Seth Andrews

Mentor: Steven Stice

BMP-2 Overexpressing Mesenchymal Stem Cells in CS hydrogels regenerate Critical Bone Defects

Authors: Seth Andrews, Albert Chang, Robin Webb, Hazel Stevens, Robert Guldberg, Lohitash Karumbaiah, Steven Stice

In the United States, over 1 million surgical procedures per year require the use of bone autografts or autografts to prevent nonunion of bone defects. While successful in regenerating lost bone, there are problems with both donor site morbidity and availability. Current bone graft substitutes, such as collagen, have problems with immunogenicity and controlled delivery of the protein. Current alternatives to this involve the delivery of recombinant Bone Morphogenetic Protein 2 (BMP-2) to the defect site, but these approaches are limited by the protein's short half-life and complications from a large bolus of the protein. We hypothesized that BMP-2 over expressing Mesenchymal Stem Cells (MSCs) seeded in Chondroitin Sulfate (CS) hydrogels would result in prolonged release of BMP-2 for defect healing.

MSCs derived from both bone marrow and umbilical tissue were transduced at MOIs of 0, 10, and 50 MOI with prEF1a-BMP-2 lentivirus. BMP-2 expression was determined via ELISA of media collected at 48, 72, 96, and 120 hours post-transduction. To compare with recombinant protein in vivo, 8 mm bilateral defects were created in the femurs of 13 week old female SCID rats. CS hydrogel seeded with untransduced MSCs (n=9), BMP2 overexpressing MSCs (n=8), or rhBMP-2 (n=9) were injected into a nanofiber mesh at the site of the defect. Other defects received a collagen sponge loaded with rhBMP-2 without the nanofiber mesh, and radiographs were taken 4, 8, and 12 weeks after defect creation.

Human umbilical MSCs transduced at MOI 10 had the most cost-effective BMP-2 production. BMP2-MSC/CS and BMP/CS induced comparable defect bridging of a critical-sized rat femoral defect within 12 weeks to FDA approved BMP/collagen, while unmodified MSCs in CS-GAG hydrogel do not. Noticeable mineralization was first detected at 4 weeks in BMP-2 over-expressing cells treated defects.

There are notable differences in BMP-2 expression between MSC sources.

Critical sized defects in mouse femurs show substantial mineralization at 4 weeks when treated with BMP-2 huMSCs/CS-GAG, rhBMP-2/CS-GAG, and rhBMP-2/collagen, and increased radiodensity at 12 weeks. These results indicate that MSC that over express BMP2 may be a therapeutic option and additional studies to compare this treatment to FDA approved rBMP2 therapies are in progress.

Regenerative Bioscience Center

RBC

Kate E. Birdwhistell

Mentor: Samuel Franklin

Oral Presentation

Class of 2022, DVM and other professional degrees

Sustained Release of Transforming Growth Factor-β1 from Platelet-Rich Chondroitin Sulfate-Glycosaminoglycan Gels

Authors: Kate E. Birdwhistell, Lohitash Karumbaiah, Samuel P. Franklin

Focal cartilage lesions of the knee are common and can be a source of significant pain and dysfunction for patients. Current clinical therapies result in a substandard cartilage with poor long-term performance. Augmentation of these therapies with biologics such as platelet-rich plasma (PRP) may improve their efficacy. The objective of this study was to compare the release of transforming growth factor (TGF)-β1 PRP in autologous fibrin and chondroitin sulfate-glycosaminoglycan (CS-GAG) gels. PRP was prepared from nine healthy dogs using a commercially available device. Each PRP was split into 2 aliquots; one activated with bovine thrombin and $CaCl_2$ to form a platelet-rich fibrin (PRF) gel, and the other was used to rehydrate a lyophilized CS-GAG gel. Both gels were incubated in media for 13 days. Media was collected, saved, and replaced after 24 hours and then every 48 hours through day 13. Media samples were frozen at -80°C until assayed for TGF-β1 concentrations by ELISA. Overall differences between groups were compared using a 2-way ANOVA and a Wilcoxon paired samples test was used to compare treatment groups on individual days. The type of gel had a significant effect on TGF-β1 release ($p < 0.001$) with significantly ($p<0.05$) greater concentrations of TGF-β1 released from the CS-GAG gels than the PRF gels on days 3, 5, 7, 9 and 13. TGF-β1 concentrations were up to 365% more with use of the CS-GAG gels than the PRF gels on an individual day. Use of the negatively charged CS-GAG hydrogels significantly increased the duration and amount of TGF-β1 eluted from canine PRPs *in vitro*. PRP's regenerative potential is likely dependent on provision of anabolic growth factors. Therefore, a delivery method that provides sustained release of such growth factors, such as the CS-GAG gels investigated in this study, may improve efficacy *in vivo*.

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Guiqian Chen

Mentor: Hongxiang Liu

Poster Presentation

Class of 2017, DMV and other professional degrees

Neurofibromatosis 2 (Nf2) in tongue mesenchyme is a key factor for the normal development of tongue and taste papillae

Authors: Guiqian Chen, Mohamed Ishan, Xiushen Wang, Wenxin Yu, Xinwei Cao, Marco Giovannini, Hongxiang Liu

Neurofibromatosis 2 (Nf2) has a critical role during embryonic development and is especially important in regulating migration of neural crest cells, from which the tongue mesenchyme arises. Here we report that Nf2 immunosignals were primarily in mesenchyme and nerve fibers of developing mouse tongues. In Nf2 conditional knockouts (cKO) driven by *Wnt1-Cre* that exclusively labels tongue mesenchyme, we found: (i) a misshapen tongue, e.g., wider posterior region and pointed tip at E12.5-E13.5 and shorter in tongue length from E14.5 through birth; (ii) the absence of circumvallate papilla; (iii) fewer fungiform papillae. In E18.5 Nf2 cKO tongue sections versus control we observed: (a) disorganized epithelial cells with high levels of SOX9 expression; (b) greatly reduced nerve fibers; (c) fewer and atrophied fungiform papillae; (d) significantly reduced Ki67$^+$ cells, and increased apoptosis in epithelium and mesenchyme. Interestingly, numbers of early fungiform taste buds were unaltered in Nf2 cKO. The increased phosphorylation of YAP and reduced activities of transcription factor TEAD1 in Nf2 cKO suggested the involvement of the Hippo-YAP pathway. In organ cultures, Nf2 cKO tongues responded to the disruption of Shh signaling with cyclopamine and activation of Wnt/□-catenin signaling with LiCl for an enhanced papilla formation suggesting that mesenchymal Nf2 regulates Shh and Wnt/β-catenin signaling activities for proper formation of taste papillae. Together, our data demonstrate that the Nf2-mediated Hippo-YAP pathway interacts with Shh and Wnt/β-catenin for its essential role in the development of tongue and taste papillae. Sustained early fungiform taste buds in the non-innervated and atrophied fungiform papillae in Nf2 cKO strongly support the revised conception that taste bud induction is independent of innervation and structural support from papillae.

RBC Regenerative Bioscience Center

Arnav Cherian

Mentor: Hongxiang Liu

Poster Presentation

Class of 2018, Undergraduate Student

Plp1CreER-labeled Schwann cells do not differentiate to taste buds under homeostatic conditions *in vivo*

Authors: Arnav Cherian, Brett Marshall, Hongxiang Liu

Taste buds are sensory organs for taste that arise and mature in mammals at early postnatal stages, after which they undergo continuous turnover for the life of the organism. They serve to determine characteristics of ingested food, and are essential for overall quality of life. The origin of these sensory organs, as well as the progenitors responsible for their maintenance, is currently disputed. Despite the long-standing notion that taste buds are derived from local epithelium, we have used multiple lines of transgenic mice for lineage tracing analysis of neural crest (NC) cells, and found that a proportion of taste cells are NC-derived. However, the NC contributes to a plethora of tissue types, and which NC-derived precursors serve to develop and replenish taste buds is unclear. We hypothesize that Schwann cells (SCs), which are known for their role in forming protective myelin sheaths around nerves in the PNS, are a viable candidate for this role. As nerves penetrate into taste papillae and come into close contact with taste bud cells, it is likely that nerve-associated SCs are also found in close proximity, thus giving them unique access to the taste bud that other NC-derived niches do not. Moreover, there is mounting evidence that SC maintain plasticity, retaining their ability to de-differentiate and re-differentiate under certain conditions, such as nerve trauma. To examine whether SC can contribute to taste buds, we used a cocktail of antibodies to identify SC in the tongue and taste papillae. We found that pan-SC marker S100 consistently associated with betaIII-tubulin positive nerves, as did p75, and to a lesser extent myelin basic protein (MBP) and Protein Zero (P0), each of which are often found in myelinating SCs. After establishing a baseline for SCs in the WT tongue, we used the tamoxifen inducible CreER system driven by SC marker Plp1 crossed with RFP to identify SCs and SC-derived lineages. Curiously, we found that Plp1CreER labeled prospect SCs in the absence of tamoxifen. In depth analysis of younger mice revealed that Cre driven signals, although sparse, could be found as early as P1, suggesting that Cre expression was overwhelming the CreER mechanism in these animals. Despite this, we found that Cre-labeled cells were absent in the taste buds of adult mice, suggesting that Plp1Cre-labeled SCs do not contribute to taste buds in mice.

Xiaogang Cui

Mentor: Hongxiang Liu

RNA-Seq analysis for taste sensory organs in chickens: An ideal system for multidisciplinary study

Authors: Xiaogang Cui, Brett Marshall, Ning Shi, Shiyou Chen, Romdhane Rekaya, Honxiang Liu

RNA sequencing (RNA-Seq) has emerged as a powerful tool to advance studies via transcriptome profiling of cells and tissues. We recently demonstrated that molecular markers could be used to identify chicken taste buds (TBs) in oral epithelial sheets of the palate, base of oral cavity, and posterior tongue and that chicken TBs, like mammals, have specific patterning and distribution. In this study, RNA-Seq was carried out to better understand the transcriptomic architecture of gustatory tissues in chickens. For initial RNA-Seq tests, the epithelial sheet and underlying connective tissue of the palate, base of oral cavity and posterior tongue were used. Interestingly, more differentially expressed genes (DEGs) were found between the epithelium and connective tissue in the base of oral cavity as compared to the palate and posterior tongue. Thus, subsequent RNA-Seq was focused on the base of oral cavity using more specifically defined tissues: gustatory epithelium (GE, epithelial region that contains TBs), non-gustatory epithelium (NGE), and the corresponding underlying connective tissue (GM and NGM). We found 118 GE-NGE DEGs at a |FC|>2 and 4313 GE-GM DEGs, which were enriched in multiple GO terms and pathways including many biological processes. Well-known genes for taste sensation, e.g., *TRPM5*, *GNG13*, *TAS1R3*, *SCNN1G*, *SCNN1B*, *SCNN1A*, *PRKX* and *GNAT3*, were identified as highly expressed genes in the GE. The genes of components of signaling pathways (Wnt, BMP, FGF, Notch, SHH) for organogenesis were detected as highly DEGs in GE vs GM. Combining with other features of chicken TBs, e.g., uniquely patterned array and short turnover cycle, our data suggest that chicken gustatory tissue provides an ideal system for multidisciplinary study, including organogenesis and regenerative medicine.

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Madison Fagan

Mentor: Kylee Duberstein

Poster Presentation
Class of 2018, Graduate Student

Assessment of oxidative stress and muscle damage in exercising horses in response to level and form of vitamin E

Authors: Madison M. Fagan, Robert Pazdro, Jarrod A. Call, Alexander Abrams, Kylee J. Duberstein

Vitamin E is an essential antioxidant that is abundant in fresh forages and traditionally included in commercial equine feeds as synthetic all-rac-α-tocopherol acetate. Performance horses are thought to have a higher requirement due to increased oxidative stress and limited access to fresh forage. Of the eight vitamin E isomers, α-tocopherol is most abundant in tissue and itself has eight stereoisomers. Of these, RRR-α-tocopherol is preferentially transferred to tissues in the body and is the only isomer included in natural supplements. This study aimed to (1) determine if supplemental vitamin E above NRC recommendations is beneficial to exercising horses and (2) determine if there is a benefit to natural compared to synthetic vitamin E. Following a 14d washout period on a control diet with no added vitamin E, 18 horses were divided into three treatment groups and fed the control diet plus (1) synthetic low (SYN-L), 1000 IU synthetic α-tocopherol acetate/d, or (2) synthetic high (SYN-H), 4000 IU/d synthetic α-tocopherol acetate, or (3) natural (NAT), 4000 IU/d micellized RRR-α-tocopherol. After a 7d acclimation period, horses began a 6wk exercise protocol of increasing intensity, with standard exercise tests (SET) performed prior to and at the conclusion of the 6wk exercise protocol. Resting, pre-feeding venous blood samples were collected at day 14, 21, 42 and 63, as well as pre and 2hr post SETs. Data were analyzed using SAS version 9.4 proc GLM for repeated measures over time with time and treatment as variables. No differences were seen between treatment groups in serum α-tocopherol at initial and at Pre SET1 times. At all other time points, Nat horses had higher serum α-tocopherol levels as compared to SYN-H and SYN-L (P<0.05). No differences were observed between SYN-H and SYN-L groups. Plasma protein carbonylation was similar between treatment groups at all time points. Serum aspartate aminotransferase (AST, measure of muscle damage) was significantly lower post SET2 in natural horses as compared to SYN-H and SYN-L (P<0.05). From this, it can be concluded that micellized RRR-α-tocopherol was superior to synthetic all-rac-α-tocopherol in raising and maintaining serum α-tocopherol levels, whereas feeding higher levels of synthetic all-rac-α-tocopherol did not raise serum levels. Finally it was noted that elevated serum α-tocopherol levels in horses fed micellized RRR-α-tocopherol resulted in lower levels of AST post exercise following six weeks of conditioning.

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Alexandra Flemington

Mentor: Jarrod Call

Poster Presentation

Class of 2018, Undergraduate Student

Affect of Volumetric Muscle Injury on Mitochondrial Function

Author: Alexandra Flemington

Volumetric muscle injuries sustained during combat cost the Department of Defense a total of $42.4 billion a year. Currently, the specific pathophysiologyof such an injury are unknown. This lack of knowledge leads to inadequate rehabilitation and recovery for patients. The primary objective of this study is to determine how the mitochondria of these injured cells are affected. We hypothesize that by determining the mitochondrial enzyme activity, specifically citrate synthase, in the muscle during the first couple of weeks post-injury, we will gain a better understanding of what occurs physiologically following such an injury. To determine mitochondrial enzyme function, we will perform a citrate synthase assay on the muscle samples. We first homogenize each sample in phosphate buffer and then freeze-thaw each one three times. We then dilute each sample and add an oxaloacetate solution in order to start the enzymatic reaction. We will then put the sample along with the oxaloacetate solution in a spectrophotometer while the reaction is occurring. The spectrophotometer will take readings at 412 nm every 15 seconds for 3 minutes. These readings are then used to determine the activity of the citrate synthase enzyme in the mitochondria of the injured muscle. Overall, we hope to clarify how the injury affects mitochondrial function, thus indirectly studying the effect on the rate of muscle recovery. We hope to apply this new knowledge to the creation of innovative and effective rehabilitation programs for victims of volumetric muscle injuries.

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Lily Francis

Mentor: Franklin West

A Novel Porcine Model of Vascular Cognitive Impairment Demonstrates Changes in Cerebral Blood Flow and White Matter Tracts

Authors: Lily G. Francis, Erin E. Kaiser, Madelaine N. Wendzik, Simon R. Platt, Silun Wang, David C. Hess, Hui Mao, Franklin D. West

Recent studies anticipate the prevalence of dementia will increase by three hundred percent in the next three decades. Almost all aging adults develop cerebral white matter damage that is linked to poor blood supply which progresses and leads to symptoms ranging from mild cognitive dysfunction to severe vascular dementia. Although there is no preventative treatment for dementia, preliminary results of remote ischemic conditioning (RIC) show promise of decreasing white matter damage by increasing cerebral blood flow. Most animal models of dementia use rodents which are inadequate due to the extreme cerebral differences between humans and rodent species. For this reason, it is necessary a model more similar to the human brain is developed to test RIC treatment for vascular cognitive impairment (VCI). The aim of this study was to develop a chronic hypoperfusion porcine model to more adequately mimic dementia in humans. In order to decrease cerebral blood flow and induce corresponding white matter lesions, bilateral vascular constrictors were placed on the common carotid arteries (CCA). The efficacy of these constrictors and the resulting hypoperfusion model were studied using magnetic resonance imaging (MRI) diffusion tensor imaging (DTI), magnetic resonance angiogram (MRA), and arterial spin labeling (ASL) sequences pre-VCI induction and 4 weeks post-VCI induction. These sequences revealed gradual bilateral constriction of the CCAs resulted in a global decrease in cerebral blood flow and compromised white matter integrity. These findings suggest that bilateral vascular constriction of the CCAs produce a reproducible potential porcine model of VCI.

Regenerative Bioscience Center

Olivia Fuller

Mentor: Franklin West

Poster Presentation

Class of 2018, Undergraduate Student

Assessment of Cognitive and Motor Function in a Piglet Model of Traumatic Brain Injury Utilizing Spatial T-Maze Tests

Authors: Olivia R. Fuller, Madelaine N. Wendzik, Emily W. Baker, Holly A. Kinder, Franklin D. West

Traumatic brain injury (TBI) is the fourth leading cause of disability in the United States. There is currently no effective treatments to address the onset of secondary injury, tissue damage, or tissue death that results from TBI. The damages caused by a TBI often result in long-term cognitive and motor deficits. This is a large issue because children ages 0 to 5 years are among those with the highest risk for sustaining a TBI, which can leave them with life-long impairments in leaning, memory, attention and executive function. To try and mitigate this problem, studies have been conducted with animals such as rats, which don't truly represent the human condition. However, a pig is a more human-like model because their brain is similar in size, structure, composition, and follow a similar pattern of development to a human's. Our objective is to analyze changes in cognition and motor function in a piglet traumatic brain injury model with hopes of bridging the current gap in translation medicine. To assess learning and memory in the piglets, we used spatial T-maze test. In this test, pigs learn to navigate a plus-shaped maze to locate a food reward using extra-maze cues. A GaitFour pressure mat was used to assess the spatial-temporal gait biomechanics at pre, 1 day post, 3 days post, 1 week post, and 4 weeks post-TBI animals. We compared the results of the TBI treatment group (n=5) to an uninjured group (n=5). Our results showed that the TBI piglets navigated the spatial T-maze significantly ($p<0.05$) slower than the uninjured group and made more ($p<0.05$) mistakes than normal pigs during the acquisition phase of testing. TBI piglets travel at a significantly ($p<0.05$) slower velocity after reversal on days Reverse 1 and Reverse 2 as compared to normal pigs. Both TBI and normal piglets travel significantly ($p<0.05$) less distance by day Acquisition 5. Overall, this more translational model will help us to better assess the injuries and long-term deficits caused by a TBI to create more effective therapeutics to improve cognitive and motor deficits in the future.

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Forrest Goodfellow

Mentor: Steven Stice

Zika Virus Induced Mortality and Microcephaly in Chicken Embryos A comparison of Asian and African Stains

Authors: Forrest T. Goodfellow, Blanka Tesla, Gregory Simchick, Qun Zhao, Thomas Hodge, Steven Stice

The explosive spread of the Zika virus (ZIKV) through South and Central America has been linked to an increase in congenital birth defects, specifically microcephaly. Representative rodent models for investigating infections include direct central nervous system (CNS) injections late in pregnancy and transplacental transmission in immunodeficient mice. Microcephaly in humans may be the result of infection occurring early in pregnancy therefore, recapitulating the human course of ZIKV infection should include normal embryo exposed to ZIKV during the first trimester. In ovo development of the chicken embryo closely mirrors human fetal neurodevelopment and, as a comparative model, could provide key insights into both temporal and pathophysiology effects of ZIKV in particular how Asian and African linages of the virus differ.

Chick embryos were directly infected early in development with either Asian or African ZIKV strains. The prevalence of viral infection, mortality, and volume of the CNS was accessed by MRI.

High doses of virus caused embryonic lethality, where as low doses did not. In a subset of lower dosed embryos, replicating ZIKV was present in various organs including the CNS throughout development. Surviving ZIKV-infected embryos presented a microcephaly-like phenotype. Chick embryos were longitudinally monitored by MRI that documented CNS structural malformations, including enlarged ventricles (30% increase) and stunted cortical growth (decreased telencephalon by 18%, brain stem by 32%, and total brain volume by 18%), on both embryonic day 15 (E15) and E20 of development. ZIKV-induced microcephaly was observed with inoculations of as few as 2 to 20 viral particles.

The chick embryo model presented ZIKV embryonic lethal effects, and progressive CNS damage similar to microcephaly.

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Mohamed Ishan

Mentor: Hongxiang Liu

Mesenchyme-specific activation of BMP receptor ALK2, but not ALK3, results in altered tongue formation and cell differentiation

Authors: Mohamed Ishan, Guiqian Chen, Sunny Patel, Brett Marshall, Yuji Mishina, Hongxiang Liu

Taste organs, among many epithelial appendages such as feathers, hair follicles, and teeth, require mesenchymal-epithelial interactions for proper development. Bone morphogenetic protein (BMP) signaling, one of multiple molecular pathways, plays important roles in regulating the formation of taste organs. However, little is known about the involvement of receptor(s) mediating BMP signaling in taste organ development. In the present study, we used mouse models with constitutively activated (ca) BMP receptors in a mesenchyme-specific manner driven by Wnt1-Cre to examine the roles of type I BMP receptors ACVR1 (ALK2) and BMPR1A (ALK3) in the development of tongue and taste papillae. CaALK2 and caALK3 mutants at embryonic day (E) 10.5-11.5 had no observable morphological differences in the embryos and branchial arches compared to littermate controls. At E12.5-P1, caALK2/Wnt1-Cre, tongues were smaller, misshapen, and missing the pharyngeal region compared to littermate controls. In contrast, no obvious change was seen in caAlk3/Wnt1-Cre mice. We found that in caALK2/Wnt1-Cre tongues, fungiform papillae and early taste buds formed in the absence of innervation at E14.5 and E18.5 respectively. Immunoreactions on sections using the cell markers E-Cadherin and Vimentin showed that both the epithelium and the mesenchyme were disorganized in caALK2/Wnt1-Cre mouse tongue at E14.5 and E18.5. Our data indicate that BMP signaling pathways mediated by different receptors have distinct roles in lingual mesenchyme that interact with overlying epithelium for proper formation of taste organs. There are ongoing mechanistic studies exploring how the intracellular signaling pathway mediated by caALK2 is involved in the malformation of taste organs.

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LaDonya Jackson

Mentor: Lohitash Karumbaiah

Evaluating the Effects of Electrical Simulation on Neural Network Activity

Authors: LaDonya Jackson, Mohammad.S Eslampanah Sendi, Steven L. Stice, Maysam Ghovanloo, Lohitash Karumbaiah

Excitotoxic damage to cortical neural networks leads to widespread disruption of function. Although Functional Electrical Stimulation (FES) has shown promise in rejuvenating network activity, the precise mechanisms that dictate recovery are unknown. In this study, we subjected embryonic stem cell derived neurons to excitotoxic damage and evaluated the effects of Direct Current Stimulation (DCS) and/ or Low Frequency Stimulation (LFS) in mediating network recovery using Multi-electrode Arrays (MEAs).

We hypothesized that DCS coupled with LFS could rejuvenate synaptic activation and restore network activity after exposure to sub-lethal doses of the neurotoxic agent – L-Glutamate.

A high density MEA system with 64 electrodes in each well was used to record activity. At 2 weeks post-seeding we exposed the cells to 100 µM of L-Glutamate to induce neurotoxicity. We then assessed electrode specific and whole well network extracellular spiking activity along with individual spike durations and amplitudes over a period of three weeks across 8 groups: control, toxicity, LFS only, DCS only, LFS + DCS only, LFS + toxicity, DCS + toxicity, LFS + DCS + toxicity.

Neurotoxicity resulted in a reduction of neurons ($P<0.001$) and oligodendrocytes ($P<0.01$) as determined by immunohistochemical assessment; and a decline in bursting activity ($P<0.05$ and $P<0.01$), and synchrony as determined by electrophysiological assessments. The administration of LFS (10 µA, 0.1 Hz) ameliorated these effects by enhancing the bursting activity ($P<0.05$), synchrony ($P<0.001$) and mean firing rate (MFR) ($P<0.05$), when compared to both the toxicity and the no stimulation controls. DCS (10 µA) and the LFS paired with DCS failed to significantly enhance bursting, MFR or synchrony. LFS, DCS and LFS paired with DCS all significantly increased the frequency of neuronal membrane oscillations ($P<0.01$), and the neuronal excitability ($P<0.05$).

LFS had a more potent effect on synaptic activation and repair. This finding can aid in the optimization of FES to rejuvenate neural network activity after insult. Since neurodegenerative disorders such as Traumatic Brain Injury (TBI) all share a common pathway of neural network disruption we are currently examining the effects of electrical stimulation in a moderate-severe rodent TBI model. A custom wireless headstage and EnerCage system was designed to record electrophysiological signals and continuously administer LFS or DCS to awake and freely moving rats "on-demand" and in response to motor evoked neuronal activity. On-going studies will evaluate the therapeutic effects of electrical stimulation *in vivo* to mediate synaptic activation and repair, and restore lost function post-TBI.

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Brian Jurgielewicz

Mentor: Steven Stice

NPEX treatment improves spatiotemporal gait parameters in a middle cerebral artery occlusion porcine stroke model.

Authors: Brian Jurgielewicz, Steven Stice, Franklin West, Robin Webb, Kylee Duberstein, Erin Kaiser, Simon Platt, Samantha Spellicy

Decreasing ischemic stroke induced neurological deficits and disability is an imperative and necessary objective of stroke therapy. In order to abate the induced motor deficits, it is essential to optimize a translatable animal model, verify functional assessments, and to create a novel regenerative therapy. The porcine brain is similar to humans in anatomy, composition, and size compared to the previously used rodent model making it a more ideal model. As a burgeoning field, human stem cells and derivatives are believed to have a therapeutic effect. We hypothesize that neural stem cell exosomes can alter the ischemic area to enhance motor functional recovery after brain ischemic attack. The current study was undertaken to determine the effects of administering these neural stem cell products, reducing injury mechanisms and gait deficits in the porcine stroke model. Comparing 72 spatiotemporal and pressure gait parameters at 1, 2, 4, 8, and 12 weeks post MCA occlusion, we observed numerous differences. By week one post surgery, we observed a change in stride frequency, stride length, paw print area, hind reach, and total scale pressure. We can conclude gait analysis is a sensitive quantifiable assessment of motor activity and can be used to monitor the effects of a neural stem cell derived factor in a pig stroke model.

RBC Regenerative Bioscience Center

Meghan Logun

Mentor: Lohitash Karumbaiah

Oral Presentation
Class of 2020, Graduate Student

Small Molecule GAG-Antagonist Surfen Decreases Glioma Cell Infiltration *In Vitro* and Attenuates Tumor Growth in Rat Model of Glioma

Authors: Meghan Logun, Akshaya Naranyanan, Wujun Zhao, Leidong Mao, Lohitash Karumbaiah

Glioblastoma multiforme (GBM) is a stage four astrocytoma comprising the majority of primary malignant brain tumor diagnoses in the United States. Conventional therapies are ineffective, leading to patient death within 15 months of diagnosis. Chondroitin sulfate proteoglycans (CSPGs) and their glycosaminoglycan (GAG) side chains are key components of brain extracellular matrix (ECM) implicated in promoting tumor invasion and spread. We hypothesize that glioma cell invasion is triggered by selective expression of oversulfated CS-GAGs in the tumor microenvironment and that preventing tumor cell interactions with CS-GAGs will dampen glioma invasion and spread. Microfluidics devices with mono- and oversulfated CS-GAG matrices were used to evaluate F98 rat glioblastoma cell infiltration *in vitro.* Cell infiltration was compared across unsulfated hyaluronic acid (HA) and 4,6-sulfated CS-GAG (CS-E) matrices, and media only controls. The small molecule GAG-antagonist (surfen) was introduced to inhibit cell interaction with sulfated GAGs and evaluate if inhibition subsequently halted cell infiltration within hydrogel matrices. Focal adhesion protein colocalization was quantified within antagonist-containing and control hydrogels to determine influence of CS-GAGs on migratory cell phenotype. *In vivo* tumor inductions in Sprague Dawley rats were performed stereotactically to induce frontal lobe tumors accurately mimicking human GBM. F98 cells either in media only or media containing surfen were inoculated at a depth of ~3 mm to evaluate effects of surfen on glioma formation and invasion over 21 days *in vivo.* MR imaging was used to track progress and quantify tumor volume and angiogenesis.

Our results demonstrated enhanced preferential cell migration into hydrogel matrices containing disulfated CS-E compared to unsulfated hydrogels (p<0.05). F98 cells invading into CS-E hydrogels displayed enhanced colocalization (p<0.05) of focal adhesion proteins compared to cells within unsulfated hydrogels. This effect was significantly reduced in cells within CS-E hydrogels containing surfen (p<0.05). F98 cells inoculated within rats developed into diffusely invasive tumors after 14 days, but when inoculated in media containing surfen tumors were contained to more defined margins and smaller gross size after 7 days (p<0.05). These results suggest that sulfated CS-GAGs may directly induce tumor invasion, and this signaling mechanism can be can be disrupted by surfen to restrict invasion.

Our results suggest that heightened presence of extracellular CS-E induces enhanced cellular migration in a GAG sulfation-dependent manner, and perturbing cellular interactions with CS-E has consequences for gross tumor formation and invasion. Identification of the role of CS-GAGs in glioma behavior would advance our understanding of glioma invasion, and contribute to design of novel therapeutic interventions.

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Brett Marshall

Mentor: Hongxiang Liu

SOX10-Cre labeled neural crest derived cells are absent in taste buds of zebrafish

Authors: Brett J. Marshall, Rebecca Ball, James Lauderdale, Robert Kelsh Wenbiao Chen, Hongxiang Liu

Taste buds were initially defined as being derived from local surrounding epithelial cells. However, multiple lines of evidence strongly suggest that the underlying connective tissue, which is primarily neural crest (NC)-derived, contributes to a significant population of taste bud cells. To determine the extent to which the NC contributes to taste buds in a species-specific manner, we utilized *SOX10-Cre* zebrafish to label NC cell lineage and identify labeled cells in taste buds. In zebrafish, numerous taste buds develop within days post fertilization (dpf) and are easily identified. In this study, we first used *SOX10-eGFP* zebrafish to validate that SOX10 expression was limited to the NC. We found that eGFP$^+$ cells were highly restricted to the neural keels of embryos between 12-13 hours post fertilization (hpf), and these cells' migration was tracked into the cranial region by 14-15 hpf. Importantly, while eGFP$^+$ cells were extensively distributed in the connective tissues of the lips and oral cavity, they were absent in taste buds at 5.5 and 15 dpf. The lack of active SOX10 expression in taste buds allowed us to utilize *SOX10-Cre* to conduct NC lineage tracing and test NC contribution to taste buds. To this end, we crossed *SOX10-Cre* fish with a double reporter fish containing a Cre-driven switch from constitutively expressed GFP to RFP. We found that in contrast to our results in mice, *SOX10-Cre* labeled (NC-derived) cells were not observed in taste buds, yet were abundant within the connective tissue around the oral cavity at 15 dpf. These data suggest that unlike mice, taste buds in zebrafish are not from the NC. Thus, careful considerations should be taken when applying principles across species with regards to the origin of taste buds.

RBC Regenerative Bioscience Center

Ana Maslesa

Mentor: Luke Mortensen

Oral Presentation

Class of 2019, Undergraduate Student

Bone characterization in the treatment of Hypophosphatasia with mesenchymal stem cells

Authors: Ana Maslesa, Emily Pendleton, Luke Mortensen

We present the use of second-harmonic generation (SHG) images to characterize bone morphology using Hypophosphatasia (HPP) as a disease model. Hypophosphatasia (HPP) is a rare genetic disorder caused by mutations to the tissue-non-specific alkaline phosphatase (ALP) gene. Diminished ALP activity prevents the enzyme from dephosphorylating inorganic pyrophosphate (PPi), a potent inhibitor of mineralization, resulting in disarticulated collagen and porous bones. Current treatments only alleviate symptoms in the long bones of patients with HPP and do not address premature loss of teeth and craniosynostosis. A promising treatment is mesenchymal stem cell (MSC) therapy, which has been used in clinical studies along with myeloablation and full bone marrow transplants. However, many patients in need do not qualify for bone marrow transplants as they are too sick for such a harsh, risky procedure. While it is established that HPP reduces mineralization in bone, the effect of HPP on other parameters of bone formation, such as bone microarchitecture, is unknown. First, a method for describing pores had to be established. SHG images were used to examine the collagen microstructure in cranial bones of both healthy and HPP mice. Image J was then used to analyze characteristics of bone such as pore size, number, and spacing. As the surface of the skull is curved, the image was first flattened by removing tilt. Next, a sub-region of interest was selected and a projection was created. This image was processed using several different methods and then various thresholds were added to each processed image. Automated pore counts on a small subset of data were compared to manual counts to optimize the way pores are analyzed. The percentage of manually counted pores that was automatically counted and their standard deviation were both factors in choosing a method. It was determined that using sequential image processing was the best protocol for describing pores.In future studies, this method of describing pores will be used to assess the impact of MSCs in their local environment. We predict that bones will have smaller, fewer pores and denser collagen fibers when a mouse is treated with MSC therapy. This data will be used to determine the effectiveness of MSC therapy for HPP and will establish SHG as a means for characterizing bone morphology for bone diseases.

Marie McKenzie

Mentor: Steven Stice

Using Metabolomic Profiles to Determine Developmental Neurotoxicity of Chlorpyrifos in Neural Progenitor Cells With or Without Media Preconditioned with C3A Hepatocytes

Authors: Marie McKenzie, Mayowa Amosu, Xian Wu, Shelley Scoville, Xiaoming Bian, Kun Lu, Steven Stice, Mary Alice Smith

Many pesticides utilize neurotoxic mechanisms to be effective, but a secondary consequence may be toxicity to the developing human brain. In vitro testing can provide critical information modeling developmental outcomes on a cellular level. To address these concerns about neurotoxicity of chlorpyrifos our objectives were to 1) determine the effects of chlorpyrifos on developing neurons, and 2) further develop our system by adding a metabolism component using C3A liver cells. Viability assays were conducted to determine the dose range and appropriate doses. After exposing neural progenitor (hN2^{TM}) cells to a chlorpyrifos for 48 hours, media were collected and derivatized for metabolomic analysis. In addition, C3A cells were treated with chlorpyrifos, and the conditioned media was then applied to hN2^{TM} cell cultures for 48 hours. The media was removed, and metabolites derivatized for GC/MS. Cells demonstrated biomolecular alterations in response to chlorpyrifos exposure at 0.3, 3 and 30 µM concentrations. Using MetaboAnalyst for pathway analysis, major pathways affected in hN2 cells directly exposed to chlorpyrifos were phenylalanine metabolism, fatty acid biosynthesis, and cyanoamino acid metabolism. When hN2 cells were exposed to conditioned media from C3A cells treated with chlorpyrifos, the major pathways affected were valine, isoleucine, and leucine biosynthesis and degradation, and aminoacyl-tRNA biosynthesis. In summary, chlorpyrifos affects amino acid and fatty acid metabolism in developing neuronal cells. An indicator of the biological impact of chlorpyrifos on neural progenitor cells was calculated by taking the sum of the absolute values of significant differences identified through t-tests of metabolomics profiles. Conditioned media from chlorpyrifos treated C3A cells had a tenfold or greater biological impact on hN2 cells than direct exposure to chlorpyrifos, suggesting the metabolites are more biologically active than the parent compound.

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Adir Mohaban

Mentor: Luke Mortensen

Small Molecule-Stimulated MSC Adhesion to Enhance Atopic Dermatitis Therapy in Companion Animals

Authors: Adir Mohaban, Bobby Leitmann, Luke Mortensen

Decades of research have shown the potential of stem cells therapies as a remedy for chronic illnesses due to their anti-inflammatory and immunomodulation capabilities. While there is much hope for viable stem cell therapies, a major limitation can be linked to inefficient cell homing due to poor expression of surface adhesion receptors. Surface modification of MSCs may allow for more efficient homing. In a previous study, pretreating MSCs with Ro-31-8425, a molecule shown to upregulate the CD11a gene which codes for surface receptors, led to an increase in static adhesion to plates coated in intercellular adhesion molecule 1 (ICAM-1). ICAM-1 is expressed in increased levels by endothelial cells at sites of inflammation. In this study, we hope to expand on this by observing static and shear adhesion levels of pretreated MSCs to canine endothelial cells expressing ICAM-1. This will be accomplished by inducing shear stress over MSCs firmly adhered to endothelial cells (static adhesion) or by flowing MSCs over an endothelial cell monolayer (flowing adhesion) at measured levels of shear stress, using a syringe pump and a parallel plate flow chamber. White blood cells will be used as a positive control for comparison. We expect to observe increased adhesion of pretreated MSCs to the endothelial cells. This would indicate the possibility of using MSCs pretreated in Ro-31-8425 as a viable therapy for diseases causing inflammation, such as atopic dermatitis. We hope to move on to clinical trials with diseased canines once we determine the efficiency of pretreated MSC homing.

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Maria Naskou

Mentor: John Peroni

Platelet Lysate as a Novel Serum Free Media Supplement for the Culture of Equine Bone Marrow Derived Mesenchymal Stem Cells

Authors: Maria C. Naskou, Scarlett M. Sumner, Merrilee Thoresen, Ian Copland, John Peroni

Mesenchymal Stem Cells (MSCs) produced for clinical purposes rely on culture media that includes fetal bovine serum (FBS). FBS unfortunately is xenogeneic, and thus has the potential to significantly alter the MSCs phenotype, rendering these cells immunogenic. This may result in the rejection of MSCs by the host immune system following administration, even when autologous MSCs are used. Platelet lysate (PL) is considered a possible alternative to FBS that has shown promising results in human and equine medicine. Our goal was to evaluate the use of equine platelet lysate pooled (ePL) from donor horses in place of FBS to culture and expand equine MSCs. We hypothesized that ePL, produced following apheresis, will function as the sole media supplement to accelerate the culture and expansion of equine bone marrow derived MSCs without altering their phenotype and their immunomodulatory capacity.

Platelet concentrate was obtained from five equine blood donors via plateletpheresis and ePL was produced via freeze-thaw and centrifugation cycles. Population doublings (PD) and doubling time (DT) of bone marrow derived MSCs (n=3) at P2 to P5 cultured with media supplemented with either 10% FBS or ePL, was calculated using established mathematical equations. Cell viability was assessed via a Live/dead assay and immunophenotypic analysis for the expression levels of MHC-II, CD90, CD105, CD45, CD34 and CD44 markers was performed with flow cytometry. To assess the ability MSCs to modulate inflammatory responses, equine monocytes were stimulated with LPS *E.Coli* and co-incubated with MSCs cultured in the two different media formulations. Following eighteen hours of incubation, cell culture supernatants were collected and assayed for the production of the pro-inflammatory cytokine tumor necrosis factors-alpha (TNF-α).

Our results revealed that MSCs cultured in ePL media exhibited increased PDs and decreased DT compared to those in FBS. Moreover, MSCs cultured in ePL showed comparable viability and expressed similar levels of MSCs markers compared to FBS. MSCs cultured in ePL expressed lower levels of CD34 and CD45. Finally, MSCs cultured in ePL efficiently suppressed the release of TNF-α when exposed to LPS stimulated monocytes.

Our data demonstrate that ePL supports the proliferation, viability and immunomodulatory capacity of MSCs without altering their phenotypic profile. Thus, ePL has the potential to be used for the expansion of MSCs before clinical application, avoiding the concerns associated with the use of FBS.

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Maria Naskou

Mentor: John Peroni

Equine platelet lysate gel: a novel matrix for mesenchymal stem cell delivery

Authors: Maria C. Naskou, Ian B. Copland, Jacques Galipeau, John F. Peroni

Mesenchymal Stem Cells (MSCs) are multipotent, self-renewing cells that have been implicated in orchestrating the repair of musculoskeletal injuries. In order to influence the healing response, MSCs need to remain in the site of injury for several days to weeks depending on the application and the tissue of interest. Research conducted in tendon has demonstrated low cell retention and survival following injection of MSCs. The lack of a robust demonstration of MSC localization in the injured tissue over time and of the correlation between MSC retention and repair outcomes precludes the optimization of cell therapies. The overall goal of this study was to investigate a homologous platelet derived gel as a matrix that promotes viability, proliferation and function of MSCs. Platelet Lysate is a biological product, rich in growth factors, that we regularly manufacture after obtaining a platelet concentrate via apheresis performed in equine blood donors. We hypothesized that equine platelet lysate gel (ePL gel) supports the viability, proliferation and immuno-modulatory function of MSCs.

A tunable gel matrix was obtained from platelet lysate by the addition of calcium chloride with a final concentration of 20 mM. One million of equine bone-marrow derived MSCs were encapsulated in ePL gel and viability was assessed via a live/dead assay over a 5-day period. Images were obtained by using a laser scanning confocal microscopy. To assess the ability MSCs to modulate the inflammatory response, equine monocytes were stimulated with LPS E.Coli 0111:B4 (50 ng/ml) and co-incubated with either ePL gel or MSCs encapsulated in ePL gel. Following sixteen hours of incubation, cell culture supernatants were collected and assayed for the production of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α).

Our results indicate that MSCs incubated in the lysate at the time of gel formation demonstrate high viability over a 5-day period. Moreover, MSCs encapsulated in the gel exhibit escalating proliferative rate over a 7-day period. We determined that monocyte production of TNF-α declined by 50% in the presence of the gel. Furthermore, it appeared that the inclusion of MSCs in the gel resulted in a more efficient suppression of TNF-α.

These results open the opportunity to further investigate the biological characteristics of a platelet lysate gel in the context of optimizing MSC retention into injured tissues. Future studies include *in vivo* experiments evaluating the potency of ePL gel as a matrix for the efficient delivery of MSCs to injured musculoskeletal tissues.
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John Newman

Mentor: Elizabeth Howerth

The Effects of Aging on Neuroblast Proliferation in the Lateral Ventricle Region: A Canine Model

Authors: John Newman, Elizabeth Howerth

Through the studies of legendary neuroscientist Santiago Ramón y Cajal, it was previously thought that the adult brain lacks the ability to regenerate neurons. In modern science, adult neurogenesis has been well established in the hippocampal region of rodents. Since this landmark, many studies of adult neurogenesis in the hippocampus of gyrencephalic species, such as bovine, have been conducted. However, the ontogeny of neurogenesis in the canine brain, particularly in the lateral ventricle region, is poorly understood. Data concerning the importance of the lateral ventricle and its accompanying cerebrospinal fluid in trauma repair, nutrient delivery and waste removal is burgeoning. Therefore, it is vital to elucidate the neurogenic properties of this region, and the tissue surrounding it. Here, we evaluate the relationship between age and proliferation of neuroblasts, or immature neuronal precursors, in the Subventricular Zone (SVZ) of the lateral ventricle region of the canine brain. The SVZ is a proven hotspot for robust neuroblast proliferation, and thus an excellent target for evaluation. Also, considering the canine brain is similar to humans in that it contains many gyri, there is ample opportunity for neuroblasts to migrate through the SVZ via the Rostral Migratory Stream (RMS). We hypothesize that as age increases, the robustness of neurogenesis in the canine brain will decrease, as indicated by decreasing numbers of DCX and Ki67 immunopositive cells. To address this, formalin fixed brains from dogs of various ages submitted for postmortem examination and with known pathologies were evaluated. Immunohistochemical staining of transverse sections of forebrain for doublecortin protein (DCX) and Ki67 were performed in order to assess the density of migrating neuroblasts, and general cell proliferation, respectively.

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Anna S. Nichenko

Mentor: Jarrod Call

Oral Presentation

Class of 2019, Graduate Student

Autophagy Related Ulk1 is Necessary for the Recovery of Mitochondrial Function after Skeletal Muscle Injury

Authors: Anna S. Nichenko, W. Michael Southern, Anita Qualls, Corey C. Spencer, Jarrod A. Call

The objective of this study was to determine if autophagy is necessary for the recovery of mitochondrial function after muscle damage. Autophagy is a highly conserved cellular process for the degradation of dysfunctional or damaged organelles (e.g.,mitochondria). We have previously demonstrated that traumatic muscle injury impairs mitochondrial content and is accompanied by an induction of autophagy. However, it is unclear: 1) if only traumatic muscle injury induces autophagy, 2) if autophagy induction is contingent on mitochondrial dysfunction, and 3) if autophagy is necessary for the functional recovery of muscle strength and mitochondrial function. To determine the relationship between muscle damage, mitochondrial function and autophagy induction, ten week old C57Bl/6 mice were randomly assigned to the following groups: eccentric contraction-induced injury (physiological), freeze injury (traumatic), or contractile fatigue (non-damaging control). Injured and contralateral uninjured tibialis anterior and extensor digitorum longus muscles were harvested at 0, 6 hours, 1, 3, and 7 days. Mitochondrial function was assessed via state 3 mitochondrial respiration rates from permeabilized muscle fiber bundles, and autophagy induction was measured via Beclin1, an autophagic protein activated downstream of Ulk1, and total LC3 protein content. There was no effect of contractile fatigue on mitochondrial function or autophagy induction at any time point (P>0.19). Eccentric contraction-induced injury did not elicit mitochondrial dysfunction in the injured muscle at any time point (P>0.20), but injured muscle did have greater Beclin1 content at 3 and 7 days (~2 fold, P=0.03). This suggests that physiological muscle damage may induce an autophagy response even in the absence of overt mitochondrial dysfunction. In contrast, freeze injury caused severe mitochondrial dysfunction immediately through 3 days after injury (16-53% of contralateral control, P=0.04), and was accompanied by a robust autophagy response in both Beclin1 and LC3 protein content (peak 7 at days ~40 fold ~14 fold P<0.01, respectively) To determine if this robust autophagy response was necessary for the recovery of mitochondrial function, we subjected skeletal muscle-specific Ulk1 knockouts (Ulk1 KO) and their littermates controls (WT) to freeze injury and assessed the recovery of muscle strength and mitochondrial function at 7 days. There was no difference in muscle strength between Ulk1 KO and WT mice prior to injury (P=0.47), but at 7 days muscle strength in Ulk1 KO mice had recovered to only 12% of preinjury strength, significantly less than WT mice (30%, P<0.01). There was no difference in mitochondrial function in the uninjured muscle from Ulk1 KO and WT mice (P=0.35), although at 7 days, mitochondrial function was an astonishing 32% of uninjured in ULK1 KO mice compared to 64% of uninjured in WT mice (P=0.01). In conclusion, autophagy is induced following both traumatic and physiological muscle injury and Ulk1 is required for the recovery of mitochondrial function after traumatic muscle injury.

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Austin Passaro

Mentor: Steven Stice

Neural exosome-mediated enhancement of *in vitro* angiogenesis in a three-dimensional microfluidic culture system

Authors: Austin Passaro, Steven Stice

The nervous and vascular systems are closely associated in the human body, both spatially and functionally, and have been exhibited to interact significantly through paracrine and juxtacrine signaling. These interactions are important for proper neural and vascular functioning and result in emergent functions that would otherwise not be possible, including blood-brain barrier regulation and maintenance of the adult neural stem cell niche, among others. The importance of these functions is clearly emphasized by the many pathological states associated with neurovascular disruption. Elucidating the cellular and molecular mechanisms underlying neurovascular interactions has many implications for a wide range of applications, from clinical treatment and the development of therapeutics to tissue engineering and the development of cellular machines. Recently, exosomes, small (~30-150nm) vesicles secreted by most cell types that are known to have vital roles in intercellular signaling and communication, have been increasingly explored as a novel mechanism of neurovascular interactions. In this study, we employ a three-dimensional microfluidic culture system to determine the effects of neural exosomes on angiogenesis of embryonic stem cell-derived endothelial cells, demonstrating that neural exosomes promote angiogenesis *in vitro*, and discuss potential mechanisms for this effect, including Notch signaling and transfer of microRNA. Ultimately, exosome-mediated modulation of angiogenesis represents a novel mechanism for neurovascular patterning and co-differentiation during neuro- and vasculogenesis that could be used to develop improved clinical treatments and therapeutics or create functional neurovasculature for tissue engineering applications.

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Sunny Patel

Mentor: Hongxiang Liu

Poster Presentation
Class of 2018, Undergraduate Student

Bone morphogenetic protein receptor Alk2 mediates intracellular signaling in neural crest derived tongue mesenchyme for the formation of taste organs

Authors: Sunny Patel, Mohamed Ishan, Guiqian Chen, Yuji Mishina, Hong-Xiang Liu

The development of taste organs, including the tongue, taste papillae and taste buds, requires epithelial-mesenchymal interactions which is governed by multiple signaling pathways. Among these signaling pathways bone morphogenetic proteins (BMP) signaling plays a significant role the taste organ development. BMPs are vital in both organogenesis of embryos and homeostasis of adult tissue. Mouse knockout models of different components of the BMP signaling result in embryonic lethality, highlighting the importance of the signaling. Among the many BMP receptors, it is not clear which is specifically involved in the context of the cells/tissue for organogenesis. Present study was developed to define the regulatory roles of Type 1 BMP receptor ALK2 in the development of taste organs. Using immunohistochemistry, we identified the ALK2 expression in the different tissue compartments of the tongue organ. The immunosignals of ALK2 emerged in the nerve branches at the base of tongue swellings at E11.5. At E12.5, ALK2 immunosignals were found in the tongue mesenchyme in addition to the nerve fibers. At E13.5-E15.5, ALK2 immunosignals were also seen in the tongue epithelium. *Wnt1-Cre* driven conditional knockouts of *Alk2* (*Wnt1-Cre/Alk2 cKO*) in the tongue mesenchyme resulted in a tongue with the development of various fungiform papillae at E12.5. *Wnt1-Cre/Alk2 cKO* additionally brought about tongues with ankyloglossia at E12.5. Moreover, fungiform papillae on the mutant tongue had a changed dissemination and shaped in the medium furrow, which is typically a region without gustatory papillae. Our information demonstrates an important role of BMP signaling in the proper formation of taste organs including the tongue and papilla patterning.

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Emily Pendleton

Mentor: Luke Mortensen

Bone Characterization in the Treatment of Hypophosphatasia with Mesenchymal Stem Cells

Author: Emily Pendleton, Ruth Barrow, Ana Maslesa, Trey Powell, Kayvan Tehrani, Luke Mortensen

We present the use of second-harmonic generation (SHG), X-ray imaging and skeletal staining to characterize bone morphology in a murine Hypophosphatasia (HPP) model. HPP is a devastating genetic disease caused by limited bone mineralization due to impaired alkaline phosphatase (ALP) function. The currently approved treatment is frequent administration of bone targeted recombinant ALP, but it does not treat all symptoms, including craniosynostosis. Therefore, an alternative therapy is needed. Mesenchymal stem cells (MSCs) have been used in previous clinical case studies along with myeloablation and full bone marrow transplants leading to improved bone mineralization, but this grueling procedure has long term complications, and patients with the greatest need are often too sick to be eligible. Therefore, our current efforts aim to develop an engineered MSC therapy to treat all HPP patients. Efforts are ongoing to evaluate the effects of MSC transplantation on our quantitative parameters in the calvarial bones and the femur. The results of our studies could transform therapy for HPP and other congenital bone diseases.

Regenerative Bioscience Center

Trey Powell

Mentor: Luke Mortensen

Oral Presentation

Class of 2020, Undergraduate Student

Investigating the use of mRNA Transfection to treat Hypophosphatasia

Authors: Trey Powell, Kayvan Tehrani, Emily Pendleton, Luke Mortensen

Hypophosphatasia (HPP) is a rare genetic disease that impairs the process of bone mineralization due to low activity of tissue-nonspecific alkaline phosphatase (TNAP). Severe hypophosphatasia also manifests with complications including hypercalcemia, vitamin B6-dependent seizures, and craniosynostosis. Many patients suffering from these symptoms die from respiratory failure. Other forms of HPP include perinatal, childhood, adult, and odonto-HPP with symptoms ranging from still birth to loss of teeth. Current treatment for HPP, enzyme replacement therapy, hinders the livelihood of HPP patients, as it requires daily intravenous treatment, and does not impact craniofacial or tooth defects. Mesenchymal stem cells derived from bone tissue have shown promise to treat HPP with a low allogenic transplantation rejection rate, and yield increased bone mineralization and muscle mass in patients receiving MSC therapy. To enhance mesenchymal stem cell (MSC) therapy as a treatment for HPP, I studied the application of mRNA transfection in MSCs using the CRE lox system. The transfection of mRNA may be used to alter protein expression in therapeutic MSCs to increase cell production of alkaline phosphatase or enhance transplanted cell engraftment. Here, mRNA transfection was used to express fluorescent proteins. Three experiments analyzed the transfection optimization and indicated that 1:3 and 1:5 mRNA to transfection reagent ratios produced the greatest overall cell fluorescence. Our results indicate the power of mRNA transfection to alter MSC behavior, which will be useful for in vitro and in vivo studies.

Regenerative Bioscience Center

Anita Qualls

Mentor: Jarrod Call

Poster Presentation

Class of 2019, Undergraduate Student

Early Rehabilitation to Augment Skeletal Muscle Function Following Volumetric Muscle Loss Injury

Authors: Anita Qualls, Anna S. Nichenko, W. Michael Southern, Zach Agan, Gordon L. Warren, Sarah M. Greising, Jarrod A. Call

Large scale traumatic orthopedic injuries or necessary surgical removal of skeletal muscle represent volumetric muscle loss (VML) injuries, and these injuries result in extensive long-term dysfunction. Injuries such as VML pose specific limitations to recovery of function because extensive *de novo* muscle fiber regeneration is inadequate following VML and long-term functional limitations present as vast reductions in muscle strength and joint range of motion. Current clinical rehabilitation approaches following VML injury are limited, as there is no standard guideline for surgical care or rehabilitation guidelines. The objective of this study was to investigate the extent to which the remaining muscle tissue after VML injury adapts to early rehabilitation electrical stimulation. Adult male mice underwent an ~20% multi-muscle VML injury to the posterior compartment and were randomized to rehabilitation twice per week beginning 72 hours post-injury. Mice were divided into two rehabilitation groups: passive ankle range of motion (ROM) serving as a clinical control model, and range of motion with intermittent electrical stimulation (ROM-E). Passive torque about the ankle joint, a measure of passive muscle stiffness, and sub-maximal isometric torque of the plantarflexor muscles was recorded during each therapy session. Cohorts of mice completed 1, 2, or 4 months of therapy prior to a final *in vivo* assessment of peak isometric torque and contractile fatigue resistance of the plantarflexor muscles. The remaining tissue after VML responded well to therapy as passive stiffness decreased 25% during each therapy session for both ROM and ROM-E mice ($P<0.01$) and sub-maximal torques generated at 20 Hz stimulation improved ~20% week-to-week in ROM-E mice ($P<0.04$). Peak isometric torque was 18%, 22%, and 14% greater in ROM-E mice compared to ROM mice at 1, 2, and 4 months, respectively ($P≤0.04$). Contractile fatigue resistance was 37% greater in ROM-E mice compared to ROM mice at 4 months ($P<0.01$). The lack of *de novo* regeneration was confirmed by large mass deficits in the injured limb compared to the contralateral uninjured limb (25% deficit, $P<0.01$) for both ROM and ROM-E mice, although there was a trend for greater injured limb muscle mass in ROM-E mice ($P=0.08$). In conclusion, early rehabilitation techniques combining range of motion with electrical stimulation may improve the function of the remaining tissue after VML injury. Future testing is needed to assess the metabolic component of rehabilitation intervention as well as the potential consequences of musculoskeletal co-morbidities, such as physical inactivity, associated with VML injury.

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Kelly Scheulin

Mentor: Franklin West

Nanoparticles Transport of FDA-Approved Drugs Across the Blood Brain Barrier in a Porcine Stroke Model

Authors: Kelly M. Scheulin, Anil Kumar, Madelaine N. Wendzik, Holly A. Kinder, Xueyuan Yang, Emily W. Baker, Erin E. Kaiser, Jen Xie, Franklin D. West

Stroke is the leading cause of disability and the fourth leading cause of death amongst adults in the United States. Despite the devastating effects of stroke, there is only one FDA-approved treatment, which in itself is only advantageous in a small fraction of strokes. Our research team has developed two enabling technologies 1) a highly lipophilic, biodegradable nanoparticle (NP) delivery platform that has a high zeta potential and the novel ability to cross the blood brain barrier (BBB) and 2) a novel translational pig stroke model. We hypothesize that this novel NP technology will be able to deliver FDA-approved, hydrophilic drugs past the BBB into the damaged brain tissue in our pig model, which will help reduce the cytotoxic environment for future stem cell transplant therapy. In preliminary studies, we have determined that the FDA-approved drugs, Co-Q-10 and Tetracycline, can be successfully incorporated into our novel NP vehicle (at concentrations of 304.92 ug/mL, 186.24 ug/mL, respectively). To trace NPs delivered into the injured brain tissue, we successfully incorporated the fluorescent Cyanine 5.5 dye into NPs. We have demonstrated that intravenous injection of NPs leads to high Cyanine 5.5 fluorescence concentrations in the brain, heart, lungs, kidneys, liver and spleen in a piglet neural injury model. These results suggest that NPs can cross the BBB and transport FDA-approved drugs to the injured site of our novel pig model. This study will provide a novel approach to stroke treatment and will likely lead to more success in human clinical trials.

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Justin Sharma

Mentor: Steven Stice

Oral Presentation

Class of 2018, Undergraduate Student

Human neural stem cell derived exosomes for pre-clinical trials in porcine stroke models

Authors: Justin Sharma, Robin Webb, Steven Stice

Stroke is currently the second most fatal cause of death globally, and the leading cause of physical disability. Several potential stem cell therapies are in consideration to address the urgent need for stroke treatment, but only one therapeutic has received FDA approval for clinical trials.

Standard small molecule pharmaceuticals can bypass the blood brain barrier to deliver treatment to damaged cerebral tissues, but these drugs are limited in their protective and regenerative properties. Although stem cells offer restorative abilities, whole cells cannot bypass the blood brain barrier. Recent studies suggest stem cell derived vesicles, termed exosomes, possess beneficial properties and deliver their contents directly to the site of injury.

Exosomes are small lipid vesicles containing proteins and RNA that are actively secreted by several cell types and circulate in all body fluids to communicate with other cells. Via a simple intravenous injection, exosomes can deliver therapeutics in the form of proteins or RNA for the purpose of treating brain injury. The lipid bilayer capsule of the exosomes facilitates the movement of contents from the blood stream to damaged tissues.

The objective was to collect neural stem cell (NSC) exosomes for evaluation in a porcine middle cerebral artery occlusion model. Gathering information on vesicle concentration and average exosome output by human NSCs in a controlled environment enables the scale up of production necessary for the sale of a biopharmaceutical. The success of exosome products demands optimization of more efficient purification methods. By examining shortcomings in conventional methods of cell culture, ArunA Biomedical can improve yield and minimize costs of NSC exosome production.

The success of a production run supports the large-scale manufacturing processes of exosomes in quantities relevant for large animal studies. These processes will be critical as these new technologies are transferred to biomanufacturing under quality control systems required for human clinical trials of exosome therapeutics.

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Aditya Sood

Mentor: Steven Stice

Poster Presentation

Class of 2019, Undergraduate Student

Analyzing TGF- β Regulation of the Progenitor Motor Neuron/Motor Neuron Glial Fate Switch

Authors: Aditya Sood, Tarun Daniel, Raymond Swetenburg, Steven Stice

In the developing primitive spinal cord, termed the neural tube, motor neuron progenitor cells (pMN) undergo a temporal change in potency, first generating motor neurons (MN) and, later, oligodendrocyte precursor cells (OPC). Oligodendrocyte transcription factor 2 (Olig2) is a critical fate determinant central to this fate switch. In vivo, Olig2 promotes self-renewal, and primes cells for neurogenesis, ultimately resulting in the generation of MN while maintaining a subset of undifferentiated pMN. pMN then co-express the transcription factor Homeobox protein Nkx-2.2 alongside Olig2, which together drive much of the genetic glial machinery, marking the initiation of oligodendrogenesis. The underlying mechanism(s) involved in this fate switch, namely the transcription and expression of Nkx2.2 in pMN, are poorly understood. Previous studies suggest that a soluble, secreted transforming growth factorβ family protein can regulate similar neural progenitor fate switches. Sonic hedgehog (Shh), a morphogenic inducer of both Olig2 and Nkx2.2 transcription, is also an important component in driving this initiation event, though alone it is insufficient in triggering glial initiation. Using an in vitro, pluripotent stem cell (PSC)-based model of neural tube development, we hypothesize that TGF-β primes pMN for the glial fate switch by altering the post-transcriptional state of Olig2. Further, we expect Shh to drive Nkx2.2 transcription. Ultimately, our studies will aid in developmental and disease modeling, stem cell manufacturing, drug and toxicity testing and regenerative medicine.

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Michael Southern

Mentor: Jarrod Call

Oral Presentation

Class of 2018, Graduate Student

Mitochondrial Abnormalities May Contribute to Skeletal Muscle Weakness and Poor Regeneration in Fukutin-Deficient Muscular Dystrophy Mice

Authors: W. Michael Southern, Anna S. Nichenko, Aaron M. Beedle, Jarrod A. Call

The dystrophin-glycoprotein complex (DGC) provides a link between extracellular and intracellular structures of the muscle cell. We have previously demonstrated that muscle-specific deletion of the fukutin gene [Myf5/*Fktn* KO mice (KO)] causes DGC disruption. KO mice present moderate to severe muscular dystrophy characterized by muscle weakness and delayed regeneration following muscle injury. The objective of this study was to determine the extent to which mitochondrial dysfunction contributes to the muscle weakness and delayed regeneration in these mice. We hypothesized that mitochondrial dysfunction contributes to muscle weakness in KO mice and that improving mitochondrial content would benefit muscle regeneration following muscle injury. We administered daily injections of saline or AICAR (500mg/d/kg), an agent previously shown to improve mitochondrial quality in dystrophic muscle, to both KO (saline n = 9, AICAR n = 6) and littermate (LM) control (saline n = 9, AICAR n = 8) mice. Two weeks after onset of treatment, the left hindlimbs (anterior and posterior compartments) of all mice were injured with cardiotoxin to induce muscle regeneration, while the right hind limbs served as contralateral uninjured controls. Mice were sacrificed 2 weeks post-injury. Body mass and gastrocnemius mass normalized to body mass was 21% and 13% lower in KO mice compared to LM controls (body mass: *P*<0.001, gastrocnemius: *P*<0.002). Pre-injury peak isometric torque about the ankle joint was 38% lower in KO mice compared to LM *(P*=0.044*)*. Interestingly, muscle strength was not fully recovered in any group of mice 2 weeks post-injury, but AICAR-treated mice had 25% greater muscle strength following injury compared to salinetreated mice, independent of genotype (*P*=0.036). Mitochondrial respiration of injured and uninjured permeabilized fibers was also assessed 2 weeks post-injury. Mitochondrial respiration was 23% lower in KO mice compared to LM mice, independent of treatment and injury *(P*=0.023). Further analysis is required to determine if this is due to lower mitochondrial content or mitochondrial dysfunction. While there was no effect of AICAR on mitochondrial respiration, AICAR-treated mice had ~14% greater levels of COXIV protein content compared to salinetreated mice, independent of genotype and injury (*P*=0.015). This suggests a longer duration of AICAR treatment may be necessary for gains in mitochondrial protein contents to be realized in terms of mitochondrial respiration. In conclusion, this data suggests that fukutin deficiency is associated with mitochondrial abnormalities that may contribute to skeletal muscle weakness and poor regeneration. AICAR treatment may facilitate strength recovery following muscle injury, although it is unclear the extent to which AICAR affects mitochondrial function following injury.
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Samantha Spellicy

Mentor: Steven Stice

Poster Presentation

Class of 2020, Graduate Student

Behavioral Improvements Following Neural Stem Cell Derived Exosome Therapy in a Porcine Model of Stroke

Authors: Samantha Spellicy, Brian Jurgielewicz, Erin Kaiser, Robin Webb, Michael Bowler, Holly Kinder, Simon Platt, Franklin West, Steven Stice

Stroke remains the 5th leading cause of death in adults in the United States, with the incidence projected to almost double by 2030[1,2]. Despite thousands of ongoing clinical trials for potential stroke therapies, currently only one FDA approved small molecule therapy exists to date. We proposed to use neural stem cell exosomes, a type of cell derived nanovesicle, as a potential novel therapeutic to combat behavioral and motor impairments following middle cerebral artery occlusion (MCAO) in a porcine model of stroke. First, we isolated the exosomes from neural stem cell spent media using an ultracentrifugation and filtration protocol and then characterized their size, morphology, and concentration using nanoparticle tracking analysis. We then IV administered the exosomes, or a sham saline solution, at 2, 14, and 24 hours' post MCA occlusion. We then performed open field and novel object recognition behavioral tests at 3 days post stroke, 1 week post, 3 weeks post and 12 weeks post, and used behavior tracking software to quantify differences between pigs in the exosome treatment and sham groups. During open field testing, we found that by 1 week post MCAO, sham pigs had statistically significant decreases in velocity and distance traveled in the testing arena, while the exosome treatment group did not. We also saw during open field testing, the amount of distance traveled by exosome pigs at 12 weeks was significantly greater than the distance traveled by sham pigs. We did not find any statistically significant differences in the novel object recognition test between the sham and exosome treatment pigs at any of the 5 tested time points. Overall, we found that neural stem cell derived exosome therapy following a right sided MCA occlusion in pigs aided in preventing a significant decrease in velocity and distance traveled during open field testing compared to sham, and had a significant positive influence on these parameters at certain time points compared to sham.

Min Sun

Mentor: Lohitash Karumbaiah

Evaluating Electrospun Nanofibers as 3D Scaffolds for Regenerative Therapy

Authors: Min Kyoung Sun, Indra Apsite, Leonid Ionov, Lohitash Karumbaiah

With multi-syringe pump and adjustable 3D printed needle holder, distance between needles and Spraybase® electrospinning equipment, and the flow rate were controlled. Electrospun fibers were collected on grounded rotating drum. Combination of polycaprolactone (PCL) and poly(N-isopropylacrylamide) (PNIPAM) polymer solutions were used to create multiple layers of substrates. Nanofiber layers were pressed together using 20 tons hydraulic press. Using a cylindrical punch, nanofiber substrates were cut into uniform circular discs of 1 mm diameter. They were placed on a 12-well plate and soaked in either methanol or 70% ethanol overnight, and rinsed thoroughly with PBS. ~200,000 human NSCs in 100uL expansion media were seeded onto each disc and allowed to adhere for an hour at 37℃/ 5% CO_2. Then 1mL of expansion media was added to each well and replaced every two days for seven days. For Hoechst/PKH26GL staining, cells were labeled before seeding and fixed in PBS containing 4% paraformaldehyde and 0.4M sucrose at the end of seven days for imaging. For the alamarblue assay, the old media was replaced with new media with 10% alamarblue reagent at the end of seven days, and incubated for 4 hours. Supernatant was collected and transferred to 96 well plate to measure the fluorescence. Hoechst/PKH26GL staining of HIP NSC seeded on unpressed electrospun PCL, pressed PCL and PCL/PNIPAM bilayer showed qualitative differences in cell adhesion. Ongoing quantitative assessments will determine the extent of NSC adhesion and neuronal differentiation if any. We expect the electrospun nanofiber scaffolds with PCL/PNIPAM to induce enhanced NSC adhesion, which could potentially be applied to the fabrication of clinically translatable solutions to bridge peripheral nerve gaps.

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Pavan Suryadevara

Mentor: Sheba MohanKumar

High fat diet affects serum fatty acid profiles differentially in diet-induced obese and dietary resistant rats: Reversal by Metformin

Authors: Pavan,Suryadevara, Priya Balasubramanian, Andrew Shin, Poliyur MohanKumar and Sheba MohanKumar

Obesity is associated with increased adipose tissue deposition and altered stress axis activity. The hypothalamus serves as the center for stress axis activation and we have demonstrated previously that free fatty acids are capable of affecting the hypothalamus to alter stress axis activity. In this study, we hypothesized that fatty acid profiles are altered in diet-induced obesity. This could be a reason why the stress axis is affected in obese animals. To test this, we used a diet induced obese (DIO) and a dietary resistant (DR) rat model and compared it to normal Sprague Dawley (SD) rats. DIO and DR rats were placed on chow or a high fat diet for 1 or 6 weeks. Finally a group of high fat fed DIO and DR rats were treated with a low dose of metformin (100mg/Kg BW) to see if this could reverse the effect on serum free fatty acids. Serum from trunk blood was analyzed using GC-MS for fatty acids such as Oleic, linoleic and arachidonic acids. Basal free fatty acid levels were higher in DIO rats compared to SD rats, while DR and SD rats had comparable levels. When placed on a high fat diet, DIO rats had 3 fold increases in oleic acid after 1 and 6 weeks exposure compared to their chow fed counterparts, while there were modest increases in arachidonic acid. DR rats had no significant changes in fatty acid levels after 1 week of high fat, but there were marked increases in oleic acid levels after 6 weeks of high fat diet.   Metformin treatment was capable of reducing linoleic acid after 1 week and reduced oleic and linoleic acid levels after 6 weeks of treatment in DIO rats. These results indicate that DIO rats have higher circulating free fatty acids that could have metabolic implications and treatment with metformin is capable of reducing these levels possibly producing beneficial outcomes.

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Raymond Swetenburg

Mentor: Steven Stice

A Computational and Experimental Approach to Elucidating Progenitor Cell Fate Decisions

Authors: Raymond L. Swetenburg, Steven L. Stice

The controlled and robust derivation of specific cell types from pluripotent stem cells is critical to the promise of cell manufacturing and regenerative medicine while providing an *in vitro* platform for developmental and disease modeling, and drug and toxicity testing. The differentiation process is essentially a stepwise series of binary fate decisions made by multipotent stem and progenitor cells as they progress towards a more terminal state. Each fate decision is based on the complete internal state of the cell and the mechanical, electrical, chemical and biological cues in its surrounding microenvironment. Thus, each cell is presented with a certain amount of external information which interfaces directly and indirectly with its internal genetic landscape to generate a predictable outcome: self-renewal or differentiation. Notch-Delta signaling is a form of juxtacrine signaling fundamentally involved in the self-renewal of most dividing cells, particularly in the developing nervous system. However, Notch-Delta acts in a context-dependent manner, yielding contradictory outcomes in varying temporal contexts, specifically in the oligodendrocyte transcription factor 2- (Olig2) expressing motor neuron progenitor cell type. Therefore, we sought to probe the interplay of various genetic factors with Notch-Delta signaling in a pluripotent stem cell-derived, *in vitro* model of spinal cord development. Using a combined experimental and computational approach, we identified potential novel mechanisms and cell states, as well as a set of testable biological hypotheses which will ultimately lead to a better understanding of cellular differentiation and its implications.

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Zhonghou Wang

Mentor: Hongxiang Liu

Origin of taste buds in chickens

Authors: Zhonghou Wang, Wenxin Yu, Xiaogang Cui, Hong-Xiang Liu

Taste cells, the chemoreceptor cells in taste buds, are specialized cells that transduce gustatory stimuli into neural signals which are conveyed to the central nervous system for the sensation of taste that is essential for life and quality of life. Therefore, taste cell origin and differentiation is a fundamental issue for the development of taste organs and taste function. Studies examining taste cell origin and differentiation will significantly advance understanding of mechanisms underlying taste disorders caused by progenitor defects (radiotherapy, chemotherapy, and neuropathies), and will thus provide vital information for treatment of patients with taste disorders in many clinical settings. However, we lack basic understanding regarding the precursor source of taste bud cells. Actually there has been a debate over whether taste buds arise solely from local epithelium or have a dual origin from both neural crest and epithelium. To test the hypothesis that neural crest migrates and differentiate to taste bud cells, we are using a GFP and non-GFP chicken chimera model. In this model, neural fold which contains the progenitors of neural crest from a GFP chicken embryo was dissected and transplanted to a regular non-GFP chicken embryo. This allows us to solidly trace the lineages of neural crest. We have demonstrated that (1) transplanted GFP+ neural fold were successfully integrated into the host chicken embryo; (2) transplanted GFP$^+$ neural fold cells were expanded and migrated into the target tissue regions, i.e., primordia of upper beak and lower beak where gustatory tissue will develop. Importantly, we observed GFP$^+$ cells in the epithelium of the base of oral cavity where taste buds develop at 3 days after surgery. Studies are ongoing to harvest oral tissues from the chimera embryos at different time points to trace the GFP$^+$ cells in taste buds.

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Madelaine Wendzik

Mentor: Franklin West

Characterization of a Piglet Model of Traumatic Brain Injury Utilizing Non-Invasive Magnetic Resonance Imaging and Histological Assessment

Authors: Madelaine N. Wendzik, Emily W. Baker, Holly A. Kinder, Silun Wang, Hui Mao, Franklin D. West

Traumatic brain injury (TBI) is a major cause of death and disability in the United States, chiefly affecting children ages 0-4 years. TBI at such a young age may lead to long-term neurological deficits. Animal models not truly representative of the human condition have impeded development of a translatable TBI treatment, suggesting a more human-like animal model, such as a piglet, is necessary for developing an effective therapy. Magnetic resonance imaging (MRI) and histological assessments are pertinent in the comprehensive understanding and treatment of TBI at the tissue and cellular levels. We hypothesized that controlled cortical impact (CCI) would result in a concussive piglet TBI model with substantial changes in lesion and hemisphere volume coupled with distinct histological changes that persist over time. TBI was induced in six male piglets, with MRI scans conducted 24 hours and 12 weeks post-TBI to measure the lesion size and midline shift. Histological changes were observed by quantifying NeuN+ neurons, GFAP+ astrocytes, and Iba1+ microglia in the cortical peri-lesion area at 1 day, 1 week, 4 weeks, and 12 weeks post-TBI. Lesion size at 24 hours post-TBI was 3.44 cm^3 (± 0.52) with a midline shift of $+1.80$ mm (± 0.46). Lesion size was significantly reduced comparatively at 12 weeks to 1.95 cm^3 (± 0.44) with a significant change midline shift of -2.98 mm (± 0.29) as compared to 1 day post TBI. There was a significant ($p<0.01$) decline in NeuN+ neurons after 1-week post-TBI that persisted through 12 weeks post-TBI. GFAP+ astrocytes increased significantly ($p<0.0001$) from normal starting 1 day post-TBI through 12 weeks post-TBI. Furthermore, significant increases in GFAP expression were observed between each timepoint. Lastly, Iba1+ microglia increased significantly ($p<0.05$) at each timepoint. The observed directional change in midline shift and decrease in lesion size can be attributed to attenuated swelling and significant brain atrophy. We observed a substantial decline in NeuN+ neurons suggesting there was significant cell death in response to the injury that never recovered over a 12 week timecourse. We also observed a significant upregulation in GFAP+ astrocytes and Iba1+ microglia which suggests that TBI leads to gliosis and inflammatory response that mounts over time in response the injury. The characterization of key cytoarchitectural changes in the CCI TBI piglet model will enable more robust and predictive assessments of novel therapeutics that will likely lead to more success in human clinical trials.

Wenxin Yu

Mentor: Hongxiang Liu

Poster Presentation
Class of 2019, Graduate Student

SOX10-Cre labeled cells are distributed in mature taste buds in mouse tongue and soft palate

Authors: Wenxin Yu, Guiqian Chen, Brett Marshall, Hong-Xiang Liu

Taste buds have been described as solely arising from local surrounding epithelium. However, our recent studies using *P0-Cre*, *Dermo1-Cre* and *Vimentin-CreER* mouse models demonstrated that the underlying connective tissue, which is primarily derived from the neural crest (NC), contributes to early and mature taste buds. These data strongly suggest a potential NC origin of taste buds. In contrast, labeled cells of *Wnt1-Cre*, widely taken as the "gold standard" for NC lineage tracing, were rarely seen in taste buds. In order to resolve this dilemma and test whether the NC contributes to taste buds, we used a mouse model, *SOX10-Cre*, to specifically label NC lineages, and analyzed the distribution of labeled cells, most likely NC derived cells, in taste buds. In E8.5 *SOX10-Cre* mouse embryos, Cre immunosignals were restricted to migrating NC cells. In postnatal *SOX10-Cre/RFP* mice at different stages (2, 4, 8, 16 weeks), we observed that (1) *SOX10-Cre* labeled cells were abundant within taste buds in all three types of taste papillae (fungiform, foliate, and circumvallate) and in the soft palate; (2) *SOX10-Cre/RFP* signals in taste buds were seen in all three types of differentiated taste bud cells, identified using NTPDaseII for type I, alpha-Gustducin for type II, SNAP25 for type III. Together, our data using the *SOX10-Cre/RFP* mouse model provides strong evidence for the NC origin of a significant population and various types of taste bud cells.

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