able to sense extracellular signals and respond to them appropriately. In order for the human body to function properly, millions of cellular interactions, signaling pathways, and regulatory mechanisms must be properly balanced to ensure that cells are able to respond to changes in their environments. Many cells are able to do this through protein-lipid interactions, which can alert the cell that an external signal is present and a response is necessary. Understanding how some proteins and lipids interact can often be very challenging but is essential for elucidating signaling pathways that allow human cells to function properly.

An important class of lipids found in the cell membrane is phosphatidylinositol phosphates (PIPs). These lipids are involved in diverse cellular processes, including regulating membrane traffic, controlling ion channels, and participating in signal transduction pathways. Since PIPs are involved in so many processes in a cell, it is essential to maintain balance of different PIP concentrations to prevent cancer, diabetes, and many other health issues from developing as a result of misregulation.

One type of protein domain involved in many cellular processes is the pleckstrin homology (PH) domain. Many PH domains interact with PIPs to regulate cellular development and promote signaling transduction pathways. The human protein Akt2 (which has a PH domain) is important for promoting cell growth and reducing the rate of cell death. If Akt2 is over-activated, cells grow uncontrollably and become cancerous. This over-activation can happen if too many PIPs are present in the membrane due to unregulated PIP formation. Understanding how this PH domain is able to bind to PIPs can elucidate ways to fix misregulations that cause health issues.

Characterizing interactions between PH domains and PIPs is essential to understanding where and how misregulation can lead to disease. Solid-state nuclear magnetic resonance (ss-NMR) is an analytical technique that utilizes the magnetic properties of subatomic particles to understand the structure of molecules. By conducting ss-NMR experiments on PH domains bound to PIPs, we hope to understand which parts of the protein are involved in binding to the PIP. This research can become the basis for the development of drug molecules that target misregulated signaling pathways. In this way, our understanding of protein-lipid interactions can improve the lives of people who develop disorders or diseases from misregulated cellular processes.

#31. Data Analysis of the Retina Leads to Discovery of Novel Photoreceptor Gene

Marissa A. Ringgold, David Rehe, Anna Y. Kornienko, Tom J. Emge, John G. Brennan

Actinides are located on bottom row of the periodic table that include rare radioactive materials most of which are not found in nature. The most notable of these compounds are uranium, plutonium, and thorium. These elements are unique in that they can achieve variable valencies due to their accessible f-electron oribital shell. This means that actinides can have more components directly bound to the metal because there is space that accommodates the extra electron density. Though the public is most familiar with actinides in the context of nuclear weapons, understanding these compounds can aid in environmental restoration, nuclear decommission, and even future energy demands.

Traditionally in literature these compounds are synthesized with large stabilizing ligands that make them easy to isolate. However, understanding the how these metals bind is still relatively unexplored especially in reference to chalcogens. Chalcogens and their corresponding ligands contain elements from group 16 which include oxygen, sulfur, selenium, tellurium, and polonium. An understanding of bonding in actinide compounds is critical to the development of actinide chemistry and interpreting the complex physical properties they exhibit. Additionally, a comprehensive understanding of how and why they react is essential to capitalizing on the unique properties for use in emerging technologies.

Our research works to explain the nature of bonding between actinides and chalcogenolates specifically thorium with sulfur and selenium. Cluster compounds have been made that yield a cube-like core configuration. These cubanes, $(py)_8Th_4(\mu_3\text{-}E')_4(\mu_2\text{-}EPh)_4(\eta\text{-}EPh)_4$ (E, E′ = S, Se), were prepared from ligand-based redox reactions of elemental E′ with $Th(EPh)_4$. Essentially, a mercury catalyst inserts into the E-E bond of PhEEPh to form a single metal containing compound $(py)_3Th(EPh)_4$. From there elemental E' (S or Se) is added which inserts into the Th-EPh bond forcing the $^{-}$EPh groups to rearrange and attach as both terminal and bridging components around the cubic core. Products with all four possible E/E′ combinations (E,E′ = S,S; Se,Se; S,Se; Se,S) were isolated as solid crystals and structurally characterized.

To further investigate the unique properties of these compounds they were examined by a multitude of experiments. The first was structural characterization by single crystal x-ray diffraction in which solid crystalline material is analyzed using x-rays. The spectra contains individual bright circles on a dark background and is unique for every crystalline compound. Then, a bulk of ground crystals was heated to deliver solid solutions of ThS_xSe_{2-x}. The technique allows for a solid-state material to be made in a more efficient manner than is conventionally employed. Next nuclear magnetic resonance (NMR) spectroscopy was utilized by dissolving the crystal in solution and observing the effects of an intense magnetic field. This assessment gives information about the connectivity of the compound and stability of these compounds in liquid solutions. These results were compared with computational analysis to provide further insight.

#32. Design and Implementation of Multi-Medium Unmanned Aerial Vehicles

Alexandria Pinto, Xin Ai and Li Cai

Stem cells are cells that have the ability to become any type of cell, such as a skin cell, liver cell, or photoreceptor cell, through a process called differentiation. Differentiation typically starts after the cells have finished cell division. During differentiation, genes are up or down regulated (up regulated genes being present more frequently and down regulated genes present less frequently) during transcription of DNA to RNA, based on the physical and chemical conditions outside the cell. Topoisomerase II β (TOP2B) is an enzyme that participates in the unwinding of DNA, an important step to allow transcription to occur. Our lab has found that the *Top2b* gene is expressed in retinal cells that have finished cell division, suggesting that the TOP2B enzyme has a function in retinal cell differentiation.

To analyze the function of TOP2B in the retina, we utilized single cell RNA sequencing data. Single cell RNA sequencing (scRNA-seq) data allows users to analyze mRNA expression for a single cell in complex tissues with multiple cell types, such as the retina. The retina contains six different types of cells – bipolar, Müller glia, ganglion, horizontal, amacrine and photoreceptor cells. During development, differentiation determines which cell type of the retinal tissue each individual stem cell will become. scRNA-seq data allows scientists to discover genetic differences between cell types in a tissue. In the case of the retina, the cell type of particular interest is the photoreceptor cells (rods and cones), which are responsible for converting incoming light into a signal that will be passed to the brain resulting in vision. If we can understand how photoreceptor cells develop and differentiate, we can find targets to use in genetic therapies for diseases such as macular degeneration and color-blindness.

We identified genes that have been impacted by *Top2b* expression utilizing publicly available scRNA-seq mice retinal datasets from multiple developmental time points. Using bioinformatics software packages, we separated different cell types by clustering cells based on individual gene expression. We found gene *Fam19a3* upregulated with *Top2b* expression and correlating strongly with cone photoreceptor cells, suggesting *Fam19a3* is somehow related to the development of cones. This research allows for the identification of genes that are involved in differentiation, which scientists can then use to artificially up or down regulate the gene's expression, and thus see the impact on the organism as a whole. In the future, our lab will be down regulating *Fam19a3's* expression and in this way further identify its role in cone cells and vision.

#33. Altered Orexin Cell Function in Obese Female Rats with a History of Binge-like Eating

Aristedes Costeas, Andrii Hlyvko, Dr. Marco Maia, Dr. F. Javier Diez

There have been great strides in the development of unmanned systems in recent years, particularly regarding Unmanned Aerial Vehicles (UAV) as it is an area that has exciting applications and could benefit from a variety of improvements. Another segment that has seen an abundance of innovation recently is Unmanned Underwater Vehicles (UUV). But, they are normally studied separately and designed with a high degree of specialization to the corresponding medium. There is much to gain from the assimilation of these two disciplines into one that encompasses multiple mediums.

Multi-medium vehicles, which are capable of operating in multiple fluid mediums such as air and water, are a new paradigm in unmanned systems and the multirotor platform is an exceptional candidate configuration. Vehicles that can swim and fly are few and those that do exist are not maneuverable enough to accomplish complex missions. Bridging the efforts made in UAV and UUV to present a vehicle that is capable of both aerial and underwater navigation is of interest. Benefits include rapid deployment for both air/underwater missions (ie.search/rescue), point-to-point underwater mapping and object recognition without needing to consistently overcome opposite water currents.

Research efforts in this area include improving the vehicle control strategy and optimizing the overall system as well as investigating different multirotor configurations and scaling. The two main configurations that are currently being investigated are the quadcopter and the coaxial multirotor vehicles. Each comes with its own benefits and drawbacks, but both offer intriguing solutions to the multi-medium vehicle problem. The quadcopter platform offers increased maneuverability and superior stability as well as a lower prototyping and manufacturing cost. On the other hand, the coaxial platform, although more difficult to control and stabilize, offers many efficiency benefits and longer flight times which directly translates to longer missions. Scalability of these vehicles is also often a challenge especially when trying to reduce the size of these UAV's due to the introduction of low Reynolds number flows which behave very differently and are much less documented than high Reynolds number flows. For reference, the Reynolds number is a dimensionless value that measures the ratio of inertial to viscous forces and describes the degree of laminar to turbulent flow. In order to investigate this space, multiple micro UAV's have been created and are currently being tested in our lab.

#34. Classifying DNA Reads to be of Prokaryotic or Eukaryotic Origin Using Machine Learning Algorithms

Samuel Liu, Morgan H. James, Sarah Walsh, Hannah E. Bowrey, Nicholas T. Bello, Gary Aston-Jones

Binge eating disorder (BED) is characterized by a progressive escalation of intake of highly palatable food and increased responsivity to food-associated cues that induce craving and overeating. In this way, many of the clinical characteristics of BED closely resemble symptoms of drug abuse disorders, pointing to possible commonalities in the neural circuitry underlying these disorders. We and others have reported that the hypothalamic orexin (hypocretin) system is critically involved in the expression of highly-motivated drug seeking behavior, however its role in compulsive food seeking is not well understood. Here, we tested the hypothesis that orexin-1 receptor signaling mediates food motivation in female rats with a history of binge-like eating. In addition, we examined the role of obesity on our outcomes.

Using a within subjects design, female Long-Evans rats were assessed for baseline economic demand for sucrose using a novel behavioral economics paradigm. Binge-like eating was induced by exposing rats to sweetened fat (vegetable shortening/10% sucrose) for 30 min, twice/wk for 4wk, before being re-assessed for sucrose demand and following injections of the orexin-1 receptor antagonist SB-334867 (0,10,30mg/kg, ip). Rats were then exposed to a high fat diet (HFD; 45% fat) for 8wk, and the experiment was repeated. Sweetened fat binge intake was measured following SB dosing. Binge eating increased sucrose demand only after HFD- exposure, which was dose-dependently reversed by SB. Binge intake was not altered by HFD exposure. SB also dose-dependently decreased sweetened fat binge intake after HFD exposure. We also found increased orexin cell number within the lateral hypothalamus area. Our findings indicate an interaction between binge-like eating and excessive weight gain with respect to motivation for food. This effect was blocked by an orexin-1 antagonist, highlighting the orexin system as a potential novel target for pharmacotherapies for controlling overeating episodes in individuals with obesity. Ongoing studies are investigating the effect of excessive weight gain and binge-like eating on other properties of the orexin system.

#35. Predicting and Testing Variant HCV Proteases for Selected Substrate Sequences via Computational Design

Chahna Patel, Yannick Mahlich, Yana Bromberg

Organisms were classified as eukaryotes or prokaryotes depending on their DNA's AT content and the specific 3mers. In the world of bioinformatics, there has been a huge explosion in genomic data. However, with this expansion in data there also comes cross contamination which can affect the results of other bioinformatic studies. A scientist looking to only conduct experiments on bacterial DNA (prokaryotes) might have their results altered if there is cross contamination of fungi DNA(eukaryotes). Designing a tool that can classify eukaryotes from prokaryotes based on aspects of their DNA will allow them to be filtered from genomic data. The process consists of first downloading whole genome sequences of prokaryotes and eukaryotes from NCBI. These genome sequences are then spliced into 100-200 base pair fragments, and each fragment's AT content and 3mer content are calculated. A data file with all of this information is put into the machine learning tool SciKit from Python. The AT content and 3mer content are considered "features" that the machine learning tool can use for classification. In this tool, the best machine learning model is developed through testing and training the data set, and determining which features and number of features give the best result. This machine learning model is used as the final predictor, that can distinguish eukaryotes from prokaryotes.

Chahna Patel is a rising junior majoring in Biomedical Engineering, with an interest in bioinformatics and medical devices. She conducted research in bioinformatics in The Bromberg Lab this summer with Yana Bromberg, an assistant professor in the Department of Biochemistry and Microbiology. Her passion for biology, math and computer science led her to be interested in the field of bioinformatics. She is the Internal Vice President of the Rutgers SASE (Society of Asian Scientists and Engineers) chapter and also a member of Rutgers ENABLE. She has utilized the opportunities given by Douglass Residential College, by participating in an externship at Picatinny Arsenal through DRC her Freshman Year. Her future aspirations include attending graduate school and working in the biotech industry.

#36. Injectable Solution of a Green Tea Compound as a Potential Preventative Measure for Cartilage and Connective Tissue Damage

Jeremy Mahr, Joseph Lubin, Sagar Khare

Proteases are enzymes that perform proteolysis: the process of breaking down other proteins. They are found in all organisms and are essential for diverse biological functions. Trypsin, for example, helps to break down the proteins in your food into smaller fragments so that your body can use them. We can use this ability of proteases to break down other proteins for applications in human health and wellness. Htra1, for example, is an enzyme capable of cleaving tau fibrils that build up in the brains of Alzheimer's disease patients. Studying how proteases are able to determine where and where not to cut helps us better understand their biological roles while building better enzymes to cut targets of interest. Currently, we are in the process of computationally designing proteases using a computer program called Rosetta, that we predict are capable of cleaving selected protein targets, or substrates. These designed enzymes are all sequence substitutions of the HCV protease. Based on our biophysical models constructed on Rosetta, we select the proteases that appear to best bind specific target residues in order to cleave them at the right location. Then, we experimentally validate the selected proteases by observing if they cleave as expected. This allows us to test our predictions, and if necessary, make further refinements to our detection and identification system.

#37. Polymerized Hemoglobin for Enhanced Oxygen Transport in Liver Bioreactor

Mary Pat Reiter, Shawn Ward, Michael Pellegrini, Moti L Tiku, Adrian B Mann, Joseph W Freeman

Cartilage and connective tissue damage can be the result of any number of conditions including genetic disorders, athletic injury, and degenerative diseases. The focus of this research was for treatment of osteoarthritis of the articular cartilage in the knee and the sprains and strains of connective tissue in patients with genetic disorders such as Ehlers-Danlos syndrome. Osteoarthritis (OA) is a debilitating disease defined by degradation of joint tissue resulting in significant pain and hindered joint mobility diminishing patients' quality of life. OA's impact on articular cartilage is both marked by areas of thinned cartilage and exposed bone and a decrease in the mechanical properties of the remaining cartilage. Sprains and strains are a serious concern for injury-prone patients especially those with connective tissue disorders since such conditions cause joint instability and reduced tendon and ligament strength making healing difficult. Few treatments exist for people who have osteoarthritis or connective tissue disorders making development of prevention and treatment methods a critical goal of healthcare, tissue engineering, and biomechanics research.

Green tea's effects on the human body have been studied for decades and sworn by for over a millennium, but recent research has shown that its most abundant catechin, Epigallocatechin Gallate (EGCG), is responsible for many of its benefits. EGCG is a polyphenolic compound and has been shown to alter the levels of inflammatory cytokines as well as cross-link with collagen, a major component of articular cartilage and connective tissues. Crosslinking is the process of bonding two or more molecules chemically which may alter the material and mechanical properties of the given sample.

Our research aims to test whether treatment with this green tea polyphenol could increase the mechanical properties of cartilage and connective tissue. Two animal experiments were designed to test this hypothesis: a nanoindentation study of *in vivo*-treated rat hindleg tibial articular cartilage and a tensile study of *in vitro*-treated rat tail tendons. Nanoindentation and tensile testing both record load and displacement at given time points which can be used to calculate elastic modulus, hardness, and ultimate tensile strength which are three mechanical properties in which we were interested. By identifying how these mechanical properties change, we can gain an understanding of the mechanisms by which the alterations occur. Our research on EGCG's strengthening effects using mechanical testing is a crucial step for developing a preventative treatment for these two conditions. As this research continues with similar studies, our lab looks ahead to how the treatment can best be utilized and in what novel ways it can improve the lives of patients with other conditions.

#38. Human induced pluripotent stem cells as a model for study of Duchenne Muscular Dystrophy

Kalina Andrysiak, Józef Dulak

Duchenne Muscular Dystrophy is an X-linked recessive genetic disease caused by a mutation in DMD gene. DMD gene is the largest known human gene encoding protein called dystrophin. This protein is a component of dystrophin-associated glycoprotein complex connecting cytoskeleton and extracellular matrix and is primarily located in muscle cells. Its proper function is related to strengthening muscles and protect them from damage. Lack of dystrophin leads to progressive skeletal muscle degeneration and weakness, which usually begins around the age of three. By the early teens, the heart and respiratory muscles are also affected what is manifested by problems with breathing and cardiomyopathy - the leading cause of death of DMD patients (usually before 30 years of age). There is no known cure for Duchenne muscular dystrophy. Currently available treatment aims to control symptoms and lessen the severe muscle degeneration to improve quality of life.

To study the mechanisms of the disease in vitro human induced pluripotent stem cells (hiPSC) are used. The hiPSC technology was developed in 2007 by Shinya Yamanaka, who showed, that the introduction of four transcription factors into adult somatic cells can convert them to stem cells with a potency of differentiation into cells of the three germ layers. iPSC generated from somatic cells of patients with DMD can be differentiated to skeletal muscle cells or cardiomyocytes and provide the model for in vitro studies.

Currently, in addition to the traditional 2D cell culture system, which is relatively easy and has well-known methodology, new cell culture models are being sought that would better reflect physiological conditions and have greater environmental control. 3D cell culture systems (spheroids and organoids) allow to study cell-cell and cell-ECM interactions, but on the other hand, it is difficult to produce them at a bigger scale, control the conditions in the inner parts and there is a lack of fluid perfusion in such cell cultures. Therefore, new tool called “organs-on-a-chip” was developed. There are usually based on advanced microfluidic devices allowing the flow of medium, parameters control, mechanical and electrical stimulation and better imaging of the cells located inside. This technology seems to be promising, also in terms of DMD modeling, as it can provide a tool for better understanding of the mechanisms of the muscle cell degeneration.

#39. Developing Robust and High-Performance Functional Materials for Clean Energy Applications

Yang Fang, Jing Li Research Group

Currently, our research focuses on the design, synthesis, characterization and clean-energy related applications of two important material classes: Metal-Organic Frameworks (MOFs) and inorganic-organic hybrid semiconductors. MOFs (also referred to as coordination polymers) are crystalline solids consisting of discrete metal cations or clusters bridged by organic linkers to form one-dimensional (1-D), two-dimensional (2-D) or three-dimensional (3-D) extended networks. The combination of organic and metal functionalities with permanent porosity and nearly infinite structural tunability lead to very interesting and diverse properties making them promising for a number of applications including but not limited to: gas storage and separation, luminescence based sensing and imaging, catalysis, optoelectronics and energy storage. The current topics of our MOF research include (a) energy-efficient capture and separation of various chemical species in gas, vapor or solution phase, (b) selective detection and sensing of harmful molecules such as high explosives, volatile organic compounds and heavy metals, and (c) development of alternative lighting phosphors free of rare-earth-elements (REEs). Our research on hybrid semiconductors aims at developing crystalline materials that incorporate organic and inorganic components into a single crystal lattice. Such a combination not only leads to modified and enhanced properties due to the individual components, but also new features and unique phenomena that are not possible by either component alone. We have discovered several new classes of hybrid nanostructured crystals that are comprised of sub-nanometer sized semiconductor motifs of II-VI, III-VI, VI-VI and I-VII (inorganic component) and various ligands with mono- or multi-binding sites (organic component). These hybrid materials possess a number of enhanced properties compared to their parent bulk semiconductors, including broad and systematic band-gap tunability, high absorption coefficients and significantly improved photoluminescence. They also possess rich **structural** chemistry and exhibit interesting structure-related thermal properties.

#40. Hydrogel Electrode Coatings for Improving Treatment of Neurodegenerative Disorders

Erika Davidoff and Jay C. Sy

Many chronic neurodegenerative disorders, including epilepsy, Parkinson's disease, dystonia, OCD, chronic pain, and PTSD, can be treated by implanting electrodes into the brain that deliver electric current to counteract abnormalities in neuron signaling. Unfortunately, friction between the stiff metal implant and surrounding brain tissue triggers a foreign body response, causing inflammation, which can lead to further tissue damage, and gliosis, the formation of scar tissue around the electrode that insulates the electrode and prevents it from functioning. Many researchers have suggested using soft coatings made of hydrogels—water-rich networks of chain-like polymer molecules—to lessen the foreign body response. However, existing coatings are made from gels held together by covalent bonds, which irreversibly break under high strain. We are working with non-covalently linked gels, which use other types of molecular interactions to form. Our gels are shear-thinning and self-healing, meaning that they disassemble to absorb strain energy and reassemble when the strain is lifted. Coatings made of these gels are more effective and more durable. We are also developing a custom bioreactor to test the effectiveness of these coatings in cultured cells, reducing the number of expensive and lengthy animal studies needed to evaluate different coatings. Combined use of our unique device and established animal model studies is helping us identify novel hydrogel coating systems that better protect brain tissue from the negative effects of implanting electrodes, enabling the implant treatment to work longer and more efficiently.

#41. Designing New Inhibitors for Mycobacterium Tuberculosis Using Bioinformatics

Eujin Lim. Dr. Phalguni Ghosh, Dr. Sutapa Ghosh, Dr. Brian Lavey, Dr. Rowley

Traditional protocols like 2D approaches for drug discovery, it does not consider the 3D the active site of the protein, thus requiring more resources and time. With the use of bioinformatic software to study the 3D structure, specific amino acid interactions can now be identified. Also by following this approach, we reduce the amount of time and resources during the discovery process. With the use of bioinformatic tools, users can make more accurate design choices compared to 2D Structure Based Drug Design, and reduced the resources needed to synthesize the ligands using other methods.

Tuberculosis is currently the ninth deadliest disease in the world, killing 1.4 million people yearly. The mycolic acid coating the outside of the bacteria is what makes the tuberculosis difficult to treat. With the addition of mutations in the genes of the bacteria, traditional first line drugs, such as Rifampicin and Isoniazid, are rendered useless. The goal of my research was to build a new inhibitor for an enzyme found in Mycobacterium tuberculosis called fatty acid degradation D32, better known as FadD32. This enzyme contributes to the condensation of mycolic acid, which gives its antibiotic resistance property. With the use of bioinformatic tools in our disposal, we were able to replicate the known crystallized structure of FadD32; and docked with other ligands that were designed. This allowed us to analyze the binding sites and conformational change. This information will help us to provide a foundation of new molecules that have never been tested to our knowledge before. If this research is successful, then the inhibitor could potentially be further synthesized into a drug to treat patients with M. tuberculosis.

#42. Delayed Differentiation of iPSC-Derived Induced Neurons of a Tuberous Sclerosis Complex Patient

Natalie Samper, Valentina Dal Pozzo, Gabriella D'Arcangelo

The Tuberous Sclerosis Complex (TSC) is a genetic disorder associated with the growth of benign tumors on various parts of the body and has an incidence of 1 in every 6,000 live births. It often causes neurological disorders, including epilepsy, intellectual disabilities and autism that correlate with brain tumors or tubers present in the majority of patients. Dr. D'Arcangelo's lab previously established induced pluripotent stem cell (iPSC) lines from a TSC patient carrying a genetic mutation of the *TSC2* gene and an unaffected sibling control. We then generated induced neurons (iNs) from the iPSC samples using a virus. In this model system the iPSCs are converted directly into excitatory neurons over the course of few days. Cell types can be identified by immunofluorescence using the antibody markers like HuC/D, which label the neurons in the culture, and DAPI stain, which labels all the cells in culture. These fluorescent labels can be visualized using a confocal microscope imaging system. The iN cultures were used in this study to identify cellular abnormalities in TSC neurons. In particular, we discovered that patient cells become neurons less readily than healthy control cells.

This work will lead to further studies to understand the functional significance of the observed phenotype and to develop new therapies to improve the treatment of TSC patients.

#43. Changes in Orexin/Hypocretin Activity Associated with the Expression of Cocaine Anticipatory Behavior

A. J. Chang, S. L. O'Connor, M. H. James, H. E. Bowery, K. Peng, J.E. Fragale, J Nauman, G. Aston-Jones

Drug addiction, or Substance Use Disorder [SUD], is a chronically relapsing disorder that has an estimated 20.3 million sufferers in America. This disorder develops as recreational drug use leads to uncontrolled consumption and certain diagnostic criteria emerge.

With an estimated 1.5 million cocaine users in America, cocaine remains a serious public health problem. During its initial use, cocaine produces pleasant effects like decreased anxiety and social inhibitions, heightened alertness, euphoria, and increased self-perception of mastery. But as addiction and dependency develop, the pattern of intake transitions from regulated to uncontrolled, high-dose binges that can last from hours to days. Disruptions in sleep and circadian rhythmicity, and several addiction-related behaviors emerge which exhibit a clear circadian regulation.

We and others have demonstrated that the hypothalamic orexin (hypocretin) system is a critical regulator of drug-seeking behavior. Orexin neurons exhibit significant diurnal fluctuations in cell number and activity; both measures are significantly higher during the active period relative to the inactive period. Here, we tested whether these regular fluctuations in orexin function are disrupted by cocaine self-administration.

Through the use of animal models, we showed that rats exhibited an increase in general activity in the period immediately preceding cocaine self-administration, indicating that cocaine may act as an entrainable cue. Furthermore anticipatory activity was also a strong predictor of cocaine-seeking behavior, suggesting that interventions that reduce anticipatory activity may be effective at reducing cocaine-seeking behavior. We also demonstrated that cocaine self-administration disrupts regular diurnal fluctuations in orexin function, implying that this system may contribute to circadian regulation of drug-seeking behavior and that the orexin system may be a potential pharmacological target to treat circadian disruption associated with cocaine abuse.

#44. Identifying Cannabinoid Interaction within the CB1-Receptor

Joshua Alb, Phaguni Ghosh, Brian Lavey

Cannabinoids have shown the capacity and ability to treat numerous diseases and ailments such as pain, grand mal epileptic seizures, anxiety, and migraines. These small molecules interact with known cannabinoid receptors known as cannabinoid 1 (CB1) receptor and cannabinoid 2 (CB2 receptor). These cannabinoids can function as either an agonist, inverse agonist, or antagonist. Although their function is known, the binding interactions of the agonists & antagonists at a molecular level in the CB1 protein pocket is not well defined.

Our research focused on what amino acid residues interacted with inverse agonists/ antagonists versus agonist binding cannabinoids. Using a series of bioinformatic tools we were able to replicate the interaction of known cannabinoids binding to the recently crystallized CB1 receptor complexes. Our focus was to analyze antagonist, inverse agonist and agonist binding cannabinoids by docking them into their respective crystallized sites. We then took the analyzed residues from both categories (agonist & antagonist) and developed a compare and contrast list. This list generated an idea of which residue interaction was shared between agonists and antagonists, and which was not.

The next step was to then assess the unknown ΔG value that was generated from this study and compare it to their known IC50 value. The purpose of this was to examine if our study correlated with current results. The combination of these two helped us generate theoretical hypothesis of what would constitute a psychoactive cannabinoid versus what would constitute a non-psychoactive cannabinoid. This can be useful in the emerging field of cannabinoid based pharmaceuticals in the assessment of unknown cannabinoids and what their potential use might be in treatment of various disorders.

#45. High Resolution AFM Studies of Ionic Liquid Interfacial Structure

Krystal House, Robert Hayes

How can scientists build better batteries? One important, yet often overlooked strategy is to design better electrolytes – that is, the liquid in the battery with both dissolved cations (positive) and anions (negative) used to conduct electricity. This research focuses on a new, emerging class of electrolytes called ionic liquids (ILs). Unlike traditional electrolytes, ILs are highly appealing because they consist entirely of ions without solvent and so are intrinsic conductors of electricity. However, little is known about the way electrolyte ions arrange in 3D near solid surfaces, especially for ILs.

Here, we use advanced atomic force microscopy (AFM) experiments to study IL structure. An AFM operates akin to an atomic scale record player, but instead of playing music, a very sharp tip can image IL ions and measure the forces between them near a variety of solid surfaces and solution conditions. Data will be presented for ILs adjacent to model surfaces (e.g. mica), at both high and low temperature. The results show new molecular design rules for IL integration in battery and surface-science applications.

#46. Collagen at the Nanoscale: High Resolution AFM Experiments

Jonathan Roth, Robert Hayes, Jean Baum

Atomic Force Microscopy (AFM) is a powerful and versatile experimental technique that allows for high resolution imaging and material characterization. AFM works similar to a record player, but instead of musical notes, a sharp tip can visualize the structure, dynamics or physical properties of many systems from single atoms to biological molecules. In this project, AFM is used to study the protein collagen. In the human body, collagen plays a critical role in many tissues (skin, bones, blood vessels), conferring structural integrity and elasticity; fibril tensile strength is greater than steel, such that when healthy, they can withstand enormous forces without breaking. However, this behavior is still poorly understood at the nanoscale. We have used two new complementary experimental platforms from physical chemistry to study collagen: Fast Force Mapping (FFM-) & Amplitude Modulation (AM-) Atomic Force Microscopy (AFM). These experiments have yielded exquisite data with spatial resolution approaching the size of atoms (< 1 nm) and map variations in stiffness (Young's Modulus) and adhesion force.

#47. Regulation of Macrophage Phenotype by Farnesoid X Receptor during Nitrogen Mustard-Induced Lung Injury

Tanvi Banota, Alexa Murray, Debra L. Laskin

The immune system functions to protect our bodies from foreign objects, pathogens, and toxins through several lines of defense, one of which employs macrophages, or white blood cells, to help mitigate injury. Nitrogen mustard (NM) is a toxicant known to cause acute lung injury which progresses to fibrosis, the thickening of tissue. Following NM exposure, there is a sequential accumulation of pro-inflammatory/cytotoxic macrophages and anti-inflammatory/wound repair macrophages in the lung.

In these studies, we analyzed mechanisms regulating the activation of these macrophages, focusing on the role of pulmonary lipids, which are dysregulated following NM exposure. FXR is a receptor involved in lipid balance and has also been shown to regulate inflammatory responses. Previous studies showed that the expression of FXR was increased in lung macrophages following NM exposure.

To analyze the role of FXR in macrophage activation, we used mice that did not have the FXR gene (FXR-/-) and compared them to wild type mice. Both groups were treated with PBS (control saline solution) or NM via airway instillation. Lung tissue and fluid were collected 14 days later.

To assess the damage in the lung samples, we used techniques to quantify protein in the fluid and evaluate staining in tissue sections to look for particular inflammatory markers and overall tissue structure changes. We saw that NM caused histopathologic alterations in the lung including an increase in inflammatory cells and damage and thickening of the lung tissue. These changes were more prominent in FXR-/- mice. Additionally, in FXR-/- mice, but not WT mice, we observed evidence of fibrosis. This correlated with exacerbated increases in protein and cell content, indicating high levels of inflammation. Immunohistochemistry, a technique to stain macrophages in the tissue, showed that expressions of HO-1, a marker of inflammatory stress, and ARL11, a marker of pro-inflammatory macrophage activation, were increased in FXR-/- mice when compared to WT mice.

These findings demonstrate that FXR modulates the response of macrophages to NM and is involved in controlling inflammation. These findings may be useful in the development of therapeutics aimed at mitigating lung injury and inflammation.

#48. Convergence of Neural Responses: a Mechanism for Variant Independent Sound Representation in the Zebra Finch Auditory Forebrain?

David Natanov, Mingwen Dong, Mimi Phan, and David Vicario

Discovering the mechanisms behind how the auditory system can recognize complex sounds with natural variations is essential for understanding speech processing and other complex forms of vocal communication. Zebra finches are the best-developed model animal for studying the neural basis of communication; adult male zebra finches sing highly structured and unique learned songs that are used for individual recognition and mate selection. Similarly to how humans can understand the same word when produced by multiple speakers with very different voices, zebra finches are capable of recognizing an individual by their song even if the song is produced slightly different each time. As a result, it is possible that some portion of zebra finch's brain loses the ability to tell apart variations of the same sound, a process that we call variant-independent sound representation. Past studies have shown that neural responses in the caudomedial neostriatum (NCM), a higher auditory area of the zebra finch brain, play an important role in remembering individual songs. We developed a hypothesis that certain neurons in NCM lose the ability to tell apart variants of the same song as these variants are repeatedly played to the zebra finch. To investigate this hypothesis, I used a K-nearest neighbor (KNN) machine learning algorithm to measure how neural responses to different variants change with exposure to the variants. After undergoing a electrode implantation surgery, several zebra finches were played either blocked repetitions of certain song variants (blocked), randomly ordered song variants (shuffled), or variants of 2 different songs in random order (contrast). After processing the raw neuron firing patterns, I used the KNN algorithm to determine the distinctiveness each neuron's response to a given stimulus. Each electrode's neural responses were split into a training and testing set. After the KNN algorithm was trained using the training set data, the algorithm's accuracy at predicting the stimulus that caused a given neural response was used as a metric for neural discriminability. A high decoding accuracy score indicates that the neurons can more easily tell apart the sound. We found that the decoding accuracy to variant sounds jumped up in all conditions as the bird was first exposed to the sound, and then gradually fell as the bird realized that all the sounds heard were variants of the same song. In other words, the neuron's responses to variant songs converged and became more similar, making the KNN algorithm worse at telling apart the sounds. We also found that birds were best able to tell apart the sound blocked condition, but realized the songs were all variants of the same song more quickly in the shuffled condition. We also found the effect to be strongest in a small category of neurons that quickly adapted to the variant songs. Lastly, we also found some interesting differences between the two hemispheres of the bird's brain in processing these stimuli, which mirrors how human brains process language.

#49. TGF-β1 Modulates Gs Protein Mediated cAMP Generation in Human Airway Smooth Muscle (HASM) Cells

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RATIONALE: G protein-coupled receptor signaling regulates many human airway smooth muscle (HASM) functions which are often associated with bronchodilator therapy for obstructive lung diseases such as asthma and chronic obstructive pulmonary disease. The G protein signaling network modulates airway smooth muscle relaxation through the β2-adrenergic receptor (β2AR), Gs, and adenylyl cyclase axis.
Our previous studies have shown that levels of pro-fibrotic cytokine, transforming growth factor beta 1 (TGF-β1), attenuates β2AR-agonist-induced relaxation responses in HASM cells with isoproterenol (ISO) via a Smad2/3 pathway. We have shown that TGF-β1 inhibits the downstream pathway effects of cAMP. However, TGF-β1 showed little effect on forskolin (FSK)-stimulated cAMP levels in HASM cells and the specific mechanism of this phenomenon still remains clear. We hypothesize that TGF-β1 modulates Gs protein function of the β2AR receptor in HASM cells, thereby decreasing ISO-induced cAMP levels.

METHODS: HASM cells were stimulated in serum-free F12 media with TGF-β1 (10 ng/mL) overnight. Subsequently, HASM cells were treated with cholera toxin (CTX) (0.25 μg/mL; 30-60 min), the β2-agonist ISO (1 μM; 5 min), or the adenylyl cyclase direct activator, FSK (10 μM; 15 min). HASM were lysed and intracellular cAMP levels were determined by chemiluminescent immunoassay. In addition, phosphorylated myosin light chain (MLC) and total MLC were determined by immunoblot after HASM cells were stimulated with CTX (0.25 μg/mL; 30-60 min) or ISO (1 μM, 10 min) in the presence and absence of carbochol (Cch) (20 μM, 12 min) or TGF-β1. Data is represented as mean ± SEM/SD.

RESULTS: CTX induced cAMP production in a time-dependent manner (15-90 min; 0.25 μg/mL). Overnight TGF-β1 treatment blunted CTX-induced intracellular cAMP generation compared to CTX stimulation alone, particularly at 45 minutes. TGF-β1 treatment caused a 60% reduction in ISO-induced cAMP generation compared to the vehicle-treated control. TGF-β1 did not alter FSK-simulated cAMP generation. ISO treatment reduced Cch-induced pMLC, but failed to attenuate TGF-β1-induced pMLC. CTX reduced TGF-β1-induced pMLC compared to TGF-β1 alone.

CONCLUSION: These data show that TGF-β1 attenuates intracellular cAMP levels in HASM through modulation of Gs dependent signaling, as well as contractile agonist-induced phosphorylation of myosin light chain. Taken together, this data and our previous data suggest that TGF-β1 modulates not only remodeling associated with asthma, but also attenuates the actions of bronchodilators in asthma therapy.

#50. Exploring the use of Eye-Tracking as a Method to Capture Students' Knowledge Acquisition in a Virtual Science Inquiry Investigation

Rachel Dickler, Janice Gobert, and Ozge Yasar

Eye-tracking has been used as a measure of students' knowledge acquisition processes within online science environments but has yet to be used to study students' science inquiry processes. In the present study, we explored the use of eye-tracking as a methodology to examine students' knowledge acquisition processes as they conducted virtual investigations within the science inquiry environment, Inq-ITS. Inq-ITS consists of virtual labs in which students are automatically evaluated on central science inquiry practices (i.e. forming questions, conducting experiments, analyzing and interpreting data, arguing using evidence) using machine learning and knowledge engineering techniques. The findings suggest that eye-tracking may be an effective method for unpacking the knowledge acquisition strategies of students with varying levels of inquiry proficiency. Implications in terms of using eye-tracking to deeply assess and guide science inquiry investigations are discussed.

#51. "If They Gunned Me Down:" The Effect of Stereotypicality of Images on Blame

Analia F. Albuja, Samuel Kline, Shana Cole

The shooting of Michael Brown last year sparked a wave of attention on current race relations in America, including representations of Black victims in the media. After the news broke, the media circulated an image of Brown wearing baggy clothing, with his hands positioned in what some called a gang sign. Adopting the phrase "If they gunned me down," young Black people questioned how the media would portray them if they were killed. Thousands of Black youth took to Twitter and posted two different photographs of themselves—one that embodied the negative portrayal of Blacks in the media and another that did not. By juxtaposing the images, the movement contended that the media's choice to display the former leads to greater blame for Black victims and perpetuates a dangerous racial narrative.

Though past research has explored the role of skin tone and prototypically—characteristics that vary between individuals—in assessments of blame, the present work sought to hold differences in physiognomy constant and vary secondary features of an individual like dress and posture. Two studies tested the hypothesis that Black men would be blamed more when wearing "stereotypical" versus nonstereotypical clothing.

Study 1a tested the effect of image stereotypicality on victim blame. Participants (N = 239) read a news story in which responsibility for a car accident was ambiguous. The story was paired with either a stereotypical or nonstereotypical image of the victim, and participants assigned blame to the individual. The results revealed a significant difference between the conditions, $t(235) = -2.29$, $p = .023$. Despite reading the same account of the incident, participants blamed the victim more when they saw the stereotypical ($M = 26.46$) versus nonstereotypical image of him ($M = 18.76$).

Study 1b tested this effect on perpetrator blame. Participants in this study (N = 226) followed the same procedure as in Study 1a, with the exception that the image was labeled as the driver (i.e., the perpetrator) and participants assigned blame to him. Again, a significant difference was found between conditions, $t(142) = -2.12$, $p = .036$. Participants blamed the perpetrator more when they saw the stereotypical ($M = 78.34$), versus nonstereotypical ($M = 69.98$) image of him.

After the death of Trayvon Martin, Fox News host Geraldo Rivera said, "I think the hoodie is as much responsible for Trayvon Martin's death as George Zimmerman was." The present results lend some support to this notion, suggesting that the stereotypicality of a person's clothes influences how much he is blamed. The same person was blamed more for the same behavior when his image was more strongly associated with negative stereotypes about Blacks. Countless recent news stories underscore the timeliness of this work and the important implications for media representations of Blacks.

#52. Emulsion Stability

Hams Elshaikh, Medhavi sehgal, Dina Alyelgad, Dr. Alex Bertuccio

Emulsions are homogenous mixtures of two immiscible liquids in which one liquid is finely dispersed in another liquid and are commonly found in a variety of household goods including: shampoo, soaps, lotions, food, and cosmetic creams. Consumer products manufactured today are often left on the shelf for an extended period of time, and it is therefore important for these emulsions to have long shelf lives so they are fully used before they phase separate. By nature, emulsions are thermodynamically unstable, since they are mixtures of immiscible liquids, and therefore, will eventually phase separate. However, the shelf life of an emulsion can be altered and extended by adding chemicals, altering the ratio of oil and water, and by changing the concentration of the chemicals added. One common ingredient in emulsions – used to increase emulsion stability- is a surfactant. Surfactants are amphiphilic molecules which have both a hydrophilic group and a hydrophobic group. The hydrophilic head attracts to the polar water molecules and the hydrophobic region attracts to the non-polar oil molecules. These surfactants screen water and oil molecules from full contact and reduce the driving force for phase separation. Here, vegetable and deionized (DI) water were used as a simple oil/water system to investigate the effects of oil to water ratio, surfactant type and surfactant concentration on emulsion stability. The separation time was measured and defined as the time necessary for the volume of oil to return to 90% of its original volume. Various oil to water ratios were prepared, and it was found that a 1:3 oil to water ratio had the longest separation time of 8.0 minutes. It was determined that vegetable oil/water emulsion stability was dependent on surfactant concentration and surfactant type.